# HYPOGLYCEMIC, HYPOLIPIDEMIC AND BIOCHEMICAL EFFECTS OF *TITHONIA DIVERSIFOLIA* AQUEOUS ROOT EXTRACT IN WESTERN DIET FED WISTAR ALBINO RATS.

BY

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE DEGREE OF THE DEPARTMENT OF MEDICAL BIOCHEMISTRY, MOI UNIVERSITY.

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## DECLARATION

## **Declaration by the Candidate**

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# **DEDICATION**

This thesis is dedicated to my loving husband Edwin and our lovely children Patience, Praston and Powell.

## ABSTRACT

**Background:** Diabetes and obesity pose risks of severe complications, including cardiovascular diseases and cancer. The Luo community in Kenya uses Tithonia diversifolia root preparations for hypoglycemic and hypolipidemic effects, necessitating scientific validation.

**Objectives**: To determine the hypoglycemic and hypolipidemic effects of aqueous root extract of *Tithonia diversifolia* and its biochemical effects on the liver and kidney of western diet-fed Wistar albino rats.

**Methods**: This was a laboratory-based study in wistar albino rats with elevated fasting blood glucose and lipids. The rats were put on a western diet composed of rodent chow enriched with 21% animal lard and 0.15% cholesterol for 35days. Thirty-five male rats weighing 180-200g were selected, acclimatized for one week, and randomly grouped into 7 groups of normal control(G1), those fed on western diet for 35 days(G2), the ones fed on western diet for 35days and given 10mg/kg atorvastatin in the last 7 days(G3), those fed on western diet for 35days and given 0.5mg/kg glibenclamide in the last 7 days(G4), the ones fed on western diet for 35days and given 200mg/kg(G5), and 400mg/kg of the extract (G6) respectively in the last 7 days (G7). Blood fasting glucose levels were determined weekly by obtaining blood from the tail.

In contrast, lipid profile, kidney and liver function were determined using blood obtained by cardiac puncture at the end of the experiment. Data was stored in SPSS version 20 and analyzed for means, post-hoc Least Significance Difference, and Duncan's tests to compare the pairs of groups. A p-value  $\leq 0.05$  was considered statistically significant.

**Result:** The fasting blood glucose levels gradually increased in all the groups between weeks one to four though maintained normal range for wistar albino rats (3.95±1.31 mmol/L). There was a significant reduction (p=0.000) in serum cholesterol (normal 1.06-3.25mmol/L) when the negative control group(G2) was compared to the groups that received 200mg/kg(G5) mean 2.0mmol/L, 400mg/kg of the extract(G6) mean1.2mmol/L and 10mg/kg Atorvastatin(G3) mean 2.0mmol/L. For triglycerides (0.5-2.9mmol/L) p=0.036 the mean values in G5, G6 and G3 were 1.0, 1.0 and 0.4mmol/L respectively. There was a significant increase (p=0.000) in urea levels (normal levels 3.9-8.9mmol/L) and a significant reduction (p=0.011) in creatinine (17.68-61.88umol/L) among groups given 200mg/kg and 400mg/kg extract compared to positive control. There was no significant increase in levels of aspartate aminotransferase (p=0.264) and alanine aminotransferase (p=0.264), whose normal levels are 34-109U/L and 198.68  $\pm$ 15.66U/L respectively, but there was significant (p=0.000) difference in alkaline phosphatase(95-611U/L) after administration of 200mg/kg of extract daily for seven days. Reverting to rodent chow for seven days(G7) did not significantly change all the parameters except creatinine and urea, where the changes were significant compared to the positive control group.

**Conclusion:** Aqueous root extract of *Tithonia diversifolia* administered at 200mg/kg and 400mg/kg is safe for the liver and demonstrated hypoglycemic and hypolipidemic activity similar to standard drugs glibenclamide and atorvastatin, respectively. However, it seems to be associated with glomerular damage, as evidenced by the elevated levels of urea.

**Recommendation**: Further studies are recommended in order to establish proper dosage and kidney toxicity.

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# LIST OF ACCRONYMS

ANOVA	Analysis of variance
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ALP	Alkaline phosphatase
b.w	body weight
CHD	Coronary heart disease
СНО	Cholesterol
CVD	Cardiovascular diseases
HDL	High-density lipoprotein
REC	Research Ethics Committee
LD	Light-darkness
LDL	Low-density lipoprotein
LSD	Least Significance Difference
SPSS	Statistical Package of Social Sciences
TD	Tithonia diversifolia
TGs	Triglycerides
T2DM	Type-2 diabetes
WHO	World Health Organization

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#### **CHAPTER ONE**

#### **1.0 INTRODUCTION**

## 1.1 Background

Diabetes mellitus (DM) is characterized by abnormally elevated blood glucose concentrations that are associated with increased mortality, microvascular and macrovascular complications (Nathan, 1993). It is defined by increased glucose levels and glycosuria resulting from impaired pancreatic  $\beta$ -cell function and insulin resistance (Liu *et al.*, 2021). In contrast, obesity is characterized by elevated levels of very low-density lipoprotein, low-density lipoprotein, and total serum cholesterol. Hyperlipidemia is associated with lipid disorders and is a well-known contributor to atherosclerosis, serving as a primary risk factor for cardiovascular diseases (DeFronzo *et al.*, 2015). Both elevated blood glucose concentrations and lipid levels have long-term consequences, leading to organ damage, dysfunction, and failure (Daryabor *et al.*, 2020).

Diabetes mellitus and obesity have shown an alarming increase worldwide and are considered the fourth or fifth most important cause of death globally. These two conditions are linked to severe complications such as peripheral vascular diseases, coronary artery problems, stroke, diabetic neuropathy, blindness, and kidney failure. The predisposing factors to the rapid rise of these conditions are sedentary lifestyles, nutrition transitions, urbanization, and aging (Tuso, 2014).

It is projected that by 2045, the global diabetic population will exceed 693 million individuals (Reddy & Mahesh, 2018). The global burden caused by obesity and overweight is on the rise, leading to significant economic, social, and health consequences. Approximately 2.8 million

people worldwide perish annually due to obesity-related illnesses. Excess weight increases triglycerides, total cholesterol, low-density lipoprotein (LDL), or "bad cholesterol," and reduces high-density lipoprotein (HDL), known as "good cholesterol," increasing the risk of type 2 diabetes and cardiovascular diseases (CVDs). This happens because increased body fat hinders insulin from effectively incorporating glucose into cells, leading to elevated blood glucose levels (Okube & Omandi, 2019). Approximately 80% of fatalities related to diabetes mellitus can be attributed to cardiovascular diseases, mainly as a result of coronary artery disease (Hayat *et al.*, 2004).

Diabetes mellitus has remained one of the most expensive and burdensome chronic illnesses, increasing public health concerns. Among individuals above 60, stroke and coronary/ ischemic heart disease (CHD) are emerging as the number one cause of more than a quarter of deaths in this age group (Zhou *et al.*, 2020). In Africa, this represents a remarkable shift as coronary heart disease and stroke, which were not known until recently, have presented mortality rates and prevalence comparable to high-income countries. African countries are thus undergoing a shift in the epidemiological change in lifestyle diseases while retaining a high burden of infectious diseases (Boutayeb, 2006).

In Africa, particularly sub-Saharan Africa, Diabetes mellitus and obesity are the leading causes of mortality among persons aged 30 years and above. Moreover, the epidemiologic burden of these two conditions threatens to impose a significant economic burden on middle and low-income countries. Common risk factors for Diabetes mellitus and obesity in Africa include obesity, sedentary lifestyles, unhealthy diets high in sugar and saturated fats, genetic predisposition, and socioeconomic factors. Diabetes mellitus and obesity can lead to atherosclerosis, especially stroke and cardiovascular disease risk factors, notably

hypertension (Jagannathan *et al.*, 2019). Risk factors for cardiovascular diseases associated with these conditions are reportedly rising in Kenya (Watetu *et al.*, 2019). Urbanization, which has been rapidly advancing in Kenya, may adversely affect physical health due to dietary shifts in urban areas. Consequently, the rural-to-urban transition may increase obesity and overweight levels nationwide, although Kenya is expected to face a dual burden of malnutrition in the near future (Peters *et al.*, 2019).

According to the STEP-wise survey in Kenya, the prevalence of Diabetes mellitus in adults is estimated at around 4.56%, affecting approximately 750,000 individuals and resulting in 20,000 annual deaths. Generally, 87.8% of Kenyans have never been screened for Diabetes mellitus, and fewer than half (40.1%) are receiving treatment among those diagnosed. The disease significantly impacts healthcare expenditures and has become a significant medical and economic burden in Kenya (Kweyu *et al.*, 2022).

Data on the prevalence of dyslipidemia and its association with poor blood pressure control among obese patients in Kenya are scarce (Nderitu, 2020). A recent household survey in Kenya revealed that 27% of the adult population is affected by obesity (Wamai *et al.*, 2018). A "hyperlipidemia transition" is taking shape in Kenya, forming part of a double burden of diseases as the country grapples with infectious diseases that, although in decline, still dominate Kenya's disease burden (Roth, 2018).

Commonly used remedies for managing Diabetes mellitus include oral hypoglycemic medications and insulin administration. Studies have identified several side effects of these oral hypoglycemic drugs, such as hemolytic anemia, weight gain, fluid retention, peripheral

edema, central nervous system disorders, dermatological reactions, nausea, and bloating (Isitua *et al.*, 2018).

Furthermore, the cost of insulin and other medications used to manage this condition is considered high, especially for low-income communities. Consequently, many patients in third-world countries are compelled to turn to traditional medicines suspected of having some therapeutic value (Inteso & Isaacs, 2021). Throughout history, humanity has relied on phytomedicine to manage hyperglycemic and hyperlipidemic conditions (Amirehsani *et al.*, 2021) and hypertension (Huan *et al.*, 2021) because medicinal plants are known to be cost-effective, readily available, safer, and better tolerated, less likely to produce side effects, and have therapeutic potency and effectiveness (Erion *et al.*, 2016).

The goal of the present study was to evaluate the hypoglycemic and hypolipidemic activity of the aqueous root extract of *Tithonia diversifolia* in Western diet-fed Wistar albino rats, comparing them with glibenclamide and atorvastatin, which are the standard drugs for Diabetes mellitus and obesity, respectively.

The plant's safety and toxicity have been confirmed in its leaf extracts. Higher doses of more than 400 mg/kg of the aqueous leaf extract of *Tithonia diversifolia* caused significant changes in biochemical, histopathological, and hematological parameters in Wistar rats after 14 days of treatment (Fakunle & Abatan, 2007). In contrast, a 100 mg/kg dose was well-tolerated in rats after seven days of treatment (Adebayo *et al.*, 2009). However, a 200 mg/kg dose led to heart and liver damage, as indicated by elevated alkaline phosphatase levels in these tissues (Mabou *et al.*, 2018). Additionally, continuous administration of 10 mg/kg of the same

extract for 90 days increased alkaline phosphatase levels and reduced white blood cell counts in rats (Passoni *et al.*, 2013).

In summary, these findings suggest that *Tithonia diversifolia* is relatively well-tolerated in Wistar rats when administered orally at lower doses (<100 mg/kg) for a short-term period (less than seven days). However, higher-than-required doses can cause adverse side effects, such as kidney damage and hepatic dysfunction. *Tithonia diversifolia* is widespread in tropical and subtropical climates, and its leaves have traditionally been used by indigenous people to treat various ailments and diseases (Mabou *et al.*, 2018). In Kenya, the Luo communities traditionally use boiled roots to maintain normal blood sugar and lipid levels. The claim regarding the hypoglycemic and hypolipidemic activity of this plant's roots formed the basis of this study, as it has not been scientifically tested, proven, or documented. Therefore, this study aimed to investigate the hypoglycemic and hypolipidemic effects of the aqueous root extract of *Tithonia diversifolia* in Western diet-fed Wistar albino rats, along with its impact on the liver and kidney.

## **1.2** Statement of the problem

Approximately 200 million persons worldwide suffer from Diabetes mellitus, which is expected to reach 300 million by 2025. The major reason for this global increase in Diabetes mellitus is primarily associated with obesity. The International Diabetes Federation (IDF) has reported that the prevalence of diabetes mellitus has reached epidemic levels globally. The IDF estimates that in 2010, 285 million adults had diabetes, an increase of 39 million people from 2007. The projection for 2030 is 439 million (Waly *et al.*, 2010)

In sub-Saharan Africa, Diabetes mellitus is estimated to affect 40.7 million people by 2045, up from 15.9 million in 2017. This burden is exacerbated by estimates showing that more than two-thirds of individuals in sub-Saharan Africa with diabetes go undiagnosed. Due to the increasing demand and insufficient funding for diabetes care, the quality of diabetes care in sub-Saharan Africa is below average. Additionally, diabetes mellitus and obesity represent a significant "double burden" of infectious and chronic diseases (Mercer *et al.*, 2019).

The World Health Organization estimates that the prevalence of Diabetes mellitus in Kenya is at 3.3% (Chege, 2010), and this figure is predicted to increase to 4.5% by 2025 (Mcferran *et al.*, 2008). Studies have shown that Kenya has a prevalence of 14.2% in the general population, 2.2% in rural areas, and 12.2% in urban residents (Christensen *et al.*, 2009), partly due to sedentary lifestyles.

The number of individuals with Diabetes mellitus and obesity continues to rise due to population growth, urbanization, aging, and the increasing prevalence of obesity and physical inactivity (Kiptisia *et al.*, 2020).

The impact of hyperglycemic and hyperlipidemic treatment on patients, economies, and societies, particularly in less developed and developing countries cannot be overstated. For many patients in Kenya, the cost of treatment for the mentioned conditions is prohibitively high, posing financial challenges for their families. As a result, many of these patients fail to adhere to treatment measures, putting them at a higher risk of developing end-organ damage. If the cost of prevention can be kept sufficiently low, it can lead to a long-term reduction in medication costs. A research study estimates that a hyperglycemic patient spends an average of 349,000 shillings on medications per year (Hall *et al.*, 2011). Due to inflation over time,

this cost is likely even higher now. The management of Diabetes mellitus and obesity remains a global crisis, and to date, a fully effective and successful medication has not been discovered (Henry *et al.*, 2020).

Available remedies for diabetic conditions include various oral hypoglycemic agents like metformin, sulfonylureas, glucosidase inhibitors, troglitazone, and insulin, among others. However, these modern remedies are known to cause adverse side effects such as lactic acidosis, diarrhea, and liver problems (Rashid & Abdelgadir, 2019).

Phytomedicine has become increasingly common in primary healthcare, especially in developing countries, with many people assuming that they are safe simply because they are natural, even though there is a lack of scientific evidence to confirm their safety for consumption (Chan, 2003). For instance, bitter melon (*Momordica charantia L.*) is used to treat fever and malaria (Adoum, 2009), but its green seeds are known to be very toxic, leading to a rapid drop in blood glucose and potentially inducing hypoglycemic coma (Li *et al.*, 2004) due to structural similarities to animal insulin in bitter melon extract components (Basch *et al.*, 2003).

While several published studies have investigated the hypoglycemic and hypolipidemic effects of the leaf and stem extracts of *Tithonia diversifolia*, more studies need to be done on the root extract's effects. However, some Luo communities in Kamagambo Sub-location, Rongo District, Migori County, Kenya, believe that the aqueous root extract of *Tithonia diversifolia* has the potential to reduce blood sugar levels in hyperglycemic individuals and lower fat content in obese individuals. This study aims to scientifically validate this claim

and assess whether it may cause any harm to organs such as the liver and kidney through the analysis of liver enzymes and some kidney biomarkers.

Due to the financial burden of managing diabetes mellitus and obesity, especially in developing countries, it can be challenging for communities to maintain a steady supply of drugs. Consequently, many diabetic and hyperlipidemic patients may not adhere to treatment measures, putting them at a higher risk of developing end-organ damage.

Furthermore, due to the high cost and unwanted side effects associated with current hypoglycemic and hypolipidemic therapies, there is an increasing consumption of herbal remedies by communities who believe these products are safe for treating and managing diseases simply because they are natural. However, herbal formulations, which may be assumed to be safe, can contain toxins, and their long-term use may lead to hepatotoxicity, nephrotoxicity, neurotoxicity, cardiotoxicity, and skin toxicity (Fatima & Nayeem, 2016). For example, the aqueous leaf extract of *Tithonia diversifolia* is reported to cause significant changes in biochemical, histopathological, and hematological parameters in Wistar rats after 14 days of treatment at doses higher than 400 mg/kg, indicating its toxicity with prolonged use at this concentration (Fankule & Abatan, 2007).

## **1.3** Justification of the study

Herbal remedies are favored for their ready availability, cost-effectiveness, efficiency, potency, low cost, improved tolerance, perceived minimal side effects, accessibility, and recyclability (Maiti & Kesari, 2011). The use of *Tithonia diversifolia* aqueous root extract in this study is based on the traditional practice among the Luo community, where it is used to manage diabetes mellitus and obesity. However, there is no scientific documentation

supporting these claims of hypoglycemic and hypolipidemic activity and their safety, which underscores the rationale for the present study. The entire plant, crude extracts, or purified constituents are used in the native system of medicines, which have eventually evolved into contemporary therapeutic sciences (Rehman et al., 2017). The crude root extract was employed in this study because the communities predominantly use boiled preparations for disease treatment and management. Experimental assessment of the toxicity of herbal plants is vital to ensure safety before human exposure. Historically, oral administration is the most suitable and commonly used route for studying toxicity. The oral route of administration aligns with the traditional use of herbal plant roots. Diabetes mellitus and obesity are affecting an increasing number of patients and significantly diminishing their quality of life (Ciulla *et al.*, 2003). Modern medications for managing these conditions are prohibitively expensive for many patients, and most of these medications are associated with adverse side effects (Schattner, 2022). Incorporating herbal medicine into the modern healthcare system may significantly enhance overall healthcare. This study assessed key parameters mainly affected during hyperlipidemic and hyperglycemic states, including body weight and blood sugar levels. Additionally, the biochemical changes in the liver and kidney were examined in Western diet-fed Wistar rats following treatment with the aqueous root extract of *Tithonia* diversifolia.

While many studies have been published focusing primarily on the leaf extract of *Tithonia diversifolia* to demonstrate its hypoglycemic and hypolipidemic properties, a segment of the Luo Nyanza community in Kamagambo sub-location, Rongo district, Migori County, Kenya, believes that the aqueous root extract of *Tithonia diversifolia* has the potential to reduce blood sugar levels in diabetic individuals and lower fat content in obese individuals. The current

study aimed to scientifically validate these claims and assess whether the extract may cause harm to the liver and kidney by analyzing liver enzymes and selected kidney biomarkers.

## 1.4 Research Questions

- 1. Does Tithonia diversifolia aqueous root extract possess hypoglycemic effects?
- 2. Does Tithonia diversifolia aqueous root extract possess hypolipidemic effects?
- 3. How does ingesting aqueous root extract of *Tithonia diversifolia* affect biochemical markers of kidney and liver functions?

## 1.5 Objectives

## **1.5.1 Broad objective**

To evaluate the hypoglycemic, hypolipidemic effects of *Tithonia diversifolia* aqueous root extract, and kidney and liver functions on western diet-fed Wistar albino rats.

## 1.5.2 Specific objectives

- 1. To qualitatively determine the phytochemical composition of the aqueous root extract of *Tithonia diversifolia*.
- 2. To determine the hypoglycemic effect of *Tithonia diversifolia* aqueous root extract on western diet-fed Wistar albino rats.
- 3. To determine the hypolipidemic effect of *Tithonia diversifolia* aqueous root extract on western diet-fed Wistar albino rats.
- 4. To determine the biochemical effects of *Tithonia diversifolia* aqueous root extract on kidney and liver functions in Western diet-fed Wistar albino rats.

#### **CHAPTER TWO**

## 2.0 LITERATURE REVIEW

## 2.1 The biochemical basis of obesity due to diabetes mellitus

The association between hyperglycemic and hyperlipidemic conditions is a well-known occurrence. High blood glucose directly has a significant effect on blood lipid levels, thus leading to lipid imbalance among people with diabetes (Belayneh *et al.*, 2019). Those who are hyperglycemic are more prone to have increased levels of serum triglycerides (TG), elevated levels of serum low-density lipoprotein cholesterol (LDL-C), and reduced levels of serum high-density lipoprotein cholesterol (HDL-C), in comparison to those whose blood sugar levels are normal (not diabetic) (Belayneh *et al.*, 2019)

The most common lipid abnormalities in diabetic patients include reduced high-density lipoprotein (HDL) and hyper-triglyceridemia (Hirano, 2018). Even though lipid abnormalities improve with glycemic control, normalization does not occur because there is a close connection between diabetic patients, particularly type-2 diabetes and obesity. Therefore, the management of obesity should begin with glycemic control (Garber *et al.*, 2020).

The elevated concentration of lipids (fats) in the bloodstream often occurs in Diabetes mellitus, where the levels of blood glucose are abnormally high (Ahmed, 2022). This biochemical relationship is particularly relevant in the context of diabetes, where both conditions frequently coexist. The interplay between diabetes mellitus and obesity involves several intricate processes at the cellular and molecular levels.

One of the fundamental mechanisms linking diabetes mellitus to obesity is insulin resistance. In type 2 diabetes and insulin-resistant states, the body's cells become less responsive to insulin (Schofield *et al.*, 2012), a hormone that plays a central role in glucose metabolism and lipid regulation. This resistance impairs glucose uptake by cells, leading to an accumulation of glucose in the bloodstream. In response to diabetes mellitus, the body adapts by increasing insulin release. However, this can trigger changes in lipid metabolism, including increased synthesis and release of triglycerides and other lipids into the blood.

Diabetes mellitus can also stimulate the liver to engage in gluconeogenesis, which synthesizes glucose from non-carbohydrate precursors like amino acids and glycerol (Cătoi *et al.*, 2015). The increased production and release of glucose from the liver contribute to higher levels of glucose in the bloodstream. These elevated glucose levels can, in turn, promote the release of free fatty acids (FFAs) from adipose tissue (Bays *et al.*, 2004). Increased FFAs in the bloodstream provide a substrate for triglyceride synthesis and contribute to the elevated lipid levels in obesity associated with increased blood glucose levels (Choi *et al.*, 2011).

The implications of obesity due to diabetes mellitus are profound, particularly in the context of diabetes. This combination of conditions significantly increases the risk of atherosclerosis, a condition characterized by the buildup of fatty deposits in the arterial walls. Elevated levels of triglycerides and low-density lipoproteins (LDL) contribute to the formation of atherosclerotic plaques. Over time, these plaques can narrow and harden the arteries, leading to an increased risk of cardiovascular complications such as heart disease and stroke (Saçlı *et al.*, 2018).

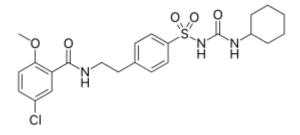
Furthermore, the presence of both diabetes mellitus and obesity can adversely affect pancreatic beta-cell function. Persistent high glucose and lipid levels can damage the cells that are responsible for producing insulin (Cerf, 2013). This dual metabolic stress further promotes insulin resistance, creating a challenging cycle in diabetes management. The risk of cardiovascular disease is significantly heightened due to endothelial dysfunction, inflammation, and oxidative stress induced by this combination of conditions (Incalza *et al.*, 2018). Abnormal lipid profiles characterized by high triglycerides, low high-density lipoproteins (HDL), and elevated LDL cholesterol levels, further increase the cardiovascular risk for persons with diabetes mellitus (Jain *et al.*, 2016).

## 2.2 Glibenclamide

#### 2.2.1 Hypoglycemic Activity of Glibenclamide

Glibenclamide (1) belongs to a group of drugs referred to as sulfonylureas. It is an antidiabetic drug that is used in the management of type-2 diabetes.

It is an effective sulfonylurea medication that promotes the control of glucose levels by acting on insulin action and insulin secretion (Monami *et al.*, 2006)



(1)

#### 2.2.2 Glibenclamide's Mode of Action

This drug functions by closing the channels of potassium ATP-sensitive on the beta cells of the pancreas. These potassium ATP-sensitive channels found on the beta cells are called sulfonylurea receptor 1 (SUR1) (Gribble & Reimann, 2003).

Glibenclamide functions by binding to and then inhibiting the channels of potassium ATPsensitive ( $K_{ATP}$ ) (Esmaeili *et al.*, 2018), the regulatory subunit sulfonylurea receptor 1 (SUR1) (Xu *et al.*, 2019) in beta cells of the pancreas.

This drug acts on the pancreatic beta-cells in order to stimulate the secretion of insulin. Under physiological conditions, the secretion of insulin from pancreatic beta-cells is mediated by increased concentration of blood glucose levels. Glucose then enters the cell through GLUT2 (SLC2A2) transporters (Berger, 2020). Once glucose is inside the cell, it is metabolized to generate adenosyl triphosphate (ATP). Increased concentration of ATP will slow down or inhibit ATP-dependent potassium channels (ABCC8), which will depolarize the cell. Depolarization leads to an opening of channels of calcium voltage-gated, this allows calcium to go into the cell. An increase in the intracellular calcium then stimulates exocytosis of the vesicle and insulin secretion. Glibenclamide stimulates insulin secretion by inhibiting ATP-dependent potassium channels (Gravielle, 2021).

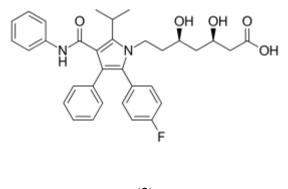
In a situation with a reduced glucose concentration level, sulfonylurea receptor 1(SUR1) remains open; this allows the efflux of potassium ions to form a -70mV potential membrane. Usually, sulfonylurea receptor one remains closed in response to increased concentration of glucose, the cell's membrane potential becomes less negative, there is a depolarization of the cell, the channels of calcium voltage-gated become open, calcium ions then go into the cell,

the raised concentration of intracellular calcium stimulates the insulin release containing granules. Glibenclamide overdoes this procedure by forcing sulfonylurea receptor 1 to close and stimulates insulin secretion, which lowers blood sugar levels to normal (Gribble & Reimann, 2003).

## 2.3 Atorvastatin

#### 2.3.1 Hypolipidemic Activities of Atorvastatin

Atorvastatin (2) and other statins are regarded as the first option for treating and managing diabetes mellitus (Grundy & Stone, 2019).



(2)

The increase in the utilization of this class of medication has been mainly attributed to the increase in cardiovascular diseases (CVD) (like atherosclerosis, peripheral artery disease, heart attack, stroke, and angina) in several countries (Einarson *et al.*, 2018). Increased levels of cholesterol (raised levels of low-density lipoprotein (LDL) specifically) are a significant risk factor for the genesis of cardiovascular diseases (Anderson *et al.*, 2016). Several studies have shown that proper use of Atorvastatin is connected to reduced levels of low-density lipoprotein and cardiovascular disease risk.

## 2.3.2 Atorvastatin's Mode of Action

Atorvastatin is a medication that reduces lipid levels incorporated in the statin group of drugs. Statins reduce unusual cholesterol and lipid levels by preventing the production of endogenous cholesterol in the liver, thus lowering the danger of developing cardiovascular disease. Specifically, statin medications compete to inhibit the activity of an enzyme called hydroxymethylglutaryl-coenzyme A (HMG-CoA) Reductase (Ahmadi, 2020). The conversion of HMG-CoA to mevalonic acid, which is a very early rate-limiting step in the biosynthesis of cholesterol, is catalyzed by hydroxymethylglutaryl-coenzyme A reductase (Johnson & DeBose-Boyd, 2018). This conversion is a very important metabolic reaction that is involved in the creation of various compounds that are used in the metabolism of lipids and their transport like the low-density lipoprotein (LDL) also called "bad cholesterol" due to the fact that it operates as a basis of cholesterol that builds up in arteriosclerotic plagues.), very-low-density lipoprotein (VLDL) and total cholesterol (Davies *et al.*, 2016).

Atorvastatin functions mainly in the liver, where reduced concentration levels of hepatic cholesterol stimulate the up-regulation of the hepatic low-density lipoprotein (LDL) receptors, which elevates the uptake of hepatic low-density lipoprotein. Atorvastatin too reduces the levels of serum triglycerides (TG), Very-Low-Density Lipoprotein-Cholesterol (VLDL-C) and Intermediate Density Lipoproteins (IDL) together with the amount of apolipoprotein B (apo B) containing particles, yet increases the levels of High-Density Lipoprotein Cholesterol (HDL-C) (Chowdhury & Chowdhury, 2015).

*In vivo* and *In vitro* studies that have been conducted in animal studies also demonstrated that Atorvastatin offers vasculoprotective activities apart from its properties of lowering lipids, which is referred to as the pleiotropic activities of statins (Rohilla *et al.*, 2016). These effects include enhanced atherosclerotic plaque stability, endothelial function improvement, inflammation, inhibition of the thrombogenic response, and reduced oxidative stress. Studies have also reported that statins allosterically bind to  $\beta$ 2 integrin function-associated antigen-1 (LFA-1), which plays a critical role in T-cell activation and leukocyte trafficking (Rohilla *et al.*, 2016).

Management of diabetes mellitus and high blood pressure management using traditional therapies is widespread in African urban and rural communities. A growing population of diabetic patients who prefer herbal therapies are motivated to do so because of several factors that are not limited to financial problems, the fact that traditional medicine is easily accessible, geographical accessibility to health facilities, indigenous knowledge of community members, inadequate healthcare systems together with the role of traditional therapists in the treatment of illnesses (Mushagalusa *et al.*, 2021).

## 2.4 Herbal medicines and disease management

Herbal medicine originated from ancient cultures where plants or their extracts were used to treat illnesses and assist bodily functions (Kooti *et al.*, 2016). About 75% of people in the world, particularly in developing and underdeveloped countries, consider herbal medicine the primary way of treating illnesses since it has better tolerability with the human body, lesser side effects, and better cultural acceptability (Rahman, 2022). For example, herbal extracts have been introduced as prescription drugs (Gunjan *et al.*, 2015).

The discovery of conventional drugs has outdone the traditional remedies, more specifically in developed countries. In resource-poor communities, particularly in Africa, herbal medicine has been considered the most crucial part of the traditional remedy, the alternative medication (Odhiambo *et al.*, 2011). The popularity of green medicine in treating and managing diseases in African populations is due to the fact that it is easily accessible and at a reduced/affordable cost compared to conventional medicine (Sam, 2019). In traditional therapy, patients get social treatment from relatives, traditional healers, and friends with good experiences. The positive outcomes of herbal therapy are because of the placebo and sometimes due to the actual efficacy of the plant. It is also worth mentioning that there is presently a renewal of herbal therapy in developed communities (Gurib, 2006). The rationale for using traditional medicine comes as no shock since it possesses numerous bioactive compounds of known therapeutic usage (Rehman *et al.*, 2022). A range of herbal extracts, together with their metabolites, can amend signaling cascades that are implicated in cardiovascular physiology. Several plants have been reported to provide a starting point in synthesizing more than fifty percent of the pharmaceutical medicines presently in use (Mushagalusa *et al.*, 2021).

#### 2.4.1 Hypoglycemic Medicinal Plants

Existing medications for hyperglycemic conditions are generally limited in value, bear the danger of undesirable effects, and are too expensive, particularly for developing countries. Consequently, a search for plant-derived hypoglycemic remedies that can be readily available and do not involve demand for expensive pharmaceutical processing becomes a very attractive area of research (Belayneh *et al.*, 2019). Approximately eight hundred and eighty plants are thought to have hypoglycemic activity, and around three hundred and forty-three plants have been reported in scientific studies (Maher *et al.*, 2021). Plants do not only contain hypoglycemic activities, but they also lead to a decline in triglyceride, alkaline phosphatase,

and cholesterol levels while increasing the content of total protein (Gopalakrishnan & Dhanapal, 2014). All these medicinal properties are quite advantageous to those who have diabetes. However, the plants believed to have hypoglycemic activities have yet to be evaluated on their mode of action, potency, and safety.

In recent times, herbal therapies have been evaluated as very important in managing diabetes mellitus globally and are used as hypoglycemic and hypolipidemic drugs. Despite the availability of hypoglycemic remedies in the pharmaceutical market, diabetes mellitus and related problems remain the leading medical crisis. Hypoglycemic activities of most of the plants are due to their potential to restore the role of the tissues of the pancreas by increasing insulin production and inhibiting glucose absorption in the intestine. Most medicinal plants possess alkaloids, carotenoids, glycosides, flavonoids, and terpenoids, among others, commonly incorporated as containing hypoglycaemic activity (Srivastava *et al.*, 2019). Several herbal medicines are used in different communities worldwide to cure numerous diseases, including diabetes (Andargie *et al.*, 2022). Some plants like *Avicennia marina*, an ethanolic extract of its leaf, have been used to minimize high blood sugar levels and oxidative stress, improve the neurobehavioral changes linked to diabetes, and protect the liver in mice (Okla *et al.*, 2019).

Studies have shown that approximately more than one thousand species of flora have been traditionally applied for managing diabetes mellitus. Some species of plants that have been reported to have hypoglycemic effects include *Asteraceae, Leguminosae (Fabaceae), Liliaceae, Moraceae, Cucurbitaceae, Lamiaceae, Euphorbiaceae, Araliaceae Calpurnia aurea and Rosaceael Acacia Arabica* (Hegazy *et al., 2013). Acosmium panamense* (Andrade-Cetto *et al., 2004). Allium sativum (garlic), Hibiscus sabdariffa L* (Su *et al., 2018).* 

*Coriandrum sativum* (Kooti *et al.*, 2016). *Securinegra virosa* (Tanko *et al.*, 2018). *Mangifera indica, Ocimum sanctu* (Vats *et al.*, 2002). *Opium graveolens* (Mans *et al.*, 2019). *Aegle marmelose*, among others. The hypoglycemic effects of these botanical remedies are due to the existence of terpenoids, phenolic, alkaloid compounds, and flavonoids.

## 2.4.2 Hypolipidemic Medicinal Plants

In the past few years, a progressive development in the field of herbal medication has been seen, and most of these medicines are becoming more popular both in developed and developing communities because of their reduced adverse effects and natural availability. A rising number of research findings have reported antihyperlipidemic activities with herbal medicines. Attempts have been made so as to reduce body weight by the use of a pharmacological intervention that possesses minimum side effects. Plants have been utilized for healing various diseases; in particular, several oriental medicinal plants have been demonstrated to have biological activity. These medicinal plants are rich sources of bioactive compounds (Alagumanivasagam & Veeramani, 2015). Therefore, they have been utilized as essential raw materials for producing conventional drugs.

Some of the hypolipidemic medicinal plants include the following: *Amaranthus Spinosus* Amaranthaceae, *Glycyrrhiza Glabra* Fabaceae, *Withania Somnifera* Solanaceae, *Chlorophytum Borivilianum* Liliaceae, *Moringa oleifera* Moringaceae, *Sphaeranthus indicus*, Asteraceae, *Rhinacanthus nasutus* Acanthaceae, *Pithecellobium Dulce benth* Leguminosae, *Hibiscus cannabinus* Malvaceae, *Eclipta prostrate* Asteraceae, *Sesbania grandiflora* Fabaceae, *Lycium barbarum* Solanaceae and *Ougeinia oojeinensis* Fabaceae (Sham *et al.*, 2014). Given that currently accessible hypolipidemic therapies do not have the desired ultimate remedy characteristics; research is expected to look for safe, effective, and inexpensive remedies. Herbal medicine is helpful in diabetes globally and has been used as hypoglycaemic and hypolipidemic drugs (Sajadimajd *et al.*, 2023). Medicinal plants for hypercholesterolemia have been shown to have no or fewer adverse effects and are economically efficient in managing obesity (Aziz *et al.*, 2023).

Several research work has recorded that dyslipidemia linked to non-communicable illnesses such as obesity and diabetes have shown to be on the rise, especially in developing countries, and steady studies are needed in order to discover native medicinal plants that can be able to alleviate, or maybe helpful in managing dyslipidemia (Tsenum, 2018). Hence, the need to conduct the present study to assess the hypolipidemic and hypoglycemic effects of the aqueous root extract of *Tithonia diversifolia* compared to the standard drugs, atorvastatin, and glibenclamide.

## 2.5 Tithonia diverfolia (Hemsley) A. Gray

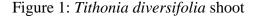
#### 2.5.1 The Plant

*Tithonia diversifolia* belongs to the Asteraceae family, genus *Tithonia* and species *T*. *diversifolia*. Vernacular/common names: (English): Mexican sunflower, Tithonia, tree marigold; (Kikuyu): Maruru; (Luo): *Maua makech, akech*; (Kamba): *Ilaa*; (Kisii): *Amaua maroro*; (Luhya): *Maua amalulu* (Mabou *et al.*, 2018).

*Tithonia diversifolia* (Figure 1) is a bushy perennial herb that grows approximately three meters high. The stems of this plant are slightly ridged, hairy, and hollow when young. Its leaves are arranged alternately and are borne on its stalks, which are about 2-10 cm long.

The colour of its leaves is dark-green and finely hairy. Its flowers are large and yellow and resemble that of the sunflower plant. The heads of these flowers are borne at the tops of the leafy branches, which are about 7-30 cm long. Flowering mainly occurs during the rainy seasons in East Africa (Mabou *et al.*, 2018).





*Tithonia diversifolia* is/was indigenous in Mexico but has spread around all over the world. It was introduced as an ornament and green manure in Africa and Asia. The plant grows wild in tropical and subtropical areas and has also become naturalized (Mabou *et al.*, 2018).

A study by Masood *et al.*, 2017 revealed that *Tithonia diversifolia* exhibits exciting healthpromoting properties that result from stimulation of its defensive cellular mechanisms that are engaged in the stress defenses at the cell and its capacity of free radical scavenger (Meshuneke *et al.*, 2020) and also in mesenchymal cells adipogenesis (Roopa *et al.*, 2021).

The phytochemicals extracted from the stem and leaf of *Tithonia diversifolia* have been traditionally applied in the treatment and management of diabetes (Yazid *et al.*, 2021), diarrhea (Gahamanyi *et al.*, 2021), menstrual pain (Obayomi *et al.*, 2021), malaria

(Ngarivhume *et al.*, 2021), hepatitis (Silva *et al.*, 2020), hepatomas (Maher *et al.*, 2021). This has been attributed to the flavonoids and terpenoids contained in this plant's aerial areas. In addition, it is established that *Tithonia diversifolia* has antimalarial, antimicrobial, analgesic, and anti-inflammatory repair activities. Herbal remedies have been culturally applied in managing several conditions and sicknesses. A study (Olukunle, 2014) reported that *T. diversifolia* aqueous leaf extract improves insulin at the cellular level since it can attenuate blood glucose concentrations in hyperglycaemic rats.

A study on ethanolic root extract of *Tithonia diversifolia* contains the following phytochemical compounds: alkaloids, tannins, phenolic compounds, flavonoids, and terpenoids (Tagne *et al.*, 2018).

Although the mechanism of action of various hypoglycemic plants is not precisely known, it is assumed that these plants have specific hypoglycemic values, which slow down the activities of some of the enzymes that are drawn in the glucose production pathway, stimulate the production of insulin and also make the target cells receptors susceptible to insulin (Olukunle, 2014).

Together with this, the hypoglycemic activities of several plants might be credited to the occurrence of trace elements like potassium, iron, zinc, copper, sodium, vanadium, nickel, and chromium, which are believed to activate the cells of the pancreas and take part in a vital role in maintaining normoglycemia (Parasuraman *et al.*, 2015). The claim on antidiabetic and hypolipidemic properties of this plant formed the basis of establishing the outcome effect of the aqueous root extract on these parameters since it has not been validated scientifically.

## 2.5.2 The traditional usage of *Tithonia diversifolia*

Scientific studies have validated *Tithonia diversifolia* medicinal properties as attributed to the presence of phytochemicals like the triterpenes, sesquiterpene lactone, and monoterpene, particularly tagitinins, which have a broad range of pharmacological effects such as anti-inflammatory, antioxidant, insecticidal, antidiabetic as well as anticancer property. Therefore, *Tithonia diversifolia* is seen to have a potential source of phytochemical compounds and antioxidant activity. The flavonoid and phenolic phytochemical compounds are secondary metabolites of plants that display outstanding antioxidant activity (Roopa *et al.*, 2021).

Through the decades, *Tithonia diversifolia* has been extensively used in diverse forms in traditional remedies for the management and treatment of numerous diseases—for example, wound treatment through administering a paste-like form made from unrefined leaves. The administration can also treat malaria of *Tithonia diversifolia* root extract. Elevated blood glucose levels is reported to be managed well using dried leaf extract (Silva *et al.*, 2020). Based on these uses, pharmacological investigations made from the earlier years have revealed that the extracts made from several parts of *Tithonia diversifolia*, like the leaves, roots, stems, and flowers, have broad pharmacological attributes, including antidiabetic, anti-inflammatory, antitumoral, immunomodulatory, antibacterial and antifungal properties (Gahamanyi *et al.*, 2021). Some of the traditional use of *Tithonia diversifolia* is presented in the table below.

Category of use	Description of traditional usage
Abscesses	The juice from the leaves and stems cleans the affected
	parts.
Diabetes mellitus	Leaf concoction is taken orally.
Malaria	Cold/hot water leaf infusion from aerial parts is
	administered orally.
Snakebite	Leaf concoction orally administered.
Bruises, wounds, and	A powdered form from the toasted leaves or powdered
skin infections	creams is applied on the affected parts.
Gastrointestinal diseases	Leaf concoction of the plant called (Likong) in Kenya
and worms in poultry	is orally administered to the livestock
Measles	A concoction made from the leaves (method of
	administration is not affirmed)
Bleeding	The leaves are crushed and applied to the cuts and
	wounds.
Infections in sexual	Cold/hot water leaf infusions are orally taken and for
organs	bathing.
Gastric ulcer	Leaf concoction is orally taken.
Diarrhea	Aqueous decoction orally administered.
Menstrual pain	Leaf concoction in water is orally taken.
Hepatitis	Orally, the decoction of stem and leaf is administered.
Disease of the nose,	The roots and leaves are boiled and taken orally
throat, and ear	
	Abscesses Diabetes mellitus Malaria Snakebite Bruises, wounds, and skin infections Gastrointestinal diseases and worms in poultry Measles Bleeding Bleeding Infections in sexual organs Gastric ulcer Diarrhea Menstrual pain Hepatitis

Table 1. The traditional usage of *Tithonia diversifolia*.

(Ajao & Moteetee, 2017).

*Tithonia diversifolia* aqueous root extract is believed to have hypoglycemic and hypolipidemic activities among the Luos of South Nyanza, though this claim is not scientifically proven. This research work aimed at validating this claim scientifically.

# 2.5.3 Hypoglycemic activity of Tithonia diversifolia

Aqueous leaf extract of *Tithonia diversifolia* orally administered at 400 mg/kg body weight led to a timely reduction of blood sugar levels in alloxan-induced diabetic rats. This effect is similar to the one yielded by glibenclamide (10 mg/kg body weight), used as a positive control. This result suggests that *Tithonia diversifolia* aqueous leaf extract promotes insulin secretion by the remnant  $\beta$ -cells of the pancreas of diabetic rats (Olukunle *et al.*, 2014).

*Tithonia diversifolia* aqueous leaf extract shows an important hypoglycemic activity on glucose tolerance (OGTT). The same extract of this plant has also been shown to contain significant antidiabetic and hypolipidemic activity in mice that have induced diabetes using alloxan by reducing lipid peroxide concentration in diabetic mice. Peroxidation of lipid products indicates oxidative damage to the pancreas and liver tissues. High blood sugar levels can increase the production of reactive oxygen species (ROS) in all tissues from protein glycosylation and glucose auto-oxidation. Diabetes mellitus causes interference in the lipid profile, particularly an increased lipid peroxide susceptibility, which is the primary cause of the occurrence of atherosclerotic state (Thongsom *et al.*, 2013). The hypoglycemic effect of *Tithonia diversifolia* is due to the presence of chemical compounds like the phenols, flavonoids, and lactones sesquiterpenes that can raise the sensitivity of insulin, which helps in the reduction of high blood sugar, reducing the intake of food and raising the body weight of hyperglycemic Wistar rats (Sari *et al.*, 2018). The average blood sugar levels in Wistar albino rats range between  $3.95\pm1.31$ mmol/L (Wang *et al.*, 2010).

#### 2.5.4 Hypolipidemic activity of *Tithonia diversifolia*

The aqueous leaf extract of *Tithonia diversifolia* given at 400 mg/kg per day considerably reduces total serum cholesterol and low-density lipoprotein (LDL) in diabetes-induced rats after treating them for 21 days. However, the serum high-density lipoprotein (HDL) significantly increases under the same conditions (Olukunle *et al.*, 2014). Saponins that are isolated from *Tithonia diversifolia* leaves significantly reduced the triglyceride levels, total cholesterol, and serum low-density lipoprotein (LDL) in normal rats at dose ranges of 60-100, 40-100, and 20-100 mg/kg body weight respectively. On the other hand, at doses of 20-100 mg/kg body weight, the serum high-density lipoprotein is also significantly lowered. These results suggest that *Tithonia diversifolia* (TD) aqueous leaf extracts, precisely the fraction that is rich in saponins, can be helpful in the management of dyslipidemia (Ejelonu *et al.*, 2017).

A study by Thongsom and his colleagues in 2013 reported that the oral administration of *Tithonia diversifolia* aqueous leaf extract in mice with diabetes for 30 days resulted in a decrease in the levels of triglyceride, LDL-cholesterol, total cholesterol, and HDL-cholesterol is reported to increase when compared diabetic control rats. The levels of lipid peroxidation, as well as reactive oxygen species, hydroxyl radical, hydrogen peroxide, and superoxide anion, are typical markers of oxidative stress in diabetes. The normal levels of serum cholesterol in Wistar albino rats range from 1.1-2.0 mmol/L, that of serum triglycerides is between 0.4-2.1 mmol/L, and normal ranges for high-density lipoprotein in Wistar rats is between 2.2-2.8 mmol/L (Boehm *et al.*, 2007).

From the above studies on the hypoglycemic and hypolipidemic activities of *Tithonia diversifolia*, the aqueous leaf extract has been evaluated. The root of this plant, claimed by the Luo community to have hypoglycemic and hypolipidemic properties, has not been evaluated scientifically. Therefore, the proposed research work targeted the roots of this plant in order to validate this claim scientifically.

# 2.5.5 Biochemical effects of *Tithonia diversifolia* in the liver and kidney

The changes made on the major organs like the kidney and the liver by the plant extract help design the therapeutic dosage required during the development of new medicines to reduce its adverse side effects on these organs. The kidneys and liver are usually the primary targets of toxicity by the plant extract because they are the ones that are associated with the degradation and excretion of many chemical substances (de Oliveira *et al.*, 2011).

The adverse effects of the aqueous leaf extract of *Tithonia diversifolia* on the kidney and liver have been investigated on Wistar albino rats after its oral administration for 14 days at a dose of 100 mg/kg and 200 mg/ kg per body weight. The observation that was made was that the graded doses of the extract caused noticeable changes to blood biochemistry by increasing the levels of alanine amino transaminase (ALT), alkaline phosphatase (ALP), and aspartate aminotransaminase (AST) which is equivalent to damage in the hepatocytes (Ajao & Moteetee, 2017). However, doses below 100 mg/kg were reported to be moderately safe in the toxicological study that was conducted by (Passoni *et al.*, 2013) after repeated oral administration of *Tithonia diversifolia* aqueous leaf extract for 90 days. Additionally, the aqueous leaf extract of *Tithonia diversifolia* contains sesquiterpene and chlorogenic acids, which can lead to severe kidney and liver damage (Passoni *et al.*, 2013).

Biochemical analysis of the blood liver enzymes helps evaluate the extent of damage to the liver caused by the extract (Elufioye *et al.*, 2009). The damaging attack on the liver by the components of the extract might be because the liver plays a vital role in the metabolism of drugs and, therefore, usually is the site for the initial pass effect for the majority of the bioactive substances (Elufioye *et al.*, 2009).

The investigation made on the biochemical effects of *Tithonia diversifolia* leaf extract on the kidney revealed a significant decrease in serum creatinine level in mice at a dosage of 300 mg/kg body weight (Dada & Oloruntola, 2016) at a dose of 600 mg/kg body weight of *Tithonia diversifolia* aqueous leaf extract, a significant increase in serum creatinine levels was revealed which implies kidney dysfunction (Ajao & Moteetee, 2017).

The biochemical changes that occurred in the liver and the kidney, as can be seen, were produced by the aqueous leaf extract of *Tithonia diversifolia*. Many studies have been published on *Tithonia diversifolia* leaf extracts only and no scientific reports on the roots; therefore, there was a need to investigate the biochemical changes in the kidney and liver that the aqueous root extract of *Tithonia diversifolia* may bring about.

# 2.6 Liver biochemical parameters

The current study investigated the biochemical changes that would have occurred in the liver and kidney via the liver and renal function tests, respectively. Clinically, liver health indicators are primarily based on the concentration of liver enzymes like alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT), which are being used in the screening of diseases of the liver (Denova-Gutiérrez *et al.*, 2021). When there is damage in the tissues of the liver, the concentrations of liver enzymes like the ALT, ALP, and AST go high in the bloodstream as they are released into the blood. Therefore, a dysfunction in the liver can be evaluated by checking the levels of these liver enzymes. Tissue damage leads to the extra release of ALT and AST in the blood circulation; hence, the levels of such enzymes are increased in the bloodstream, which are vital biomarkers of liver dysfunction.

Research has shown that when the liver is damaged, or there is a dysfunction in the liver, additional amounts of ALT and AST are released into the bloodstream, thus raising the concentration levels of serum liver enzymes. Therefore, the amount of AST and ALT in the bloodstream is directly linked to liver damage (Hasan *et al.*, 2018). ALT is the most specific and sensitive indicator in liver damage since it is predominantly produced in the liver compared to AST, which can be high in other diseases like heart disease, kidneys, and muscles. The normal levels of ALTs in adult human circulation range from 30–50 units per liter (U/L) (Ali *et al.*, 2021). In Wistar albino rats, the normal values of serum ALT range between 24-49U/L while the usual levels of AST range between 50-96U/L and that of ALP ranges from 65-193U/L (Boehm *et al.*, 2007).

# 2.7 Renal biochemical parameters

Creatinine and Urea are kidney function biomarkers that show the glomerular filtration rate (Ascher *et al.*, 2021). The main metabolic breakdown of protein in a human's nitrogenous end product is Urea. After Urea has been dissolved in the blood, it is transported and then excreted as a component of urine by the kidney. Creatinine refers to the product of the breakdown of creatine phosphate released from the skeletal muscle. Creatinine is filtered through the glomerulus, and a small amount is secreted into the glomerulus filtrate through the proximal tubules. Blood urea nitrogen and serum creatinine levels are the best guiding

principles for estimating prognosis and progression that institute nutritional limits in the kidney disease in type 2 diabetes mellitus (Kene *et al.*, 2021).

A rise in the levels of these markers shows kidney dysfunction. Serum creatinine is a substitute for general renal function by helping evaluate the glomerular filtration rate. However, it lacks the sensitivity for detecting reductions in kidney function at an early stage (Ascher *et al.*, 2021).

Serum creatinine speedily reduces to a level of about 0.25 mg/dL in the earliest month of life and again rises as one continues to grow. Healthy subjects of the ages between 20 and 70 years, on average, have normal constant serum creatinine levels of (0.63–1.16 mg/dL) for gents and (0.48–0.93 mg/dL) as the normal reference interval for ladies. In ages over 70 years, serum creatinine begins to rise once more in both genders gradually. These increases could be the first indicators to caution on the existence of probable kidney damage. Nonetheless, research has shown that these increases in serum creatinine levels may not be beneficial in detecting renal damage or impairment caused by the nephrotoxic medication (Delanaye *et al.*, 2017). In male Wistar albino rats, the expected levels of serum creatinine range between (31-48mmol/L) while that of serum urea in male Wistar rats range from 4.0-9.3 mmol/L (Boehm *et al.*, 2007).

# **CHAPTER THREE**

## 3.0 MATERIALS AND METHODS

# 3.1 Methods

#### **3.1.1** Study design

The study was a prospective laboratory-based study.

# 3.1.2 Study setting

This research study was conducted between September 2021 and December 2021. It involved a collaborative effort between the Department of Biological Sciences at the University of Eldoret and the School of Pharmacy at Kabarak University. The plant root underwent shade drying and grinding procedures within the Department of Biological Sciences, University of Eldoret facilities. Subsequently, the resulting powdered material was transported to the School of Pharmacy, Kabarak University, for further processing. The preparation of the aqueous root extract, the maintenance and handling of experimental animals, and the execution of various experimental procedures were all carried out within the premises of the School of Pharmacy, Kabarak University.

# **3.2 Materials**

#### 3.2.1. Western diet

A Western diet is characterized by elevated levels of fat, cholesterol, and fructose, resembling the dietary patterns commonly observed in human consumption of fast food. Consequently, when experimental animals are subjected to this diet, they replicate the pathogenesis of Nonalcoholic Steatohepatitis (NASH) observed in humans. It remains unclear how the various fat sources in Western diets (WD) impact the progression of non-alcoholic steatohepatitis (NASH) (Drescher *et al.*, 2019). The administration of a Western diet to laboratory animals leads to the development of insulin resistance, obesity, and NASH histological features, as demonstrated in prior research (Asgharpour *et al.*, 2016). This specific diet can increase both blood glucose and lipid levels in the experimental rats. The western diet used in this research was prepared via the guidelines of Nguyen *et al.*, (2017) on improving the rodent chow into the western diet. The rodent chow was procured from Unga Limited, Nairobi-Kenya, the composition of which is detailed in Table 2 below.

Component	% composition in rodent chow (Unga limited-Kenya)	Reconstituted composition in Western diet (Nguyen <i>et</i> <i>al.</i> , 2017) expressed in %
Carbohydrates	49	60.85
Animal fat	3	21
Protein	21	18
Cholesterol	0	0.15
Calcium	0.8	-
Phosphorus	0.4	-
Fibre	5	-
Moisture	13	-
Ash	8	-

Table 2. Comparison between the composition of rodent chow and the Western diet

#### **3.2.2.** Preparation of the western diet

According to (Franco *et al.*, 2022), one Wistar albino rat weighing between 150-200g feeds on 24g of rodent chow daily. Therefore, a total of 120g of western diet was required for every cage of five rats daily. To make 25.2 kg of western diet, 4.53 kg of animal fat was first mixed with 20.68 kg of commercial rodent chow. Then 25.16 kg of this mixture was mixed with 37.8g of cholesterol to give 25.2 kg of western diet. About 120g of this western diet was fed to the five rats in the cage daily.

#### 3.2.3. Wistar rats

A total of thirty-five male Wistar albino rats (*Rattus norvegicus*), aged two months and weighing between 180-200g, were procured from the small animal facility at Chiromo Campus, University of Nairobi, Kenya. The rats were transported in cages to Kabarak University, where they were accommodated in stainless steel cages furnished with sawdust and soft grass serving as bedding material. These animals were then categorized into seven groups of five rats. They were maintained under standard laboratory conditions, with a temperature set at  $25\pm2^{\circ}$ C. They were subjected to a 12-hour light and 12-hour dark cycle within the animal housing facility at the School of Pharmacy, Kabarak University.

Throughout the acclimatization period, which spanned one week, all the rats were provided unrestricted access to standard rodent chow (Unga Limited-Kenya) and drinking water. Their cages were routinely cleaned to remove waste, and the rats' health status was regularly monitored.

#### 3.2.4. Tithonia diversifolia (The material)

#### **3.2.4.1** Collection and Identification of Plant Material

In September 2021, fresh and healthy roots of *Tithonia diversifolia* were collected from the Kamagambo sub-location, located within the Olando shrubs near the Kanga market in Migori County, Kenya. The identification of the plant in its natural habitat was conducted by a local herbalist, and subsequently, the roots were carefully excavated, placed in polyethylene bags, securely sealed, and transported to the University of Eldoret, Kenya together with a sample shoot. At the University of Eldoret, the plant specimen was further identified by a taxonomist from the Department of Biological Sciences, and a voucher number, M.U.H/MD/0020/21, was assigned to this particular plant specimen. This specimen was duly deposited in the herbarium of the University of Eldoret for documentation and reference.

## **3.2.4.2 Preparation of the aqueous root extract**

The roots of *Tithonia diversifolia* were initially subjected to a washing process to eliminate extraneous debris. Subsequently, they were cut into small pieces and subjected to a three-week shade drying period within a well-ventilated room. The roots were finely ground into a homogeneous powder utilizing an electric mill after the drying process. This powdered material was then transferred to Kabarak University, where it was utilized to prepare the aqueous root extract. The preparation of the aqueous root extract followed a method by Alli *et al.*, 2011.

This procedure involved soaking 100 grams of the *Tithonia diversifolia* root powder in 1000 ml of distilled water. The resulting mixture was placed in an electric shaker and agitated for 12 hours at room temperature. Subsequently, the mixture was filtered through muslin cloth

and Whatman No 1 filter paper. The filtrate was subjected to drying using a rotary evaporator in a water bath maintained at 50°C until dark green syrup of the crude extract was obtained. The crude extract was then transferred into a pre-weighed crucible and left exposed to air overnight to facilitate the evaporation of any remaining water, thereby yielding the final concentrate. This concentrate was stored in an airtight container at 4°C until its use. The percentage yield of the aqueous extract was determined to be 19.4%.

This yield was calculated as follows;

% yield = 
$$\frac{\text{the weight of extract in gram}}{\text{weight of powder plant material}} x 100$$

# 3.2.4.3 Preparation of Tithonia diversifolia extract concentration

The concentration of the doses given to the rats was calculated using the formula below:

$$M_e = \frac{dM_R}{1Kg}$$

Derived from Olukunle, 2014

Where:  $M_e$  = Mass of extract given daily to the experimental rats in mg

d = desired extract dosage in mg/kg body weight

M<sub>R</sub>= Group average mass of rats in g

Table 3. Daily Mass of plant extract given to each rat in G5 and G6

Rat Groups	Average Mass of the rat	Dosage of extract Required	Daily Mass of plant extract given to each rat
G5	192.06 g	200mg/kg bw of extract	7.68 mg
G6	191.34 g	400mg/kg bw of extract	15.31 mg

# **3.2.5 Determination of Standard Drug Dosage**

The dosages of drugs used in these experiments was prepared based on the published works of (Nasri *et al.*, 2016) for artovastatin and (Samuel *et al.*, 2014) for glibenclamide Mass of standard drug orally administered to each rat daily (D) was calculated as follows;

$$\boldsymbol{D} = \frac{Average \ weight \ of \ rats \ in \ a \ group \ x \ Drug \ dosage}{1000g}$$

Mass of tablet dissolved to make 2.5mL of solution for 5 rats in a group  $(M_T)$  was calculated as follows;

$$M_T = \frac{D \ x \ Average \ mass \ of \ tablet}{Concentration \ of \ the \ drug \ component \ in \ each \ tablet}$$

Where:

D: The mass of standard drug orally administered to each rat daily

M<sub>T</sub>: The mass of tablet dissolved to make 2.5mL of solution for 5 rats in a group

Table 4. Standard	Drug Dosage
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Groups	Dosage of the standard drugs	Average mass of rat in a group	D	Average Mass of a tablet	drug concentration in a tablet	Мт
G3	10mg/kg Atorvastatin	191.86g	1.92mg	308mg	20mg	147.8mg
G4	0.5mg/kg Glibenclamide	191.76g	0.096mg	155mg	5mg	14.88mg

147.8mg of Atorvastatin powder was dissolved in distilled water to make 2.5mL of the solution daily. From this, 0.5mL (29.56mg) was orally administered to each rat in G4 daily from days 29 to 35 of the experiment.

14.88mg of glibenclamide powder was dissolved in distilled water to make 2.5mL of the solution daily. From this, 0.5mL (2.976mg) was orally administered to each rat in G4 daily from days 29 to 35 of the experiment.

# 3.3 Procedures

#### 3.3.1. Phytochemical Screening of the aqueous root extract

The stock solution for conducting phytochemical tests was prepared by dissolving 1 gram of the *Tithonia diversifolia* crude extract in distilled water, resulting in a 200 ml solution. Special care was taken to ensure that the concentration of the extract solution was suitable for facilitating the qualitative tests. The qualitative analysis of the different phytochemical components was carried out following the respective standard procedures within a fume hood for safety.

# 1. Test for Alkaloids.

#### **Preparation of Dragendorff's reagent**

In a test tube, 5.2 grams of Bismuth carbonate were introduced, followed by the addition of 4 grams of sodium iodide and 50 mL of glacial acetic acid. The resulting solution was brought to a boil for duration of 3 minutes and then allowed to stand undisturbed for 12 hours, leading to the formation of a precipitate. Subsequently, the precipitated sodium acetate crystals were filtrated using a sintered glass funnel. A 40 mL portion of the filtrate was mixed with 160

mL of ethyl acetate, followed by adding 1 mL of distilled water, thus creating Dragendorff's reagent. This reagent was then stored in an amber-colored glass bottle.

Three drops of reagent were combined with 3 mL of the plant extract for the test. The resulting solution was carefully observed for the appearance of an orange-red or reddishbrown precipitate, which indicates the presence of alkaloids (Dragendorff, 1879).

# 2. Test for Flavonoids

Shinoda Test: Three drops of concentrated HCl were added to the extract, followed by a small magnesium ribbon. The solution was observed in the formation of a pink or red colour as an indication of the presence of flavonoids (Trease and Evans, 2002).

# 3. Test for Tannins

Ferric Chloride Test: In a test tube, three drops of 1% ferric chloride (FeCl<sub>3</sub>) solution were added to 5 ml pre-boiled and filtered extract. The solution was observed for the formation of a blue-black or greenish-black colour; this colour formation indicated the presence of tannins (Harborne, 1973).

#### 4. Test for Saponins:

Froth Test: 5 ml of the extract was put in a test tube and vigorously shaken and observed for the formation of stable froth that persisted for a few minutes as an indication of saponins' presence (Sofowora, 1993).

#### 5. Test for Glycosides

Keller-Killiani Test: In a test tube containing 5 ml of Glacial acetic acid, a drop of 5% ferric chloride solution, 5 ml plant extract, and 3 drops of concentrated sulfuric acid were added. The solution was observed for the formation of a reddish-brown ring at the interface of the two layers as an indication of the presence of glycosides (Trease & Evans, 1989).

#### 6. Test for Terpenoids

Salkowski Test: 5 ml of the plant extract was mixed with 5 ml of chloroform followed by addition of of concentrated sulfuric acid carefully along the sides of the test tube. The solution was observed for the formation of a reddish-brown coloration at the interface indicating the presence of terpenoids, (Harborne, 1973).

#### 7. Test for Phenols

Ferric Chloride Test (for phenolic compounds): in a test tube, three drops of 1% ferric chloride (FeCl<sub>3</sub>) solution was added to 5 ml extract. The solution was observed for the formation of shades of green, blue, or violet, as an indication for the presence of phenolic compounds, (Harborne, 1973).

#### 8. Test for Carbohydrates

Molisch's Test: in a test tube, three drops of 1% alcoholic  $\alpha$ -naphthol solution was added to 5 ml extract, followed by slow addition of concentrated sulfuric acid along the sides of the test tube. The formation of a violet ring at the interface indicated the presence of carbohydrates, particularly sugars, (Molisch, 1937).

#### 9. Test for Steroids and Sterols

Liebermann-Burchard Test: in a teste tube containing 5 ml of the extract, three drops acetic anhydride was added, followed by slow addition of 3 drops of concentrated sulfuric acid. The solution was observed for the formation of a green color, which changes to blue and then to red, indicated the presence of steroids and sterols, (Nath *et al.*, 1946).

#### **10. Test for Cardiac Glycosides:**

Legal's Test: in a test tube, 5 ml of glacial acetic acid was added to 5 ml of the extract followed by three drops of ferric chloride solution. The solution was observed for the formation of a brown ring at the interface as an indication of the presence of cardiac glycosides, (Trease & Evans, 1989).

# 11. Test for Proteins and Amino Acids

Biuret Test: Three drops of 2% copper sulfate solution was added in a test tube containing 5 ml plant extract, followed by three drops of 10% sodium hydroxide solution. The solution was observed for the formation of a violet or lavender colour as an indication for the presence of proteins/amino acids, (Gornall *et al.*, 1949).

#### 12. Test for Anthocyanins

Acid Test: In a test tube, containing 5 ml of the extract, three drops of dilute hydrochloric acid (HCl) was added and the solution observed for the formation of a red to violet colour change as an indication for the presence of anthocyanins, (Fossen & Andersen, 2003).

#### **13. Test for Lignin**

# **Preparation of Phloroglucinol reagent**

0.5g of phloroglucinol was dissolved in distilled water to make 100 ml solution. This solution was gently warmed accompanied by continuous stirring to completely dissolve then mixed with with three drops freshly prepared 6M HCl accompanied with continuous stirring.

Phloroglucinol Test: In a test tube, three drops of phloroglucinol reagent was added to 5 ml plant extract followed by three drops concentrated hydrochloric acid. The solution was observed for the formation of a red colour as an indication for the presence of lignin, (Lin & Dence, 1992).

# 14. Test for Coumarins

NaOH Test: In a test tube containing 5 ml plant extract, three drops of 1M sodium hydroxide (NaOH) solution was added and the solution observed for the formation of a yellow colour as an indication for the presence of coumarins, (Harborne, 1973).

# **3.3.2** Groupings of the Animals

The formular used for the calculation of the sample size for all the rat groups was according to Charan & Kantharia, 2013. The male Wistar albino rats were put in cages and labeled as follows:

Group I: This was the normal control group that received drinking water in addition to the standard rat diet (rodent chow), given orally for the entire experimental period of 35 days.

- Group II: This group was the negative control that received drinking water and the Western diet throughout the experimental period (35 days).
- Group III: Fed on a Western diet for five weeks with drinking water and received 10 mg/kg of Atorvastatin (Nasri *et al.*, 2016) once a day in the fifth week.
- Group IV: Fed on western diet for five weeks with drinking water and received 0.5 mg/kg glibenclamide (Samuel *et al.*, 2014) in the fifth week.
- Group V: Fed on western diet for five weeks with drinking water and received 200 mg/kg aqueous root extract of *Tithonia diversifolia* once a day in the fifth week.
- Group VI: Fed on western diet for five weeks with drinking water and received 400 mg/kg aqueous root extract of *Tithonia diversifolia* once a day in the fifth week
- Group VII: Fed on a Western diet for four weeks with drinking water and reverted to rodent chow in the last week of the experiment.

#### **3.3.3 Drug Administration**

Stock solution of 2.5ml of the *Tithonia diversifolia* aqueous root extract (200 mg/kg and 400 mg/kg), was prepared daily and 0.5ml of this solution was given to each rat in group five and six between days 29-35. 2.5 ml Glibenclamide solution was also prepared on a daily basis and from it, 0.5ml was given to each rat in group four between days 29-35, and 2.5 ml Atorvastatin solution prepared every day and 0.5ml of this solution given to each rat in group

three between days 29-35. All the drug and extract solutions were administered orally to the rats using a 5ml syringe in their respective groups.

#### **3.4** Data collection

#### **3.4.1** Determination of rat weights

The rat weights were monitored weekly throughout the experimental period. This was done by first resetting the weighing scale and weighing an empty closed container. This was followed by putting one rat at a time in a closed container, weighing the container together with the rat, and recording the readings. This procedure was repeated severally until all the rats were weighed.

The weight of the rat was calculated as follows:

Rat weight = (Mass of the container together with the rat) - (mass of the empty container).

#### **3.4.2** Determination of fasting blood glucose level

At the commencement of the experiment, the blood glucose levels for each of the experimental rats were determined by initiating a small incision at the tip of the tail and collecting a drop of blood onto a One Touch Horizon digital glucometer test strip. This measurement process involved activating the glucometer and inserting a test strip. Once the machine was prepared, a tiny droplet of blood was applied to the strip, and the glucometer was allowed to register the blood sugar level reading. This reading was duly recorded before subsequent tests were performed using fresh test strips. Prior to blood glucose tests, the feed was withdrawn from the rats for 12 hours. Fasting blood glucose levels were assessed weekly using the One Touch Horizon digital glucometer (Parasuraman *et al.*, 2015).

#### **3.4.3** Biochemical assays

At the end of the thirty-five-day study period, all thirty-five Wistar albino rats underwent overnight fasting. Subsequently, they were, in turn, placed within a desiccator containing cotton wool soaked in chloroform, allowing them to become unconscious. Once unconscious, approximately five to six milliliters of blood samples were collected from each rat via cardiac puncture and deposited into plain test tubes. The collected blood samples were undisturbed for approximately 30 minutes to induce clotting. The serum separation from the clotted blood was carried through centrifugation at 3000 RPM for about 20 minutes (Abebe et al., 2021). The serum samples were carefully separated from the blood using a micropipette and transferred into vials. The serum lipid profile, Renal function tests (RFTs), and Liver function tests (LFTs) were subsequently analyzed using the Biobase Auto Chemistry Analyzer BK-200. The analysis encompassed various biochemical markers, including the evaluation of liver enzyme levels through measurements of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), as well as the assessment of RFTs, which involved the analysis of creatinine and urea levels (Shukla et al., 2015). Furthermore, the analysis of the lipid profile included the measurement of total cholesterol (TC), triglyceride (TG), and high-density lipoprotein (HDL) levels (Cai et al., 2021). All the biochemical analyses were conducted at the Kabarak University Medical Centre laboratory.

# **3.5** Data management plan

# **3.5.1** Data entry and storage

The numerical data from biochemical measurements obtained during the experiment were meticulously entered into a Microsoft Excel spreadsheet. Subsequently, the data underwent a thorough cleaning process involving identifying and correcting any typographical or entry errors. Finally, the cleaned data were securely stored for further analysis.

#### **3.5.2 Data Analysis**

The data entered was exported for analysis to the Statistical Package for Social Sciences (SPSS) Software, specifically version 20. Statistical analysis encompassing all seven groups was conducted employing one-way Analysis of Variance (ANOVA), subsequently followed by the application of the Least Significant Differences (LSD) method, where statistical significance was established at  $P \le 0.05$ . Additionally, Duncan's test was employed to assess the homogeneity of the various treatment groups. Each group consisted of 5 rats, and the outcomes were expressed as mean values along with their respective standard deviations, following the format described by Jayaraman *et al.* in 2018. The resulting data was effectively presented through the use of graphs and tables.

#### 3.5.3 Selection of Least Significant Difference (LSD) post hoc analysis

Opting for the Least Significant Difference (LSD) post hoc analysis is the ideal choice for this research, primarily due to its alignment with the research objectives and questions. The research focuses on customized comparisons that directly address specific research inquiries, and LSD's flexibility allows for precise refinements of treatment group comparisons. In contrast, other post hoc methods, such as Tukey's HSD or Bonferroni, may involve overly inclusive pairwise comparisons that lack the specificity required. Furthermore, given the research's emphasis on simplicity, interpretability, and effective communication, LSD offers a straightforward approach to comparing group means. Its adaptability to situations where the assumption of equal variances might not hold makes it even more favorable when compared to methods reliant on this assumption.

# **3.5.4** The selection of Duncan's test analysis

The selection of Duncan's test analysis over other methods is particularly appropriate for this research, given its direct alignment with the research objectives and questions. One of the primary research goals in this research is to discern homogeneous subsets among treatment groups and conduct customized comparisons to address specific research questions. This test excels in categorizing treatment groups into subsets with similar means, enabling a clear understanding of which groups exhibit statistically significant differences and which do not. This alignment with the research's central focus on specific treatment differences distinguishes it from other, more rigid post hoc tests. Furthermore, the Duncan's test's simplicity, ease of interpretation, and precise results make it relevant to this work.

# **3.6 Ethical Considerations**

The experimental procedures employed in this study were submitted for review. They obtained approval from the Research Ethics Committee (REC) at the University of Eastern Africa, Baraton, prior to the initiation of the experiment. The study was conducted under the ethical approval number UEAB/REC/16/03/2020.

# **CHAPTER FOUR**

# 4.0 **RESULTS**

# 4.1 Phytochemical screening on the aqueous root extract of *Tithonia diversifolia*

The qualitative phytochemical analysis of the aqueous root extract of *Tithonia diversifolia* revealed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, phenols, carbohydrates, anthocyanins, and coumarins, as depicted in Table 5 below. Conversely, glycosides, steroids and sterols, proteins and amino acids, and lignin were undetected.

	Phytochemical	Test	Observation	Indication (+:Presence), (-: Absence)
1	Alkaloids	Dragendorff's Test	Orange-red colouration	+
2	Flavonoids	Shinoda Test	Reddish colouration	+
3	Tannins	Ferric Chloride Test	Blue-black colouration	+
4	Saponins	Froth Test	Froth that persists for a few minutes	+
5	Terpenoids	Salkowski Test	Reddish-brown coloration at the interface	+
6	Glycosides	Keller-Killiani Test	Darkening of the solution	-
7	Phenols	Ferric Chloride Test	Blue-green coloration	+
8	Carbohydrates	Molisch's Test	Dark Violet ring at the interface	+
9	Steroids and Sterols	Liebermann- Burchard Test	The solution turned dark brown	-
10	Cyanogenic Glycosides	Picric Acid Test	No observable change	-
11	Proteins and Amino Acids	Biuret Test	Bluish colouration	-
12	Anthocyanins	Acid Test	Reddish colouration	+

Table 5. Phytochemical constituents of the aqueous root extract of *Tithonia diversifolia* 

13	Lignin	Phloroglucinol Test	Browning of the solution	-
14	Coumarins	NaOH Test	Yellowing of the solution	+

# 4.2 Effects of aqueous root extract of *T. diversifolia* on body weights of Western dietfed Wistar albino rats

The rats underwent a one-week acclimatization period. After this week, each rat was weighed and assigned to their treatment groups G1-G7. All groups, except the normal control group (G1) and G7, were fed a Western diet for five weeks (throughout the experimental period). The rats' weights were recorded weekly for five weeks. The feeding of the western diet was replaced with normal rat chow at the end of the fourth week for G7. Starting from week five, the treatments were administered to the corresponding groups for seven days, and the rats' weights were measured again at the end of week five. The results are detailed in Table 6 below.

Table 6: Effects of aqueous root extract of *T. diversifolia* on mean weights of Western dietfed Wistar albino rats within the five weeks

Week	Normal Control (G1)	Negative Control (G2)	10mg/kg b.w Atorvas tatin (G3)	0.5mg/kg b.w Glibencl amide (G4)	200mg/kg b.w extract (G5)	400mg/kg b.w extract (G6)	(G7)
1	187.52	186.34	187.74	187.74	188.14	187.14	188.86
2	189.46	188.72	188.94	189.25	189.2	189.18	190.1
3	190.22	191.38	190.12	190.46	190.38	190.64	191.7
4	191.2	194.84	191.86	191.76	192.06	191.34	193.8
5	192.46	197.12	189.52	191.38	191.94	190.7	193.98

The table above showed a gradual increase in rat weights for all groups between weeks one and four. By the end of week 5, the mean weights of rats decreased for those receiving 10 mg/kg atorvastatin (G3), 0.5 mg/kg glibenclamide (G4), 200 mg/kg (G5), and 400 mg/kg aqueous root extract (G6) of *Tithonia diversifolia*, compared to the normal control (G1), negative control (G2), and the rats fed a Western diet for four weeks and then switched to rodent chow in the fifth week (G7).

In the post hoc LSD analysis used to compare mean differences in rat weights between the groups, with the normal control group (G1) as the baseline, a significant reduction in average rat weights was observed (p=0.000) among the groups that received 10 mg/kg atorvastatin (G3), 0.5 mg/kg glibenclamide (G4), 200 mg/kg *Tithonia diversifolia* aqueous root extract (G5), 400 mg/kg *Tithonia diversifolia* aqueous root extract (G5), 400 mg/kg *Tithonia diversifolia* aqueous root extract (G5), 400 mg/kg *Tithonia diversifolia* aqueous root extract (G6), and those fed a Western diet for four weeks and switched to normal rodent chow in the fifth week (G7) (p=0.013). There was a significant decrease in rat weight in all the treatment groups compared to the negative control (G2) (p=0.00).

**Multiple Comparisons** Dependent Variable: Average body weight at WK5 95% Confidence Mean Std. Interval **(I) (J)** Differe Mean Err Sig. Lowe GROUPS GROUPS nce r Upper or (**I-J**) Bound Boun d NEGATIVE CONTROL 2.28 .399 1.020 .016 -1.838 -.201 (G2) 10 mg/kg b.w ATORVASTAT -2.34 -3.600 .399 .000 2.781 4.418 IN(G3) NORMAL 0.5 mg/kg b.w **CONTRO** GLIBENCLAM -0.38 -1.640 .399 .000 .821 2.458 L(G1) IDE(G4) 200 mg/kg b.w -0.12 .399 .002 .561 -1.380 2.198 extract (G5) 400 mg/kg b.w -0.64 -1.900 .399 .000 1.081 2.718 extract (G6) LSD (G7) -0.18 -1.080 .399 .013 .241 1.878 NORMAL .399 1.26 -1.020 .016 .201 1.838 CONTROL(G1) 10 mg/kg b.w ATORVASTAT -2.34 -4.620 .399 .000 3.801 5.438 IN(G3) 0.5 mg/kg b.w NEGATI GLIBENCLAM -0.38 -2.660 .399 .000 1.841 3.478 VE (G2) IDE(G4) 200 mg/kg b.w -0.12 -2.400 .399 .000 1.581 3.218 extract (G5) 400 mg/kg b.w -0.64 -2.920 .399 .000 2.101 3.738 extract (G6)

0.18

-2.100

.399

.000

1.261

2.898

(G7)

weights of Western diet-fed Wistar albino rats on the fifth week

Table 7: Post-hoc results on the effects of aqueous root extract of T. diversifolia on body

Based on observed means.

\*The mean difference is significant at 0.05 level.

In Table 7, the second column is the baseline from which all the other treatment groups in column three are compared. The level of significance is shown in column 7.

# 4.3 Effects of aqueous root extract of *T. diversifolia* on blood glucose in Western diet-fed Wistar albino rats.

The normal range for fasting blood glucose levels in Wistar rats is 3.95±1.31mmol/L (Wang *et al.*, 2010).

The fasting blood sugar levels of the rats were measured using a digital glucometer every week, and the result is shown in Table 8 below.

Table 8: Effects of aqueous root extract of T. diversifolia on blood glucosde in Western diet-

Treatment Creams	Fas	Fasting Blood glucose in mmol/L							
Treatment Groups	Week 1	Week 2	Week 3	Week 4	Week 5				
Normal Control (G1)	4.06	4.14	4.18	4.2	4.32				
Negative Control (G2)	4	4.22	4.36	4.52	4.82				
10mg/kg b.w Atorvastatin (G3)	3.9	4.26	4.27	4.28	4.18				
0.5mg/kg b.w Glibenclamide (G4)	3.94	4.04	4.12	4.22	4.12				
200mg/kg b.w extract (G5)	3.94	4	4.12	4.24	4.18				
400mg/kg b.w extract (G6)	3.94	4.18	4.24	4.25	4.08				
Rodent chow (G7)	4.02	4.04	4.16	4.62	4.84				

fed Wistar albino rats.

In Table 8 above, fasting blood sugar levels gradually increased in all rat groups from week one to week four. At the start of week 5, G3 received 10 mg/kg atorvastatin, G4 got 0.5 mg/kg glibenclamide, G5 was given 200 mg/kg *Tithonia diversifolia* root extract, and G6 received 400 mg/kg *Tithonia diversifolia* root extract, while G7 returned to rodent chow. Generally, there was a decrease in fasting blood sugar levels in the rat groups that received 10 mg/kg atorvastatin (G3), 0.5 mg/kg glibenclamide (G4), those given 200 mg/kg *Tithonia diversifolia* root extract (G5), and the ones given 400 mg/kg *Tithonia diversifolia* root extract (G6). In contrast, the normal control (G1), negative control (G2), and the rats fed a Western diet for four weeks and reverted to rodent chow in the fifth week (G7) showed an increase in their mean sugar levels at the end of week five when the experiment concluded.

A post hoc LSD result compared the mean difference in fasting blood sugar levels in the rat groups. When the normal control was used as the baseline, there was an insignificant decrease in fasting blood sugar levels in the rats given 10 mg/kg atorvastatin (G3), 0.5 mg/kg glibenclamide (G4), and 200 mg/kg *Tithonia diversifolia* root extract (G5) (p=0.190, p=0.065, p=0.190, respectively), while the rats fed a Western diet for four weeks and reverted to rodent chow in the last week of the experiment (G7) recorded a significant increase in fasting blood sugar levels (p=0.000). The rats given 400 mg/kg *Tithonia diversifolia* root extract showed a significant decrease in fasting blood sugar levels (p=0.000). The rats given 400 mg/kg *Tithonia diversifolia* root extract showed a significant decrease in fasting blood sugar levels (p=0.029). When negative control (G2) was used as the baseline, there was a significant decrease in fasting blood sugar levels in the rats that received 0.5 mg/kg glibenclamide (G4), 10 mg/kg atorvastatin (G3), 200 mg/kg *Tithonia diversifolia* root extract (G5), and 400 mg/kg *Tithonia diversifolia* root extract (G6) (p=0.000), while an insignificant decrease was shown in the rats fed a Western

diet for four weeks and reverted to rodent chow in the last week of the experiment (G7)

(p=0.849).

 Table 9: Post-hoc results on the effects of aqueous root extract of *T. diversifoila* on blood

 sugar in Western diet-fed Wistar albino rats

	iple Compari ndent Varial	ole: Blood sugar in V	Wester	n diet-fed	Wistar al	bino 1	rats at we	ek 5
	(I)	( <b>J</b> )		Mean Differenc			95% Co	nfidence rval
	GROUPS	GROUPS/ TREATMENTS	Mean	e (I-J)	Error	51g.	Lower Bound	Upper Bound
		NEGATIVE CONTROL(G2)	4.82	$500000^{*}$	.1041976	.000	95% Co           International           Sig.         International $100$ 713439 $000$ 713439 $190$ 073439 $065$ 013439 $000$ 073439 $029$ .026561 $000$ 733439 $000$ .286561 $000$ .426561 $000$ .486561 $000$ .426561	286561
		10 mg/kg b.w ATORVASTATIN (G3)		.140000	.1041976	.190	073439	.353439
	CONTROL	0.5 mg/kg b.w GLIBENCLAMIDE (G4)		.200000	.1041976	.065	013439	.413439
		200 mg/kg b.w extract (G5)	4.18	.140000	.1041976	.190	073439	.353439
.SD		400 mg/kg b.w extract (G6)	4.08	$.240000^{*}$	.1041976	.029	.026561	.453439
		(G7)	4.84	520000*	.1041976	.000	733439	306561
		NORMAL CONTROL(G1)	4.32	$.500000^{*}$	.1041976	.000	.286561	.713439
		10 mg/kg b.w ATORVASTATIN( G3)		.640000*	.1041976	.000	.426561	.853439
	NEGATIVE CONTROL (G2)	GLIBENCLAMIDE	4.12	.700000*	.1041976	.000	.486561	.913439
		200 mg/kg b.w extract (G5)		.640000*	.1041976	.000	.426561	.853439
		400 mg/kg b.w extract (G6)	4.08	$.740000^{*}$	.1041976	.000	.526561	.953439
		(G7)	4.84	020000	.1041976	.849	233439	.193439

In Table 9 above, the second column is used as the baseline to which all the other treatment groups in column three are compared. The level of significance is shown in column 7.

# 4.4 Lipid Profile

In male Wistar albino rats, the normal reference values for serum cholesterol (2.0-1.1 mmol/l), serum triglycerides (2.1-0.4 mmol/l), and serum high-density lipoprotein according to Boehm *et al.* (2007).

# **4.4.1** Effects of aqueous root extract of *T. diversifolia* on mean serum cholesterol levels The blood for the cholesterol estimation was collected at the end of the experiment via cardiac puncture and estimated using Biobase BK 200. The mean serum cholesterol levels for each group are shown in Figure 2 below.

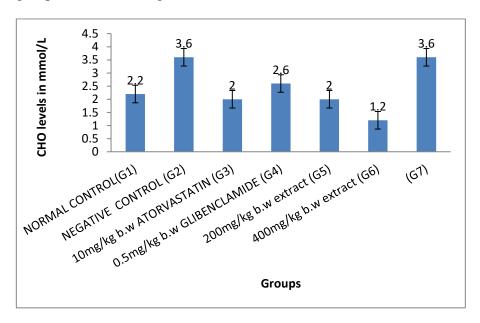


Figure 2: Effects of aqueous root extract of T. diversifolia on mean serum cholesterol levels

in Western diet-fed Wistar albino rats.

From Figure 2, there was a decrease in serum cholesterol levels in rats that received 10 mg/kg atorvastatin (G3), 200 mg/kg *Tithonia diversifolia* aqueous root extract (G5), and 400 mg/kg *Tithonia diversifolia* aqueous root extract (G6) when compared to the normal control (G1). Conversely, increased cholesterol levels were observed in rats given 0.5 mg/kg glibenclamide (G4). The rats were initially fed a Western diet for four weeks and then switched to rodent chow in the last week of the experiment (G7).

There was a decrease in serum cholesterol levels in rats given 10 mg/kg atorvastatin (G3), 200 mg/kg *Tithonia diversifolia* root aqueous extract (G5), and 400 mg/kg *Tithonia diversifolia* root aqueous extract (G6). However, the rats initially fed on a Western diet for four weeks and then reverted to a normal rat diet in the fifth week (G7) did not change in cholesterol levels compared to the negative control (G2).

In the post hoc LSD analysis, which compared the mean difference in serum cholesterol levels among the groups with the normal control (G1) as the baseline, there was an insignificant decrease in cholesterol levels in the rats treated with 10 mg/kg atorvastatin (G3) and 200 mg/kg *Tithonia diversifolia* root aqueous extract (G5) (p=0.538). However, rats given 400 mg/kg *Tithonia diversifolia* root aqueous extract (G6) recorded significantly decreased cholesterol levels (p=0.004). In contrast, the rats fed a Western diet for four weeks and switched to normal rat diet in the fifth week (G7) showed a significant increase in serum cholesterol levels (p=0.000).

When the negative control (G2) was used as the baseline, the post hoc analysis indicated a significant decrease in mean serum cholesterol levels in the rat groups that received 10 mg/kg atorvastatin (G3), 200 mg/kg *Tithonia diversifolia* root aqueous extract (G5), and 400 mg/kg

*Tithonia diversifolia* root aqueous extract (G6) (p=0.000). Meanwhile, the group of rats fed a Western diet for four weeks and switched to rodent chow in the fifth week (G7) exhibited cholesterol levels identical to the control group.

Table 10: Post hoc LSD analysis on the effects of aqueous root extract of *T. diversifolia* on serum cholesterol levels in Western diet-fed Wistar albino rats.

Multi	Multiple Comparisons							
Deper	ndent Varia	ble: Cholesterol						
	(I)		Mea	Std.	Sig	95° Confic Inter	lence	
	GROUP S	(J) Groups/Treatments	n	n Diff (I-J)	Err or	·	Lower Bound	Uppe r Boun d
	NORMA LCONT ROL	NEGATIVE CONTROL (G2)	3.60	- 1.40 *	.321	.00 0	-2.06	74
	(G1)	10 mg/kg b.w ATORVASTATIN (G3)	2.00	.20	.321	.53 8	46	.86
LSD		0.5 mg/kg b.w GLIBENCLAMIDE (G4)	2.60	40	.321	.22 3	-1.06	.26
		200 mg/kg b.w extract (G5)	2.00	.20	.321	.53 8	46	.86
		400 mg/kg b.w extract (G6)	1.20	1.00 *	.321	.00 4	.34	1.66
		(G7)	3.60	- 1.40 *	.321	.00 0	-2.06	74
	NEGATI VE	NORMAL CONTROL (G1)	2.20	1.40 *	.321	.00 0	.74	2.06
	CONTR OL (G2)	10 mg/kg b.w ATORVASTATIN (G3)	2.00	1.60 *	.321	.00 0	.94	2.26

	0.5	mg/kg	b.w	2.60	1.00	.321	.00	.34	1.66
	GLIBE	ENCLAMI	DE		*		4		
	(G4)								
	200 m	g/kg b.w e	extract	2.00	1.60	.321	.00	.94	2.26
	(G5)				*		0		
	400 m	g/kg b.w e	extract	1.20	2.40	.321	.00	1.74	3.06
	(G6)				*		0		
	(G7)			3.60	.00	.321	1.0	66	.66
							00		
Based on o	bserved	means.			•	•	•	•	•
* The mean	n differe	nce is sign	ificant	at 0.05	level.				

The second column is used as the baseline that compares other treatment groups in column three, and the significance level is shown in column 7.

A Duncan's test was employed to assess the homogeneity of the groups concerning the mean serum cholesterol levels. Duncan's multiple range tests, as a post hoc analysis, were used to discern specific distinctions between pairs of means.

The results of this test revealed that mean cholesterol values were homogeneous among the groups administered 10 mg/kg Atorvastatin (G3), 0.5 mg/kg Glibenclamide (G4), 200 mg/kg *Tithonia diversifolia* aqueous root extract (G5), and the normal control group (G1). Similarly, the negative control group (G2) and the rats exposed to a Western diet for four weeks followed by a switch to rodent chow in the fifth week (G7), also displayed consistency in cholesterol mean levels. In contrast, these groups exhibited distinctions from the rats that received 400 mg/kg aqueous root extract of *Tithonia diversifolia* (G6) and the remaining groups.

Table 11: Showing the level of homogeneity in the mean serum cholesterol levels between the groups

	TREATMENT GROUPS	Ν		Subset	
	IREAIMENI GROUPS		1	2	3
	400 mg/kg b.w extract (G6)	5	1.20		
	10 mg/kg b.w ATORVASTATIN (G3)	5		2.00	
	200 mg/kg b.w extract (G5)	5		2.00	
Duncan <sup>a,b</sup>	NORMAL CONTROL(G1)	5		2.20	
	0.5 mg/kg b.w GLIBENCLAMIDE (G4)	5		2.60	
	NEGATIVE CONTROL(G2)	5			3.60
	(G7)	5			3.60
Means for g	roups in homogeneous subsets are displaye	ed.			•
Based on ob	oserved means.				
a. Uses Har	monic Mean Sample Size = 5.000.				

A Duncan's test for assessing homogeneity categorized the treatment groups into three distinct subsets, as presented in Table 11. Rats administered 10 mg/kg Atorvastatin (G3), 200 mg/kg *Tithonia diversifolia* aqueous root extract (G5), the normal control (G1), and 0.5 mg/kg Glibenclamide (G4) did not exhibit statistically significant differences among themselves.

However, they were distinguishable from the groups of rats that were administered 400 mg/kg aqueous root extract of *Tithonia diversifolia* (G6), the rats subjected to a Western diet for four weeks followed by a switch to rodent chow in the fifth week (G7), and the negative control (G2).

# 4.4.2 Effects of aqueous root extract of *T. diversifolia* on serum High-Density Lipoprotein (HDL-C) levels in Western diet-fed Wistar albino rats.

The Serum HDL-C levels were analyzed using Biobase BK 200, and their mean levels for each rat group are shown in Figure 7 below.

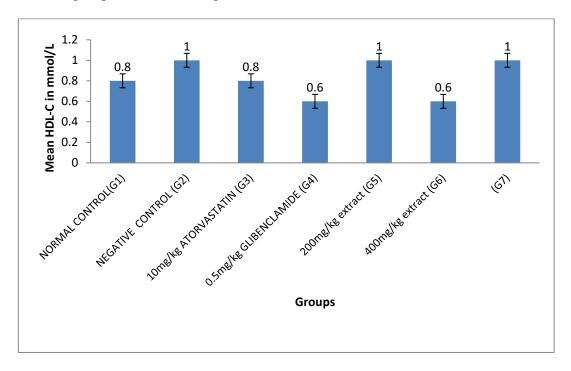


Figure 3: Effects of aqueous root extract of *T. diversifolia* on mean serum High high-density

lipoprotein (HDL-C) levels in Western diet-fed Wistar albino rats.

In Figure 3, a comparison was made between the normal control (G1) and the other treatment groups. It was observed that rats administered 0.5 mg/kg glibenclamide (G4) and 400 mg/kg aqueous root extract (G6) displayed a reduction in mean HDL-C levels, while rats that received 200 mg/kg aqueous root extract of *Tithonia diversifolia* (G5) and rats subjected to a Western diet for four weeks followed by a switch to rodent chow (G7) exhibited an increase in HDL-C levels.

When the negative control (G2) was used as the reference for comparison, it was noted that there was a decline in mean HDL-C levels in rats administered 10 mg/kg atorvastatin (G3), 0.5 mg/kg glibenclamide (G4), and 400 mg/kg aqueous root extract (G6). However, rats receiving 200 mg/kg aqueous root extract of *Tithonia diversifolia* (G5) and those subjected to a Western diet for four weeks followed by a switch to rodent chow (G7) did not exhibit any significant change in HDL-C levels.

Upon conducting a post hoc LSD analysis to compare the mean differences in serum HDL-C levels between the groups and using both the normal control (G1) and the negative control (G2) as baselines, it was evident that there were no significant alterations in HDL-C levels across all the treatment groups, as indicated by their respective p-values (Table 12).

Table 12: Post-hoc results on the effects of aqueous root extract of *T. diversifolia* on meanserum HDL-C in Western diet-fed Wistar albino rats.

M	ıltiple Com	parisons						
De	pendent Va	ariable: HDL-C						
	(T)			Maan	64.1		95% Con Inter	
	(I) GROUP S	(J) Groups/Treatmen ts	Mea n	Mean Diff (I-J)	Std. Err or	Sig.	Lower Bound	Uppe r Boun d
	NORMA L	NEGATIVE CONTROL(G2)	1.00 0	20	.239	.410	69	.29
L S	CONTR OL (G1)	10 mg/kg b.w ATORVASTATIN (G3)	.800	.00	.239	1.00 0	49	.49
D		0.5 mg/kg b.w GLIBENCLAMID E (G4)	.600	.20	.239	.410	29	.69
		200 mg/kg b.w extract (G5)	1.00 0	20	.239	.410	69	.29

	400 mg/kg b.w extract (G6)	.600	.20	.239	.410	29	.69		
	(G7)	1.00 0	20	.239	.410	69	.29		
NEGATI VE (G2)	NORMAL CONTROL (G1)	.800	.20	.239	.410	29	.69		
	10 mg/kg b.w ATORVASTATIN (G3)	.800	.20	.239	.410	29	.69		
	0.5 mg/kg b.w GLIBENCLAMID E (G4)	.600	.40	.239	.105	09	.89		
	200 mg/kg b.w extract (G5)	1.00 0	.00	.239	1.00 0	49	.49		
	400 mg/kg b.w extract (G6)	.600	.40	.239	.105	09	.89		
	(G7)	1.00 0	.00	.239	1.00 0	49	.49		
	Based on observed means. *The mean difference is significant at the 0.05 level.								

The baseline, column two, is used to compare other treatment groups in column three, and the significance level is shown in column 7.

When a Duncan's test was conducted to assess the homogeneity of the groups in terms of HDL-C mean levels, it revealed uniformity in HDL-C mean values across all the rat groups, as presented in Table 13.

Dependent	Variable: HDL-C		
	GROUPS/TREATMENTS	Ν	Subset
	GROUPS/IREAIWENIS	11	1
	0.5 mg/kg b.w GLIBENCLAMIDE (G4)	5	.60
	400 mg/kg b.w extract (G6)	5	.60
	NORMAL CONTROL (G1)	5	.80
Duncan <sup>a,b</sup>	10 mg/kg b.w ATORVASTATIN (G3)	5	.80
	NEGATIVE CONTROL (G2)	5	1.00
	200 mg/kg b.w extract (G5)	5	1.00
	(G7)	5	1.00
Means for g	roups in homogeneous subsets are displayed.	Based on o	bserved means.
a. Uses Harr	monic Mean Sample Size = 5.000.		
b. Alpha = .	05.		

Table 13: Level of homogeneity in the mean serum HDL-C levels between the groups

# **4.4.3** Effects of aqueous root extract of *T. diversifolia* on serum Triglycerides (TG) levels in Western diet-fed Wistar albino rats

The analysis of serum triglyceride levels was done using Biobase BK 200. The mean serum triglyceride levels for each group were as shown in Figure 4 below.

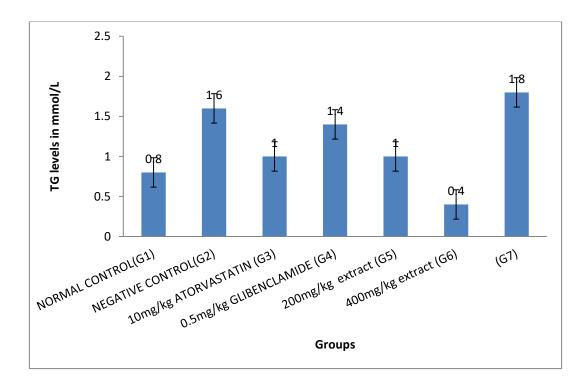


Figure 4: Effects of aqueous root extract of *T. diversifolia* on mean serum Triglycerides (TG) levels in Western diet-fed Wistar albino rats.

In Figure 4, it was observed that rats administered 10 mg/kg atorvastatin (G3), 0.5 mg/kg glibenclamide (G4), 200 mg/kg *Tithonia diversifolia* aqueous root extract (G5), and rats subjected to a Western diet for four weeks followed by a switch to rodent chow after week four (G7) exhibited an increase in mean serum triglyceride levels, in comparison to the normal control (G1). Conversely, rats that received 400 mg/kg *Tithonia diversifolia* aqueous root extract (G6) demonstrated decreased serum triglyceride levels.

When the negative control (G2) was used for comparison with the other treatment groups, it was noted that there was a decrease in mean serum triglyceride levels in all the treatment

groups except for the rats exposed to a Western diet for four weeks and subsequently switched to rodent chow (G7), which exhibited an increase in mean serum triglyceride levels.

Upon conducting a post hoc LSD analysis to compare the mean differences in serum triglyceride levels among the rat groups and employing the normal control (G1) as the reference point, it was revealed that there was a significant decrease in mean serum triglyceride levels in the group administered with 0.5 mg/kg body weight glibenclamide (p=0.034). Conversely, the group of rats subjected to a Western diet for four weeks and switched to rodent chow in the fifth week (G7) showed a significant increase in triglyceride levels (p=0.001). However, the other groups, including those administered with 10 mg/kg atorvastatin (G3) and 200 mg/kg *Tithonia diversifolia* aqueous root extract (G5) (p=0.469), and 400 mg/kg *Tithonia diversifolia* aqueous root extract (G6) (p=0.153), did not exhibit any significant differences.

When the negative control (G2) was used as the reference point, it was evident that there was a significant decrease in mean serum triglyceride levels in the groups that received 10 mg/kg atorvastatin (G3) (p=0.036) and 200 mg/kg *Tithonia diversifolia* aqueous root extract (G5) (p=0.036). Furthermore, the group exposed to a higher extract concentration at 400 mg/kg (G6) displayed a more substantial reduction in triglyceride levels (p=0.000), as indicated in Table 14.

		Multiple C	ompari	sons				
		Dependent	Variabl	e: TG				
	(I) GROUPS	(J) Groups/Treatments	Mea n	Mea n Diff (I-J)	Std. Erro r	Sig.	Con	5% fiden ee erval Up per Bou
							und	nd
	NORMA L CONTRO	NEGATIVE CONTROL (G2)	1.60	80*	.273	.007	- 1.3 6	24
	L G1	10 mg/kg b.w ATORVASTATIN (G3)	1.00	20	.273	.469	76	.36
LCD		0.5 mg/kg b.w GLIBENCLAMIDE (G4)	1.40	60*	.273	.036	- 1.1 6	04
LSD		200 mg/kg b.w extract (G5)	1.00	20	.273	.469	76	.36
		400 mg/kg b.w extract (G6)	.40	.40	.273	.153	16	.96
		(G7)	1.80	- 1.00*	.273	.001	- 1.5 6	44
	NEGATI VE	NORMAL CONTROL(G1)	.80	.80*	.273	.007	.24	1.36
	G2	10 mg/kg b.w ATORVASTATIN (G3)	1.00	.60*	.273	.036	.04	1.16
		0.5 mg/kg b.w GLIBENCLAMIDE (G4)	1.40	.20	.273	.469	36	.76
		200 mg/kg b.w extract (G5)	1.00	.60*	.273	.036	.04	1.16

 Table 14: Post-hoc LSD results on the effects of aqueous root extract of *T. diversifolia* on

 serum triglycerides in Western die-fed Wistar albino rats

400 mg/kg b.w extract (G6)	.40	1.20*	.273	.000	.64	1.76
(G7)	1.80	20	.273	.469	76	.36

In Table 14, column two serves as the reference point against which all the other treatment groups in column three are compared, and column 7 indicates the level of statistical significance.

Duncan's test has organized the treatment groups into four subsets (table 15), providing a basis for understanding the statistical similarity or dissimilarity of their mean values for the dependent variable (TG). Subset 1; normal control (G1), 10 mg/kg atorvastatin (G3), 200 mg/kg Tithonia diversifolia aqueous root extracts (G5), 400 mg/kg Tithonia diversifolia aqueous root extract (G6) contains treatments with statistically similar TG levels, highlighting that these groups share commonalities in terms of TG. In Subset 2, normal control (G1), 10 mg/kg atorvastatin (G3), 200 mg/kg Tithonia diversifolia aqueous root extracts (G5), 0.5 mg/kg glibenclamide (G4), treatments exhibit similar TG levels, but they differ significantly from Subset 1, indicating that 0.5 mg/kg glibenclamide (G4) has distinct TG levels compared to 400 mg/kg *Tithonia diversifolia* aqueous root extract (G6). Subset 3: The negative control group (G2), 10 mg/kg atorvastatin (G3), 200 mg/kg Tithonia diversifolia aqueous root extracts (G5), 0.5 mg/kg glibenclamide (G4) showcases treatments with similar TG levels within the subset, differing significantly from Subset 1, illustrating differences between the negative control group (G2) and 400 mg/kg Tithonia diversifolia aqueous root extract (G6). Subset 4; 0.5 mg/kg glibenclamide (G4), the negative control group (G2), the rats subjected to a Western diet for four weeks followed by a switch to rodent chow in the fifth week (G7) includes treatments with statistically similar TG levels within this subset but significantly different from groups in Subsets 1, 2, and 3.

Table 15: showing the level of homogeneity in the mean serum triglyceride levels between the groups

Dependent	Variable: TG					
	GROUPS/TREATMENTS	Ν		S	ubset	
	GROUPS/IREAIMENIS	IN	1	2	3	4
	400 mg/kg b.w extract (G6)	5	.40			
	NORMAL CONTROL (G1)	5	.80	.80		
	10 mg/kg b.w ATORVASTATIN (G3)	5	1.00	1.00	1.00	
	200 mg/kg b.w extract (G5)	5	1.00	1.00	1.00	
Duncan <sup>a,b</sup>	0.5 mg/kg b.w GLIBENCLAMIDE (G4)	5		1.40	1.40	1.40
	NEGATIVE CONTROL (G2)	5			1.60	1.60
	(G7)	5				1.80
Means for g	roups in homogeneous subsets a	re disp	layed. E	ased on	observed	means.
a. Uses Hari	monic Mean Sample Size = 5.00	0.				
b. Alpha = .	05.					

Therefore, Duncan's test on homogeneity produced four different subsets for the rat groups, as shown in table above.

**Liver Function Tests** 

4.5

# 4.5.1 Effects of aqueous root extract of *T. diversifolia* on serum alkaline phosphatase

### (ALP) levels in Western diet-fed Wistar albino rats

The refence values for ALP (U/L) in Wistar albino rats range between 95-611 (Loeb & Quimby, 1999).

Biobase BK 200 serum analysis on ALP levels was done, and the mean serum ALP levels for each treatment group are shown in Figure 5 below.

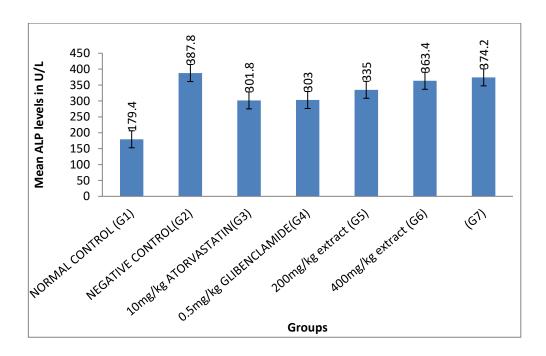


Figure 5: Effects of aqueous root extract of *T. diversifolia* on serum alkaline phosphatase (ALP) levels in Western diet-fed Wistar albino rats.

From figure 5 above, From Figure 5 above, when comparing the normal control (G1) to the other rat groups, there was a notable increase in mean serum ALP levels across all the rat groups. In contrast, when comparing these groups to the negative control (G2), a decrease in mean serum ALP levels was observed in all the rat groups, as depicted in Figure 5.

Upon employing post hoc LSD analysis to assess the mean differences in serum ALP levels among the rat groups, and with the normal control (G1) as the reference point, a significant increase in mean serum ALP levels was noted among all the rat groups, indicated by their respective p-value (p=0.000). This suggests that consuming a Western diet had a discernible impact on liver integrity.

Conversely, when the negative control (G2) was used as the baseline for comparison, all the rat groups exhibited a significant decrease in mean serum ALP levels, as detailed in Table 16 for the corresponding p-values. This implies that the interventions implemented had a beneficial hepato-restorative effect. Notably, the group of rats fed a Western diet for four weeks and then switched to rodent chow in the fifth week (G7) showed a non-significant reduction in ALP levels (p=0.184).

Table 16: Post-hoc results on the effects of aqueous root extract of *T. diversifolia* on serum

Mu	ultiple Compa	risons						
De	pendent Varia	able: ALP						
				Mean	Std.		Confi	% dence rval
	(I) GROUPS	(J) Treatments	Mean	Diff (I-J)	9.988	Sig.	Lowe r Boun d	Uppe r Boun d
	NORMAL CONTROL (G1)	NEGATIVE CONTROL(G2)	387.80 0	-208.40*	9.988	.000	- 228.8 6	- 187.9 4
L S		10 mg/kg b.w ATORVASTAT IN (G3)	301.80 0	-122.40*	9.988	.000	- 142.8 6	- 101.9 4
D		0.5 mg/kg b.w GLIBENCLAM IDE (G4)	303.00 0	-123.60*	9.988	.000	- 144.0 6	- 103.1 4
		200 mg/kg b.w extract (G5)	335.00 0	-155.60*	9.988	.000	- 176.0 6	- 135.1 4

ALP levels in Western diet-fed Wistar albino rats

	400 mg/kg b.w extract (G6)	363.40 0	-184.00*	9.988	.000	- 204.4 6	- 163.5 4
	(G7)	374.20 0	-194.80*	9.988	.000	- 215.2 6	- 174.3 4
NEGATIV E	NORMALCON TROL (G1)	179.40 0	208.40*	9.988	.000	187.9 4	228.8 6
CONTROL (G2)	10 mg/kg b.w ATORVASTAT IN (G3)	301.80 0	86.00*	9.988	.000	65.54	106.4 6
	0.5 mg/kg b.w GLIBENCLAM IDE (G4)	303.00 0	84.80*	9.988	.000	64.34	105.2 6
	200 mg/kg b.w extract (G5)	335.00 0	52.80*	9.988	.000	32.34	73.26
	400 mg/kg b.w extract (G6)	363.40 0	24.40*	9.988	.021	3.94	44.86
	(G7)	374.20 0	13.60	9.988	.184	-6.86	34.06

In the table above, column two serves as the reference against which all other treatment groups in column three are compared, and column 7 indicates the level of statistical significance.

When Duncan's test was conducted to evaluate the homogeneity of the groups concerning mean ALP levels, it revealed a similarity in ALP mean values between the groups administered 10 mg/kg atorvastatin (G3) and 0.5 mg/kg glibenclamide (G4). Additionally, rats given 400 mg/kg aqueous root extract of *Tithonia diversifolia* (G6) and those subjected to a Western diet for four weeks and reverted to rodent chow in the last seven days (G7) exhibited comparable outcomes. In contrast, the normal control (G1) and the group receiving

200 mg/kg aqueous root extract of *Tithonia diversifolia* (G5) differed from all other groups, as illustrated in Table 17.

Dependent	Variable: ALP						
	GROUPS	Ν			Subset		
	GROUPS	IN	1	2	3	4	5
	NORMAL CONTROL(G1)	5	179.4				
	10mg/kgb.wATORVASTATIN (G3)	5		301.8			
	0.5 mg/kg b.w GLIBENCLAMIDE (G4)	5		303.0			
Duncan <sup>a,b</sup>	200 mg/kg b.w extract (G5)	5			335.0		
	400 mg/kg b.w extract (G6)	5				363.	
						4	
	(G7)	5				374.	374.
						2	2
	NEGATIVE CONTROL (G2)	5					387.
							8
Means for g	groups in homogeneous subsets a	re dis	played.	Based on	observed	d means	5.
a. Uses Har	monic Mean Sample Size = 5.						
b c. Alpha =	= .05.						

Table 17: showing level of homogeneity in the mean serum ALP levels between the groups

The Dancun's test on homogeneity was, therefore, able to group the rat groups into five different subsets, as shown in Table 17 above.

4.5.2 Effects of aqueous root extract of *T. diversifolia* on serum Alanine aminotransferase (ALT) levels in Western diet-fed Wistar albino rats

The refence values for ALT (U/mL) in Wistar albino rats range between 80.08±7.49 (Marzouk *et al.*, 2011).

The mean serum ALT levels were estimated, and the result for each rat group is shown in Figure 6 below.

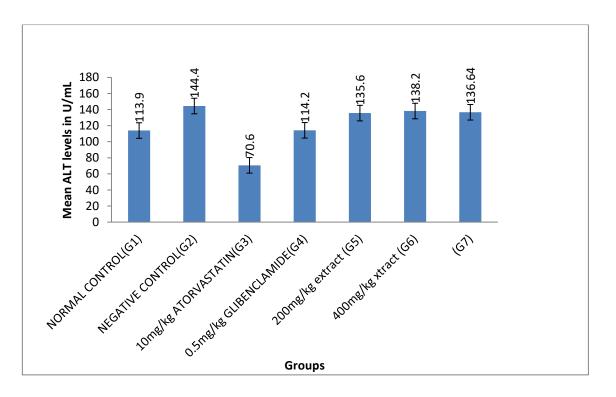


Figure 6: Effects of aqueous root extract of *T. diversifolia* on mean serum Alanine aminotransferase (ALT) levels in Western diet-fed Wistar albino rats.

When comparing the normal control (G1) to the other rat groups, there was an increase in mean serum ALT levels in all the rat groups, except for the rats that received 10 mg/kg atorvastatin (G3), which exhibited a decrease in mean serum ALT levels. Conversely, when employing the negative control (G2) as the basis for comparison, there was a reduction in mean serum ALT levels across all rat groups, as illustrated in Figure 6.

Upon conducting post hoc LSD analysis to evaluate the mean differences in serum ALT levels between the rat groups and utilizing the normal control (G1) as the reference point, it was evident that there was a significant increase in mean serum ALT levels in the rats that received 200 mg/kg aqueous root extract of *Tithonia diversifolia* (G5), 400 mg/kg aqueous root extract of *Tithonia diversifolia* (G5), 400 mg/kg aqueous root extract of *Tithonia diversifolia* (G5), and the rats exposed to a Western diet for four weeks and then reverted to rodent chow in the last week of the experiment (G7), as indicated

by their respective p-values (p=0.009), (p=0.004), (p=0.006). In contrast, the rats administered 0.5 mg/kg glibenclamide (G4) did not exhibit a significant increase in mean serum ALT levels (p=0.969). The rats that received 10 mg/kg atorvastatin (G3) recorded a significant decrease in mean serum ALT levels (p=0.000).

When the negative control (G2) was used as the baseline for comparison, there was an insignificant reduction in mean serum ALT levels in the groups of rats administered 200 mg/kg aqueous root extract of *Tithonia diversifolia* (G5), 400 mg/kg aqueous root extract of *Tithonia diversifolia* (G6), and the rats subjected to a Western diet for four weeks and then reverted to rodent chow in the last week of the experiment (G7), as denoted by their respective p-values (p=0.264), (p=0.428), (p=0.323). Conversely, the group of rats that received 10 mg/kg atorvastatin (G3) showed a significant decrease in mean serum ALT levels (p=0.000).

Table 18: Post-hoc results on the effects of aqueous root extract of *T. diversifolia* on serumALT levels in Western diet-fed Wistar rats.

Mul	tiple Compa	risons						
Dep	endent Vari	able:ALT						
				Mean Differe	Std.		Confi	% dence rval
	(I) GROUPS	(J) Treatments	Mean	nce (I- J)	Err or	Sig.	Lowe r Boun d	Upper Boun d
LS D	NORMA L CONTRO	NEGATIVE CONTROL (G2)	144.40 0	- 30.500*	7.71 3	.000	- 46.30 1	- 14.69 8
	L (G1)	10 mg/kg b.w ATORVASTATIN (G3)	70.600	43.300*	7.71 3	.000	27.49 8	59.10 1
		0.5 mg/kg b.w GLIBENCLAMID E (G4)	114.20 0	300	7.71 3	.969	- 16.10 1	15.50 11
		200 mg/kg b.w extract (G5)	135.60 0	- 21.700*	7.71 3	.009	- 37.50 1	- 5.898
		400 mg/kg b.w extract (G6)	138.20 0	- 24.300*	7.71 3	.004	- 40.10 1	- 8.498
		(G7)	136.64 0	- 22.740*	7.71 3	.006	- 38.54 1	- 6.938
	NEGATI VE	NORMAL CONTROL(G1)	133.90 0	30.500*	7.71 3	.000	14.69 8	46.30 1
	CONTRO L (G2)	10 mg/kg b.w ATORVASTATIN (G3)	70.600	73.800*	7.71 3	.000	57.99 8	89.60 1
		0.5 mg/kg b.w GLIBENCLAMID E (G4)	114.20 0	30.200*	7.71 3	.001	14.39 8	46.00 1

	200 mg/kg	b.w	135.60	8.800	7.71	264	-	24.60
	extract (G5)		0	8.800	3	.264	7.001	1
	400 mg/kg	b.w	138.20	6.200	7.71	.428	-	22.00
	extract (G6)		0	0.200	3	.420	9.601	1
	NEGATIVE		136.64		7.71		_	23.56
	CONTROL (G7)	-2	0	7.760	3	.323	8.041	1
Base	d on observed me	ans.						
*. The mean difference is significant at the .05 level.								

In Table 18, presented above, the baseline in the second column served as the reference against which all other treatment groups in column three were compared, and the significance level is indicated in column 7.

A Duncan's test was conducted to evaluate the homogeneity among the groups. It revealed similarity in ALT mean values between the negative control (G2), those administered 200 mg/kg extract (G5), 400 mg/kg extract (G6), and the rats exposed to a Western diet for four weeks, followed by a switch to rodent chow in the fifth week (G7). These groups exhibited uniformity in their outcomes but differed from all other groups.

Conversely, the normal control (G1) and the group administered 0.5 mg/kg glibenclamide (G4) displayed similarity in outcomes but were distinct from those given 10 mg/kg atorvastatin (G3). The Dancun's test on homogeneity recorded three different subsets of the rat groups, as detailed in Table 19.

Dependent	variable: ALT				
	GROUPS/Treatments	Ν		Subset	
	GROUPS/Treatments	IN	1	2	3
Duncan <sup>a,b</sup>	10 mg/kg ATORVASTATIN (G3)	5	70.6000		
	NORMAL CONTROL(G1)	5		113.9000	
	0.5 mg/kg b.w	5		114.2000	
	GLIBENCLAMIDE (G4)				
	200 mg/kg b.w extract (G5)	5			135.6000
	(G7)	5			136.6400
	400 mg/kg b.w extract (G6)	5			138.2000
	NEGATIVE CONTROL (G2)	5			144.4000
Means for g	groups in homogeneous subsets are di	splayed	I. Based on	observed m	eans.
a. Uses Har	monic Mean Sample Size = 5.				
b. Alpha =	.05.				

Table 19: The level of homogeneity in the mean serum ALT levels between the groups.

4.5.3	Effects	of	aqueous	root	extract	of	<b>T</b> .	diversifolia	on	serum	aspartate
amino	otranfera	se (A	AST) level	s in W	estern di	et-fe	ed V	Vistar albino	rats		

The refence values for AST (U/ml) in rats range between 198.68 ±15.66 (Marzouk et al.,

2011). The mean serum AST levels for each rat groups at the end of week five are as shown in figure 7 below.

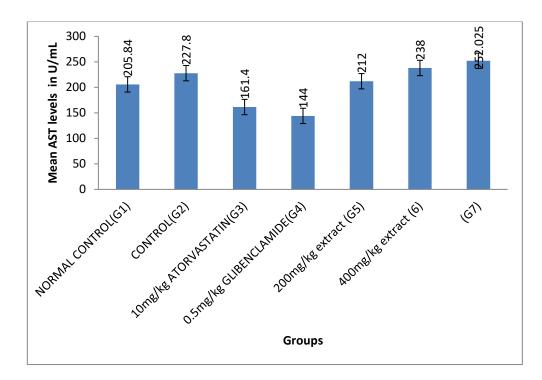


Figure 7: Effects of aqueous root extract of *T. diversifolia* on serum aspartate aminotransferase (AST) levels in Western diet-fed Wistar albino rats.

Figure 7 shows a decrease in mean serum AST levels in the rat groups that received 10 mg/kg atorvastatin (G3) and 0.5 mg/kg glibenclamide (G4). Contrariwise, the groups that received 200 mg/kg aqueous root extract of *Tithonia diversifolia* (G5), 400 mg/kg aqueous root extract of *Tithonia diversifolia* (G6), and those fed a Western diet for four weeks followed by a reverse to rodent chow for the last seven days (G7) exhibited an increase in mean serum AST levels when compared to the normal control (G1). When employing the negative control (G2) as the reference point for comparison, rats given 10 mg/kg atorvastatin (G3), 0.5 mg/kg glibenclamide (G4), and 200 mg/kg aqueous root extract of *Tithonia diversifolia* (G5) displayed a decrease in mean serum AST levels, while those given 400 mg/kg aqueous root extract of *Tithonia diversifolia* (G6) and rats subjected to a Western diet for four weeks, with

a subsequent switch to rodent chow in the last seven days (G7), demonstrated an increase in serum AST levels.

Subsequent post hoc LSD analysis was conducted to evaluate the mean differences in serum AST levels among the rat groups. When utilizing the normal control (G1) as the baseline, it was evident that rats administered 10 mg/kg atorvastatin (G3) and 0.5 mg/kg glibenclamide (G4) exhibited a significant decrease in mean serum AST levels (p=0.018 and p=0.002, respectively). In contrast, the group of rats subjected to a Western diet for four weeks, followed by a switch to rodent chow in the fifth week (G7), recorded a significant increase in mean serum AST levels (p=0.020). Those given 200 mg/kg aqueous root extract of *Tithonia diversifolia* (G5) and 400 mg/kg aqueous root extract of *Tithonia diversifolia* (G5) and 400 mg/kg aqueous root extract of *Tithonia diversifolia* (G5) and 400 mg/kg aqueous root extract of *Tithonia diversifolia* (G5) and 400 mg/kg aqueous root extract of *Tithonia diversifolia* (G5) and 400 mg/kg aqueous root extract of *Tithonia diversifolia* (G5) and 400 mg/kg aqueous root extract of *Tithonia diversifolia* (G6)

When the negative control was used as the baseline (G2), a significant decrease was observed in the rats administered 10 mg/kg atorvastatin (G3) (p=0.018), while an insignificant decrease was noted in the mean serum AST levels of the rats given 200 mg/kg aqueous root extract of *Tithonia diversifolia* (G5) (p=0.377). Conversely, those administered 400 mg/kg aqueous root extract of *Tithonia diversifolia* (G6) and the rats subjected to a Western diet for four weeks, followed by a switch to rodent chow in the last 7 days (G7), displayed insignificant increases in the levels of mean serum AST (p=0.567 and p=0.205, respectively).

Mul	tiple Compa	risons						
Dep	endent Varia	able: AST						
	(I)		M	Mean Differe	Std.	G.		nfidence erval
	GROUPS	(J)Treatments	Mean	nce (I-J)	Error	Sig.	Lower Bound	Upper Bound
LS D	NORMAL CONTRO L	NEGATIVE CONTROL (G2)	227.80	-21.960	17.585 3	.222	-58.042	14.122
	(G1)	10 mg/kg b.w ATORVASTA TIN (G3)	161.40	44.440*	17.585 3	.018	8.358	80.522
		0.5 mg/kg b.w GLIBENCLA MIDE (G4)	144.00	61.840*	17.585 3	.002	25.758	97.922
		200 mg/kg b.w extract (G5)	212.00	-6.160	17.585 3	.729	-42.242	29.922
		400 mg/kg b.w extract (G6)	238.00	-32.160	17.585 3	.078	-68.242	3.922
		(G7)	252.02	- 46.185*	18.652 0	.020	-84.456	-7.914
	NEGATI VE CONTRO	NORMAL CONTROL(G 1)	205.84	21.960	17.585 3	.222	-14.122	58.042
	L (G2)	10 mg/kg b.w ATORVASTA TIN(G3)	161.40	66.400*	17.585 3	.001	30.318	102.48 2
		0.5 mg/kg b.w GLIBENCLA MIDE (G4)	144.00	83.800*	17.585 3	.000	47.718	119.88 2
		200 mg/kg b.w extract (G5)	212.00	15.800	17.585 3	.377	-20.282	51.882
		400 mg/kg b.w extract (G6)	238.00	-10.200	17.585 3	.567	-46.282	25.882
		(G7)	252.02	-24.225	18.652 0	.205	-62.496	14.046

Table 20: Post-hoc results on the effects of aqueous root extract of *T. diversifolia* on serum aspartate aminotranferase (AST) levels in Western diet-fed Wistar albino rats.

Based on observed means.

\*. The mean difference is significant at the .05 level.

In Table 20, the second column serves as the baseline against which all other treatment groups in column three are compared, and column 7 indicates the level of statistical significance.

Upon conducting Duncan's test to assess the homogeneity of the groups in terms of AST mean levels, it was observed that there was similarity in AST mean values among the following groups: normal control (G1), negative control (G2), those administered 200 mg/kg of the extract (G5), and those given 400 mg/kg of the extract (G6). These groups exhibited no statistically significant differences among them. However, they differed from the groups of rats that received 10 mg/kg atorvastatin (G3), 0.5 mg/kg glibenclamide (G4), and the rats subjected to a Western diet for four weeks, with a subsequent switch to rodent chow in the fifth week (G7).

Tabl	e 21:	Showing	the	level	of	homoge	neity	in t	the	mean	serum	AST	levels	between	the
~***															
grou	.ps														

Dependent v	ariable: AST				
	GROUPS	N		Subset	
	GROUPS	IN	1	2	3
Duncan <sup>a,b,c</sup>	0.5 mg/kg b.w	5	144.00		
	GLIBENCLAMIDE (G4)		0		
	10 mg/kg b.w	5	161.40		
	ATORVASTATIN (G3)		0		
	NORMAL CONTROL(G1)	5		205.84	
				0	
	200 mg/kg b.w extract (G5)	5		212.00	
				0	
	NEGATIVE CONTROL(G2)	5		227.80	227.80
				0	0
	400 mg/kg b.w extract (G6)	5		238.00	238.00
				0	0
	(G7)	5			252.02
					5
Means for gr	oups in homogeneous subsets are di	isplaye	d. Based c	on observe	d means.
a. Uses Harm	onic Mean Sample Size = 5.				
b. The harmo	nic mean of the group sizes is used	•			
c. Alpha $= .02$	5.				

Therefore, Dancun's test on homogeneity grouped the rat groups into three different subsets,

as shown in Table 21 above.

## 4.6 Kidney profile

## 4.6.1 Effects of aqueous root extract of *T. diversifolia* on serum creatinine levels

Figure 8 below shows the mean serum creatinine levels for each rat group.

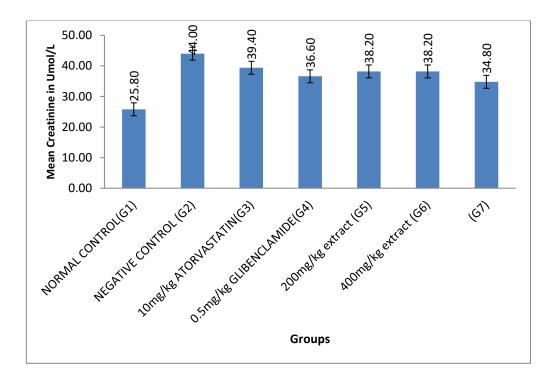


Figure 8: Effects of aqueous root extract of *T. diversifolia* on serum creatinine levels in Western diet-fed Wistar albino rats

In Figure 12, a comparison between the normal control (G1) and the other groups revealed an increase in mean serum creatinine levels in all the rat groups. Conversely, when the negative control (G2) was used to compare to the other groups, all groups exhibited a decrease in mean serum creatinine levels.

Subsequently, a post hoc LSD analysis was conducted to evaluate the differences in mean serum creatinine levels among the groups. Using normal control (G1) as the baseline, a significant increase in creatinine levels was observed across all treatment groups, as indicated by the p-value (p=0.000).

Furthermore, when the negative control (G2) was utilized as the baseline, a significant decrease in serum creatinine levels was noted in the rat groups given 10 mg/kg atorvastatin

(G3), 0.5 mg/kg glibenclamide (G4), 200 mg/kg extract (G5), 400 mg/kg extract (G6), and the rats subjected to a Western diet for four weeks, with a subsequent switch to rodent chow in the fifth week (G7). The corresponding p-values were as follows: (P=0.040), (P=0.002), (P=0.011), (P=0.011), and (P=0.000), respectively, as presented in Table 22.

Table 22: Post-hoc results on the effects of aqueous root extract of *T. diversifolia* on serum creatinine levels in Western diet-fed Wistar albino rats.

	Multiple Comparisons Dependent Variable: CREATININE										
				Mean Differe	Std.		Confi	% dence rval			
	(I) GROUPS	(J) Treatments	Mean	nce (I-J)	Error	Sig.	Lowe r Boun d	Uppe r Boun d			
LS D	NORMAL CONTRO	NEGATIVE CONTROL (G2)	44.00	-18.20*	2.137	.000	-22.58	-13.82			
	L (G1)	10 mg/kg b.w ATORVASTATIN (G3)	39.40	-13.60*	2.137	.000	-17.98	-9.22			
		0.5 mg/kg b.w GLIBENCLAMID E (G4)	36.60	-10.80*	2.137	.000	-15.18	-6.42			
		200 mg/kg b.w extract (G5)	38.20	-12.40*	2.137	.000	-16.78	-8.02			
		400 mg/kg b.w extract (G6)	38.20	-12.40*	2.137	.000	-16.78	-8.02			
		(G7)	34.80	-9.00*	2.137	.000	-13.38	-4.62			
	NEGATI VECONT	NORMAL CONTROL (G1)	25.80	18.20*	2.137	.000	13.82	22.58			
	ROL (G2)	10 mg/kg b.w ATORVASTATIN (G3)	39.40	4.60*	2.137	.040	.22	8.98			

	0.5 mg/kg b.w GLIBENCLAMID E (G4)	36.60	7.40*	2.137	.002	3.02	11.78			
	200 mg/kg b.w extract (G5)	38.20	$5.80^{*}$	2.137	.011	1.42	10.18			
	400 mg/kg b.w extract (G6)	38.20	5.80*	2.137	.011	1.42	10.18			
	(G7)	34.80	9.20*	2.137	.000	4.82	13.58			
Based on observed means.										
*. The mean diffe	*. The mean difference is significant at the .05 level.									

In Table 22 provided above, the second column serves as the reference against which all other treatment groups in column three are compared, and column 7 denotes the significance level.

Upon conducting Duncan's test to assess the homogeneity of the groups concerning serum creatinine mean levels, it was observed that there was a similarity in creatinine mean values among the following groups: rats administered 10 mg/kg atorvastatin (G3), 0.5 mg/kg glibenclamide (G4), 200 mg/kg aqueous root extract of *Tithonia diversifolia* (G5), 400 mg/kg aqueous root extract of *Tithonia diversifolia* (G5), 400 mg/kg aqueous root extract of *Tithonia diversifolia* (G5), 400 mg/kg aqueous root extract of *Tithonia diversifolia* (G6), and the group of rats that were subjected to a Western diet for four weeks and then switched to rodent chow in the fifth week (G7). These groups exhibited no statistically significant differences but differed from the normal control (G1) and the negative control (G2).

Table 23: Showing the level of homogeneity in the mean serum creatinine levels between the groups

CREATI	NINE				
	GROUPS	NT		Subset	t
		Ν	1	2	3
Duncan <sup>a,</sup>	NORMAL CONTROL (G1)	5	25.80		
b	(G7)	5		34.80	
	0.5 mg/kg b.w	5		36.60	
	GLIBENCLAMIDE (G4)				
	200 mg/kg b.w extract (G5)	5		38.20	
	400 mg/kg b.w extract (G6)	5		38.20	
	10 mg/kg b.w ATORVASTATIN	5		39.40	
	(G3)				
	NEGATIVE CONTROL (G2)	5			44.00
Means for	groups in homogeneous subsets a	e displ	layed. E	Based on	observed
means.					
a. Uses Ha	armonic Mean Sample Size $= 5.000$ .				
b. Alpha =	= .05.				

A Duncan's test for assessing homogeneity categorized the treatment groups into three distinct subsets, as presented in Table 23. The rats that were given 10 mg/kg atorvastatin (G3), 0.5 mg/kg glibenclamide (G4), 200 mg/kg aqueous root extract of *Tithonia diversifolia* (G5), 400 mg/kg aqueous root extract of *Tithonia diversifolia* (G6), and those subjected to a Western diet for four weeks, followed by a transition to rodent chow in the final seven days of the experiment (G7), exhibited similarity in their outcomes. However, they differed from the normal control (G1) and the negative control (G2).

# **4.6.2** Effects of aqueous root extract of *T. diversifolia* on serum urea levels in Western diet-fed Wistar albino rats.

At the end of week five, analysis of serum urea levels was done by Biobase BK 200, and the mean serum urea levels for each rat group were shown in Figure 9 below.

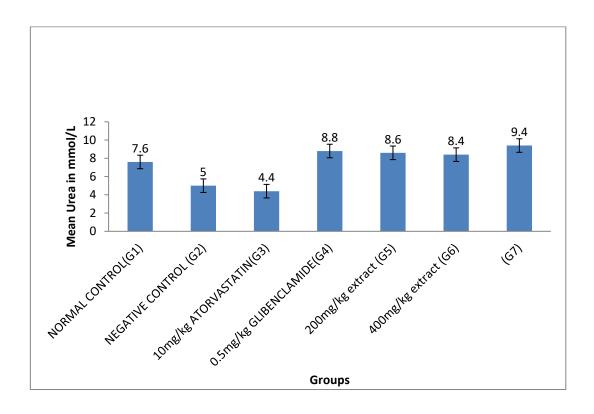


Figure 9: Effects of aqueous root extract of *T. diversifolia* on serum urea levels in Western diet-fed Wistar albino rats.

When normal control (G1) served as the reference for comparison with other rat groups, there was an increase in mean serum urea levels in all the rat groups, except for those administered 10 mg/kg atorvastatin (G3), which exhibited a decrease in mean serum urea levels.

Conversely, when compared to the negative control (G2), all the other rat groups displayed an increase in mean serum urea levels, except for those receiving 10 mg/kg atorvastatin (G3), which demonstrated a decrease in mean serum urea levels.

Upon conducting a post hoc LSD analysis to examine the mean differences in serum urea levels among the rat groups and using the normal control (G1) as the reference, there was an insignificant increase in mean serum urea levels in the rats administered 0.5 mg/kg glibenclamide (G4), 200 mg/kg extract (G5), and 400 mg/kg extract (G6) with p-values of (p=0.153), (p=0.232), and (p=0.336), respectively. Rats given 10 mg/kg atorvastatin (G3) significantly decreased (p=0.001). At the same time, those fed a Western diet for four weeks and then reverted to a normal rodent chow in the fifth week (G7) exhibited a significant increase in urea levels (p=0.036).

Using the negative control (G2) as the baseline, a significant increase in serum urea levels was observed in the rats administered 0.5 mg/kg glibenclamide (G4), 200 mg/kg extract (G5), 400 mg/kg extract (G6), and those fed a Western diet for four weeks and reverted to rodent chow in the fifth week (G7) (p=0.000). Rats given 10 mg/kg atorvastatin (G3) showed an insignificant decrease in mean urea levels (p=0.469).

Dep	endent Varia	able: UREA				1			
	(I) GROUPS	(J) Treatments	Mean	Mean Differ ence	Std. Err	Sig.	95% Confidence Interval Lower Upper		
	GROOTS			(I-J)	or		Bound	Bound	
LS D	NORMAL CONTRO L (G1)	NEGATIVE CONTROL (G2)	5.00	2.60*	.818	.004	.93	4.27	
		10 mg/kg b.w ATORVASTAT IN (G3)	4.40	3.20*	.818	.001	1.53	4.87	
		0.5 mg/kg b.w GLIBENCLAM IDE (G4)	8.80	-1.20	.818	.153	-2.87	.47	
		200 mg/kg b.w extract (G5)	8.60	-1.00	.818	.232	-2.67	.67	
		400 mg/kg b.w extract (G6)	8.40	80	.818	.336	-2.47	.87	
		(G7)	9.40	-1.80*	.818	.036	-3.47	13	
	NEGATI VE CONTRO	NORMAL CONTROL (G1)	7.60	-2.60*	.818	.004	-4.27	93	
	L (G2)	10 mg/kg b.w ATORVASTAT IN (G3)	4.40	.60	.818	.469	-1.07	2.27	
		0.5 mg/kg b.w GLIBENCLAM IDE (G4)	8.80	-3.80*	.818	.000	-5.47	-2.13	
		200 mg/kg b.w extract (G5)	8.60	-3.60*	.818	.000	-5.27	-1.93	
		400 mg/kg b.w extract (G6)	8.40	-3.40*	.818	.000	-5.07	-1.73	
		(G7)	9.40	-4.40*	.818	.000	-6.07	-2.73	

Table 24: Post-hoc results on the effects of aqueous root extract of *T. diversifolia* on serum urea levels in Western diet-fed Wistar albino rats.

The second column in the table above is the reference against which all the other rat groups in column three are compared. Column 7 indicates the level of significance.

A Duncan's test was conducted to assess the homogeneity of the groups concerning serum urea mean levels, revealing similarity in urea mean values among the rats used as the normal control (G1), those administered 0.5 mg/kg glibenclamide (G4), 200 mg/kg aqueous root extract of *Tithonia diversifolia* (G5), 400 mg/kg aqueous root extract of *Tithonia diversifolia* (G6), and those that were fed a Western diet for four weeks and then reverted to a rodent chow in the last week of the experiment (G7). These groups were not different from each other but differed from the negative control (G2) and rats given 10 mg/kg atorvastatin (G3). This homogeneity test, therefore, grouped the rat groups into two different subsets, as shown in Table 25 below.

Table 25: showing the level of homogeneity in the mean serum urea levels between the groups

UREA				
	GROUPS	Ν	Subset	
			1	2
Duncan <sup>a,b</sup>	10 mg/kg b.w ATORVASTATIN (G3)	5	4.40	
	NEGATIVE CONTROL (G2)	5	5.00	
	NORMAL CONTROL (G1)	5		7.60
	400 mg/kg b.w extract (G6)	5		8.40
	200 mg/kg b.w extract (G5)	5		8.60
	0.5 mg/kg b.w GLIBENCLAMIDE	5		8.80
	(G4)			
	(G7)	5		9.40
Means for g	roups in homogeneous subsets are display	yed. Base	ed on obser	ved mean.
a. Uses Harr	monic Mean Sample Size = 5.000.			
b. Alpha = .	05.			

#### **CHAPTER FIVE**

### 5.0 **DISCUSSION**

#### 5.1 Phytochemical of the aqueous root extract of *Tithonia diversifolia*

In the present study, a qualitative phytochemical analysis of the aqueous root extract of *Tithonia diversifolia* revealed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, phenols, carbohydrates, coumarins, and anthocyanins. Interestingly, this differs from the findings of previous studies conducted by Obayomi *et al.* (2021) and Omolola (2020) on the leaf extract, which did not report the presence of anthocyanins, coumarins, and carbohydrates. It is worth noting that differences in the phytochemical composition between the leaf and root of *Tithonia diversifolia* may contribute to variations in the effects of these extracts. The aqueous root extract contains a broader spectrum of bioactive compounds than the aqueous leaf extract. In contrast, Olayinka *et al.* (2015) reported the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, and phenols in the aqueous stem extract of *Tithonia diversifolia*, which differs from the current findings as it was reported not to contain carbohydrates, coumarins, and anthocyanins. These disparities highlight the potential variations in medicinal use and effectiveness between the leaf and root extracts.

Phytochemicals exhibit diverse pharmacological and biochemical actions, playing a crucial role in treating and managing various illnesses. They are responsible for plants' characteristic odor and color and can contribute to their toxicity and medicinal properties. The availability of these bioactive compounds, which may exhibit activities akin to conventional synthetic drugs, can be employed to predict the potential toxicity and side effects associated with medicinal plants. Furthermore, studying these phytochemicals holds promise for developing novel medicinal agents. Many herbs contain potent phytochemical compounds that can

enhance overall health and protect against various diseases. Phytochemicals, being bioactive natural plant compounds, are predominantly employed for their medicinal properties due to their therapeutic potency (Juliani *et al.*, 2017). These phytochemicals warrant in-depth investigation for the potential development of novel therapeutic agents (Dasgupta *et al.*, 2021). Various herbal sources have demonstrated the presence of potent phytochemical compounds capable of enhancing overall health and conferring protection against a spectrum of diseases. Phytochemicals, as bioactive constituents derived from plants, are primarily harnessed for their therapeutic efficacy due to their medicinal potential, (Okarter *et al.*, 2010).

### 5.2 *Tithonia diversifolia* extract and blood glucose levels

The results of the present study indicated that the consumption of a Western diet for a fourweek duration resulted in a significant elevation of fasting blood glucose levels in all rat groups fed on the Western diet. This observation underscores the capacity of the Western diet to increase blood glucose levels in Wistar rats. In the fourth week of the study, the negative control group (G2) displayed higher weight and blood glucose levels compared to the other groups, despite the uniform diet and equivalent food quantities administered to all groups. Notably, all rat groups adhered to a consistent feeding schedule and regular intervals. Given the uniformity in feeding patterns, other factors might contribute to the distinctive outcomes observed in G2. Possible explanations could include inherent physiological variations, genetic factors, or individual responses to the diet that could manifest differently in each group.

However, a marked reduction in blood glucose levels was noted upon administering the aqueous root extract of *Tithonia diversifolia* at 200 mg/kg to Wistar rats subjected to the

Western diet for seven days (p=0.000). This reduction was comparable to the effects observed with glibenclamide administration at 0.5mg/kg for seven days (p=0.000).

These findings are in line with studies conducted by Yazid *et al.* (2021) and Chunudom *et al.* (2020), which reported a significant decrease in fasting blood glucose levels in diabetic rats following daily administration of *Tithonia diversifolia* aqueous leaf extract at 200 mg/kg for 16 days (p<0.05). These findings suggest that compounds such as coumarin, anthocyanins, and carbohydrates in the aqueous leaf extract may not significantly influence the hypoglycemic effects. However, further research in this area is warranted for clarification.

The administration of 400 mg/kg of aqueous root extract after the fourth week significantly reduced fasting blood glucose levels (p=0.000). These findings align with previous research conducted by Olukunle and (2014) Mabou *et al.* (2018), who reported a significant reduction in fasting blood sugar levels in diabetic Wistar rats after administering a similar dose of the aqueous leaf extract. It is worth noting that both (Olukunle, 2014; Mabou *et al.*, 2018) administered the extract for 21 days, in contrast to the current study, which employed a 7-day administration period. Considering the nephrotoxic effects associated with these extracts, it raises the question of the optimal duration for administering these extracts to animals.

Furthermore, other studies have reported similar reductions in blood sugar levels, albeit at varying dosages. For instance, a study by Sari *et al.* (2018) demonstrated that the administration of 150 mg/kg of leaf aqueous extract of *Tithonia diversifolia* once a day for 28 days led to a reduction in blood glucose levels comparable to that achieved with glibenclamide in hyperglycemic rats.

Similarly, another study by (Yazid *et al.*, 2021) involved the administration of 600 mg/kg of body weight for 16 days, concluding that the *Tithonia diversifolia* leaf extract significantly reduced fasting blood glucose.

Based on the preceding analysis, it is evident that the aqueous root extract, administered at both 200 mg/kg and 400 mg/kg doses, exhibits a more efficient reduction in blood glucose levels over a shorter treatment duration compared to the leaf extracts, which achieved similar results but required a more protracted administration period. These variances in effectiveness can likely be attributed to differences in the phytochemical compositions between the leaf and root extracts of *Tithonia diversifolia*.

Diverse phytochemicals in a plant extract can substantially influence its pharmacological activities, particularly its hypoglycemic effects. The enhanced hypoglycemic activity observed in the aqueous root extract of *Tithonia diversifolia* may be attributed to the presence of specific bioactive compounds, including alkaloids, tannins, and anthocyanins. In previous research, alkaloids, a class of naturally occurring compounds found in various plants, have been linked to hypoglycemic effects. The mechanisms underlying alkaloid-induced hypoglycemic effects can vary, contingent upon the specific alkaloid and its plant source. These mechanisms include insulin secretion stimulation (López *et al.*, 2004), enhanced insulin sensitivity, gluconeogenesis inhibition, inhibition of gluconeogenesis by boldine (Silva *et al.*, 2023), and AMP-Activated Protein Kinase (AMPK) activation, with berberine, one of the principal alkaloids in *Rhizoma coptidis* (Shen *et al.*, 2012), being a notable example. Notably, alkaloids' hypoglycemic effects can vary widely depending on the specific alkaloid, its concentration, and the individual's overall health and metabolic profile (Derosa *et al.*, 2014).

Additionally, the presence of tannins in the roots of *Tithonia diversifolia* also contributes to its hypoglycemic activity. Tannins, a group of polyphenolic compounds found in various plant sources, primarily exert hypoglycemic effects by inhibiting carbohydrate absorption and modulating glucose metabolism (Tsujita, 2016). Moreover, tannins possess antioxidant properties capable of reducing oxidative stress and inflammation (Kumari & Jain, 2012), contributing to insulin resistance. This property is also shared with anthocyanins, which are absent in the leaf extract of *Tithonia diversifolia* (Garcia & Blesso, 2021). The presence of anthocyanins in the root extract is likely responsible for the improved hypoglycemic activity observed, as they aid in reducing oxidative damage to insulin-sensitive tissues and enhancing insulin signaling. This ultimately facilitates more efficient glucose uptake by cells and improves glycemic control (Oliveira *et al.*, 2020).

Notably, despite experiencing elevated fasting blood glucose levels, the rats did not progress to a diabetic state, maintaining an average blood glucose levels of within the normal range of 2.64-5.26 mmol/L (Wang *et al.*, 2010). The observed elevated fasting blood glucose levels in the rats may suggest a state of insulin resistance (Leibowitz *et al.*, 2018). This phenomenon can lead to higher fasting blood glucose levels without an immediate transition to full-blown diabetes. Several factors could contribute to the observed results.

Firstly, the study was conducted over a relatively short duration, and the rats may have been in an adaptation phase. It's plausible that, given more time, the insulin resistance could progress, potentially leading to diabetes. Compensatory mechanisms (Wei *et al.*, 2020) could also be at play. The rats may have initiated adaptive responses (Zhou *et al.*, 2014), such as increased insulin production, to counteract the detrimental effects of the Western diet. While these mechanisms may temporarily prevent the onset of diabetes, their sustainability over an extended period is uncertain. Moreover, the study may not have thoroughly explored all relevant metabolic factors. Assessing additional markers, such as inflammation, lipid metabolism, or oxidative stress, could provide a more comprehensive understanding of the rats' metabolic state.

It's crucial to acknowledge the limitations of using rats as models for human physiology. Rats may not fully replicate the intricacies of human metabolism, and the progression from insulin resistance to diabetes (Kucera & Cervinkova, 2014) might differ between the two species. While the elevated fasting blood glucose levels in rats subjected to a Western diet indicate metabolic changes, the absence of diabetes may be attributed to various factors, including the study's duration and compensatory mechanisms (Hannon & Arslanian, 2015). These findings emphasize the complexity of diet-induced metabolic changes and underscore the need for further research to unravel the underlying mechanisms.

In summary, the data suggest that the aqueous root extract of *Tithonia diversifolia*, administered at doses of 200 mg/kg and 400 mg/kg daily for seven days in Western diet-fed Wistar albino rats, is more effective in reducing blood glucose levels. Consequently, it may be a valuable hypoglycemic agent for managing hyperglycemic conditions.

#### 5.3 *Tithonia diversifolia* extract and lipid profiles

The current investigation has ascertained a significant elevation in the levels of serum triglycerides and cholesterol when compared to negative control. This increase was particularly pronounced in rats subjected to a Western diet for four weeks and subsequently switched back to a standard rodent diet in the fifth week, highlighting the Western diet's potential to induce obesity. However, there were no notable alterations in HDL-C levels in Wistar rats after the five weeks when contrasted with the negative control group.

The daily administration of an aqueous root extract of *Tithonia diversifolia* at a dose of 200 mg/kg to Wistar albino rats fed a Western diet for seven days during the fifth week resulted in a significant reduction in serum cholesterol (p=0.000) and triglyceride levels (p=0.036). These outcomes were comparable to the effects observed with the standard drug atorvastatin (p=0.000). These findings align with those of Nguepi *et al.* (2021), who reported a significant decrease in serum cholesterol and triglyceride levels after administering a lower dose of 120 mg/kg of leaf aqueous extract for a more extended period of 14 days.

Upon increasing the dose of the aqueous root extract of *Tithonia diversifolia* to 400 mg/kg, this study discovered a significant decrease in serum cholesterol and triglyceride levels (p=0.000). These results are in line with the earlier work by Mabou *et al.* (2018), who demonstrated a significant reduction in serum cholesterol and triglycerides (p<0.05) following the administration of 400 mg/kg of aqueous leaf extract, as well as the study by Ajao & Moteetee (2017), which used 500 mg of *Tithonia diversifolia* daily for 21 days.

Ejelonu *et al.* (2017) also significantly reduced serum cholesterol and triglyceride levels following the administration of 100 mg/kg of leaf aqueous extract of *Tithonia diversifolia* to Wistar rats once a day for 21 days. It is noteworthy that while the current study administered the extract for a shorter duration, these previous studies also obtained similar effects on cholesterol and triglycerides, indicating the effectiveness of shorter-term administration and

potentially avoiding the risk of plant extract toxicity, as suggested by the present study's findings.

The differences in the hypolipidemic effects of the aqueous leaf and root extracts of *Tithonia diversifolia* may be attributed to variations in the concentrations of phytochemicals present in the root and leaf of the plant.

The observed hypolipidemic effects of this extract may be attributed to various phytochemicals found in its roots, such as saponins, terpenoids, and phenolic compounds. To begin with, saponins, naturally occurring compounds found in various plant sources, including *Tithonia diversifolia*'s aqueous root extract, have exhibited hypolipidemic activity. They achieve this by influencing lipid absorption and metabolism due to their amphiphilic nature (Marrelli *et al.*, 2016).

This unique property allows saponins to bind to dietary fats and cholesterol in the intestinal tract, forming micelles that encapsulate fats and cholesterol. As a result, the efficient absorption of these lipids into the bloodstream is prevented, leading to reduced levels of circulating cholesterol and triglycerides. Furthermore, saponins have been shown to interfere with the activity of enzymes involved in cholesterol synthesis and absorption ( del Hierro *et al.*, 2018), further contributing to lowered cholesterol levels. Conversely, terpenoids primarily modulate lipid metabolism and cholesterol levels (Ludwiczuk *et al.*, 2017).

In conclusion, the administration of *Tithonia diversifolia*'s aqueous root extract to Wistar albino rats fed a Western diet at doses of 200 mg/kg and 400 mg/kg daily for seven days demonstrated the potential to reduce serum cholesterol and triglyceride levels in a shorter

time frame. However, further in-depth studies are warranted to substantiate these findings adequately.

### 5.4 *Tithonia diversifolia* and liver function.

The present study's results demonstrate that a four-week consumption of a Western diet by Wistar rats led to elevated levels of ALP, AST, and ALT, indicating the hepatotoxic effects of the diet. Subsequently, upon daily administration of an aqueous root extract of *Tithonia diversifolia* at 200 mg/kg for seven days, a significant reduction in serum alkaline phosphatase (ALP) levels (p=0.000) was observed. Meanwhile, there were insignificant reductions in serum alanine aminotransferase (ALT) levels (p=0.264) and aspartate aminotransferase (AST) levels (p=0.377).

These current findings diverge from the previous study by Adebayo *et al.* (2009), which employed a similar dosage and duration but utilized leaf extract. Their study showed an insignificant decrease in serum ALP levels, while the results for ALT and AST levels align with the present study. Similar outcomes have also been reported by Ejelonu *et al.* (2017).

In a separate experiment involving a dosage of 400 mg/kg of aqueous root extract of *Tithonia diversifolia* administered daily for seven days to Wistar albino rats fed a Western diet, our study revealed a significant reduction in serum ALP levels (p=0.021), with insignificant changes in serum ALT (p=0.428) and AST (p=0.567) levels.

The current study findings align with prior research conducted by Nguepi *et al.* (2021) and Oyewusi *et al.* (2019) concerning ALP levels. However, they diverge regarding serum ALT and AST levels, where their results demonstrated a significant decrease in contrast to the outcomes established by the current study. The absence of significant changes in ALT and

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AST levels in rats treated with the *Tithonia diversifolia* aqueous root extract in the present study suggests that the extract did not impact hepatocyte function in Wistar rats.

These findings indicate a hepatoprotective potential of the extract, possibly attributable to the phytochemicals contained within the plant's roots. The liver plays a pivotal role in numerous biochemical pathways related to growth, energy supply, immune response, reproduction, nutrient regulation, and overall homeostasis within the body. Its capacity to perform these functions can be compromised by exposure to various substances such as foods, herbs, and drugs, as indicated by Ejelonu *et al.* (2017).

In contrast to certain alkaloids, such as pyrrolizidines, which have the potential to be hepatotoxic and can disrupt liver enzyme systems responsible for detoxification (Neuman *et al.*, 2015), the current study on *Tithonia diversifolia* aqueous root extract yielded distinct results. In this investigation, the extract consumption reduced ALP, AST, and ALT levels. Typically, an elevation in these liver enzymes signifies liver cell damage. The cumulative evidence suggests that the phytochemicals within the aqueous root extract of *Tithonia diversifolia* may possess a hepatoprotective effect, counteracting the potential hepatotoxicity often associated with alkaloids (Mabou *et al.*, 2018). This unique outcome implies that the extract may positively influence liver health by decreasing ALP, AST, and ALT levels, indicative of enhanced liver function rather than damage.

In conclusion, the administration of *Tithonia diversifolia* aqueous root extract at dosages of 200 mg/kg and 400 mg/kg for seven days to Wistar albino rats fed a Western diet appears to be safe for the liver and may find utility in the management of liver diseases, as it did not result in an elevation of liver enzymes.

#### 5.5 *Tithonia diversifolia* and kidney function

The present study observed that feeding Wistar albino rats a Western diet increased serum creatinine levels in the negative control group. Typically, elevated creatinine levels indicate compromised kidney function, as creatinine is a waste product excreted by the kidneys (Kamal, 2014). These findings suggest that the diet had a nephrotoxic effect on Wistar rats. However, when the aqueous root extract of *Tithonia diversifolia* was administered to the rats at doses of 200 mg/kg and 400 mg/kg daily for seven days, a significant reduction in serum creatinine levels (p=0.011) was established, implying potential kidney restorative effects of the extract.

These findings contrast with Adebayo *et al.* (2009), who reported that the daily administration of 200 mg/kg of aqueous leaf extract of *Tithonia diversifolia* for seven days produced an insignificant change in serum creatinine levels. These discrepancies between Adebayo's findings and the present study could be attributed to the metabolites present in the root extract, particularly coumarins and anthocyanins, which may influence creatinine levels. Notably, these phytochemicals are absent in the leaf extract, as indicated by previous studies such as Obayomi *et al.* (2021) and Omolola (2020).

Furthermore, this study established a significant increase in serum urea levels following daily administration of aqueous root extract of *Tithonia diversifolia* for seven days at 200 mg/kg and 400 mg/kg to Western diet-fed Wistar rats (p=0.000). This result differs from Passoni *et al.* (2013), who did not observe any change in serum urea levels after administering aqueous leaf extract of *Tithonia diversifolia* at 200 mg/kg for 14 days. This study's significant increase in serum urea levels may suggest early-stage kidney dysfunction.

Simultaneously, the significant reductions in creatinine levels indicate that the interventions reduced the circulating levels of creatinine, akin to the effects of established antidiabetic and antihypertensive drugs that improve kidney function. This could suggest that the extract, much like standard drugs, possesses antioxidant properties (Wang *et al.*, 2019) and anti-inflammatory properties, such as flavonoids (Abdallah *et al.*, 2015) and phenols (Zhang & Tsao, 2016), which may protect kidney tissues from oxidative damage and inflammation.

However, the elevation in urea levels, despite the root extract interventions, may imply kidney cell damage. Comparatively, the standard drug atorvastatin administered at a dose of 10 mg/kg, as shown by Hamid *et al.* (2016) for seven days, was found to be kidney-safe, as it did not lead to increased urea levels. The rise in urea levels may be a consequence of enhanced metabolism or altered renal function, possibly influenced by the presence of other phytochemicals like alkaloids (Adamse & van Egmond, 2010) or saponins (Adeoye & Oyedapo, 2004). Further investigations must be carried out to elucidate the reasons behind these effects.

In conclusion, the administration of aqueous root extract of *Tithonia diversifolia* at doses of 200 mg/kg and 400 mg/kg for seven days to Western diet-fed Wistar albino rats led to an elevation of urea levels while seemingly reducing the levels of serum creatinine. This observation suggests some aspects of kidney damage that warrant further investigation beyond the scope of this study.

#### **CHAPTER SIX**

#### 6.0 CONCLUSION AND RECOMMENDATIONS

### 6.1 CONCLUSION

This hypoglycemic study established that the aqueous root extracts of *Tithonia diversifolia* at doses of 200 mg/kg and 400 mg/kg possess hypoglycemic activity in Wistar albino rats fed a Western diet, comparable to the effects of glibenclamide at 0.5 mg/kg. Furthermore, this study demonstrated that the administration of aqueous root extract at doses of 200 mg/kg and 400 mg/kg to Wistar albino rats fed a Western diet for seven days resulted in hypolipidemic activities similar to those of atorvastatin at 10 mg/kg. The results of the current study have revealed that *Tithonia diversifolia* at doses of 200 mg/kg and 400 mg/kg led to an increase in urea levels, indicating potential kidney cell damage. However, these doses were associated with reduced serum creatinine levels, suggesting that the extract is safe for short-term kidney use, as creatinine is highly specific to kidney function.

### 6.2 **RECOMMENDATIONS**

Based on the findings of the present study concerning the hypoglycemic and hypolipidemic effects of the aqueous root extract of *Tithonia diversifolia* in Wistar albino rats subjected to a Western diet, the following recommendations are put forth:

- 1. Further investigations should be undertaken to identify the specific active constituents responsible for the plant extract's observed hypoglycemic and hypolipidemic activities.
- 2. Safety assessments focusing on organ health, particularly the liver and kidneys should be conducted to ascertain the potential impacts of the *T. diversifolia* aqueous root extract.

3. Additional research endeavors are warranted to understand better the precise active compounds within the *T. diversifolia* aqueous root extract.

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## APPENDICES

# **APPENDIX I: SERUM ANALYSIS RESULTS**

# G11

Patient Inform						Date		Sample No	).		
Name: G1		Gender:	•	Diagnosis:		·		No.	Patient Name	Print status	*
Position No.:		Department: OF	• O	Sample Type:	serum	▼ 2021-10-19			G21	unprint	
Age: 0	0 M	Bed No.:		Doctor:					G22 G23	unprint	
rige.		bed no							G23 G24	unprint unprint	
Name	Result	Note	RefVal	ue	Unit	Symbol			G25	unprint	
ALB	32.8	1	3.55.		mg/dL				G31	unprint	
ALP	197	1	4513		U/L				G32	unprint	
AST CHO	200.0	1 1	031		U/L mmol/L				G33	unprint	
CHO	117.0	1	2.340		mmol/L		E		G34 G35	unprint unprint	
CREA	23.4	1	9717		umol/L			10	000	unprinc	-
DBIL	0.67		07		umol/L			Reaction	Curve		
HDL-C	0.53	↓	0.772		mmol/L mmol/L			A	Guive		
LDL-C	0.09	1	03.35		mmol/L			3.000			
TBIL	3		220.5		umol/L			2,625			
TG	0.72		0.71.3	1	mmol/L		-				
空腹血糖	<b>^</b> (		Add Items					2.250			
APTT TT			Manually		irrent 👿 Pictu	we blede		1.875			
FIB		Add automati	r items	. U				1.500			
HBsAg HBsAb				) AI	I 📃 In O	rder		1.125			
HBeAg	-	Add Calculate	d Items	© Ra	inge			0,750			
Profile Items				U Na							
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查(本 血糖	-			Find	Serial L		101	0.000	5 10 15	20 25 30 35 40	>
血脂	-	Add Profile	Items								

Program Input	Print Curves Patient Informatio Name: G11 Position No.[1	n	ery Export d Gender:	•	Maintenance Diagnosis: Sample Type:	•			Sample No.	lo. Patient Name G21	Print status unprint	.0/19/2021 4:28 PM	
Item Setting	Age: 0 Y C		Bed No.:		Doctor:		ID:		8 9 10	G22 G23 G24	unprint unprint unprint		
Reagent Data Processing Maintenance User Setting Monitor	Name           CL         CC           CREA         DBIL           DBIL         DBIL           HDL-C         TBIL           TG         UREA           ALT         AA           Na         Profile Items           HBADS         HBADS           HBADS         HBADS           HBADS         HBADS			tems	5 7 2.25 1 5 5 7 3 46		ler	TCP	11 12 13 14 15 16 Reaction 2,675 2,259 1,675 1,500 1,275 0,375 0,000	625 631 632 633 634 635 0 Curve	unprint unprin		

G12		
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		and to them current oser.1000
		Time:10/19/2021 4:31 PM
	Print Curves QC Result Query Export data Data Maintenance Test Results Correction	
	Patient Information	Sample No.
	Date	Sample No.
Program Input	Name. 612 October.	No. Patient Name Print status
	Position No. 2 Department: OPD  Sample Type: serum  2021-10-19	1 mercy cherono unprint
		2 G11 unprint
Item Setting	Age: 0 Y 0 M Bed No.: Doctor: • ID:	3 G12 unprint
Actin Setting		4 G13 unprint
- <u>193</u>	Name Result Note RefValue Unit Symbol	▲ 5 G14 unprint
	ALB 34.0 ↑ 3.55.5 mg/dL	6 G15 unprint
Reagent	ALP 173 ↑ 45135 U/L	7 G21 unprint
	AST 191.3 ↑ 031 U/L CHO 2.51 2.345.2 mmo//L	8 G22 unprint
	CHO         2.51         2.345.2         mmol/L           CL         108.7         ↑         95105         mmol/L	9 G23 unprint
	CREA 25.3 1 97177 umo/L	10 G24 unprint 11 G25 unprint *
Data Processing	DBIL 0.48 07 umoVL	
	HDL-C 0.62 ↓ 0.772.25 mmol/L	Reaction Curve
	K 5.57 ↑ 3.55.1 mmol/L	3.000
Maintenance	LDL-C         0.00         03.35         mmo//L           TBIL         4         220.5         umo//L	
	TG 0.85 0.71.7 mmol/L	2.625
		2250
105	空版血糖 APTT TT E Add Items Manually @ Current III Dicture Mede	1,575
User Setting	Current V Picture Mode	1.500
	FIB Add automatic items All In Order	
- <u>88</u>	HBsAb	1.125
Monitor	Range	0.750
Monitor	Profile Items Update Calculation	0.375
	LPF Update Calculation Print Serial port IP/TCP	
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Exit	血脂 Add Prome nems	

	Print Curves Patient information	-				Test Resu		n	Sample N	10.	Time:1	0/19/2021 4:31	PM	
Program Input	Name: G12		Gender:	•	Diagnosis:				No.	Patient Name	Print status	^		
	Position No.: 2	D	epartment:	OPD -	Sample Type:	serum	▼ 2021-10-	19 🔍 🔻	1	mercy cherono	unprint			
	Age: 0 Y C	) M	Bed No.:		Doctor:		- ID:		2	G11 G12	unprint unprint			
Item Setting									4	G12 G13	unprint	E		
	Name	Result	Note	RefVa	lue	Unit	Symbol	*	5	G14	unprint			
	CL	108.7	Ť	9510		mmol/L			6	G15	unprint			
Reagent	CREA	25.3 0.48	1	9717 07		umol/L			7	G21	unprint			
1000	DBIL HDL-C	0.48	1	07		umol/L mmol/L			8	G22 G23	unprint unprint			
2	K	5.57	1	3.55		mmol/L			10	G23 G24	unprint			
ata Processing	LDL-C	0.00		03.3		mmol/L			11	G25	unprint	-		
ata Processing	TBIL	4		220.		umol/L		E	Reaction	n Curve				
~ ~ /	UREA	0.85		1.78		mmol/L mmol/L			4					
1	ALT	128.4	î	031		U/L			3.000					
Maintenance	AA	1							2,625					
	Na	142		1351	46	mmol/L		*	2,250					
12	空腺血糖 APTT	<u>^</u>	•	Add Items										
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	FIB HBsAg		Add autom	atic items					1.500					
- 88	HBsAb				© AI	i in	Order		1.125	T				
	HBeAg	-	Add Calcul	ated Items	© Ra	nge			0.750					
Monitor	Profile Items								0.375					
<b>(11)</b>	LPF 查体	â L	Update Ca	aculation	Print	Seria	al port	IP/TCP						
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Exit	血脂	-	Add Floi	ine merrito										

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	Time:10/19/2021 4:33 PM
Print Curves QC Result Query Export data Data Maintenance Test Results Correction Patient Information Patient Information Name: G13 Gender: Diagnosis: Date No. Patient Name	9 Print status
Position No.3         Department:         OPD         Sample Type:         serum         2021-10-19         Image:         1         mercy cheron         2         G11         3         G12         G11         3         G12         G13         G13	
Name         Result         Note         RefValue         Unit         Symbol           Reagent         ALB         224         1         25-56         routh         6         6         6         6         6         6         6         6         6         6         6         6         6         6         7         6         7         6         7         6         7         6         7         6         7         7         224         7         0         7         7         23         8         6         23         7         6         7         7         23         8         6         7         6         7         7         23         1         6         7         7         23         1         0         7	upprint upprint unprint unprint unprint unprint unprint vupprint v

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			57.0									
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Program Input	Name: G13	Gender:	<ul> <li>Diagnosis:</li> </ul>	-	Date		No.		Print status		•	
<b>/</b>	Position No. 3	Department: OPD	<ul> <li>Sample Type</li> </ul>	serum 🗸	2021-10-19		1		unprint			
Item Setting	Age: 0 Y 0 M	Bed No.:	Doctor:	-	ID:		2	G11 G12	unprint unprint			
	News	u N-1-	D-8/sks	11-18	Question 1		4	G13 G14	unprint unprint			
	Name Resu CL 117.0	1	RefValue 95105	Unit mmol/L	Symbol		6	G15	unprint			
Reagent	CREA 27.1 DBIL 0.71		97177 )7	umol/L			7	G21 G22	unprint			
H-1	DBIL 0.71 HDL-C 0.41		0.772.25	umol/L mmol/L			9	G22 G23	unprint unprint			
	K 5.36		3.55.1	mmol/L			10	G24	unprint			
ata Processing	LDL-C 0.00 TBIL 3		03.35 220.5	mmol/L umol/L		-	11	G25	unprint		-	
2.1	TG 1.00		0.71.7	mmol/L		-	Reaction	Curve				
6	UREA 7.8 ALT 108.9		1.78.3 )31	mmol/L U/L		_	3.000					
Maintenance	AA 1			0/L			2.625					
	Na 142		135146	mmol/L		*	2,250					
13	空粮血糖 APTT	Add Iten Manual					1.875					
User Setting	APTT TT E FIB		• C	urrent 🛛 🗹 Pictur	e Mode		1.500					
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Programmput	Position No.					2021-10-19		No.	Patient Name	Print status	<u>^</u>	
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· · · · · · · · · · · · · · · · · · ·		32.2 197	1 <u>3.5</u> 5		mg/dL U/L			7	G21	unprint		
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		2.15	↓ 2.34-		mmol/L			9	G23	unprint		
4		118.1 26.2	↑ 951 ↓ 971		mmol/L umol/L			10	G24	unprint		
Data Processing		20.2	07	"	umol/L			11	G25	unprint	*	
	HDL-C	0.58	↓ 0.77-	2.25	mmol/L			React	ion Curve			
		4.55	3.56		mmol/L				î			
Maintenance		0.00	03.3		mmol/L			3.000	°  -   -   -		+-+	
Waintenance		4 0.68	220		umol/L mmol/L		_	2.62	5		+-+	
			-		mmove			2.250			<u> </u>	
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ng	Age: 0 Y	ом	Bed No.:		DOCIOI.	•	D.		3	G12 G13	unprint unprint		=	
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	CL	118.1	1	9510		mmol/L			6	G15	unprint			
	CREA	26.2	Ţ	971	77	umol/L			7	G21	unprint			
_	DBIL HDL-C	1.82		07	0.05	umol/L mmol/L			8	G22 G23	unprint			
_	K	4.55	+	3.55		mmol/L		_	10	G23 G24	unprint unprint			
sing	LDL-C	0.00		03.3		mmol/L			11	G25	unprint		-	
ang	TBIL	4 0.68		220		umol/L mmol/L		=	Reactio	on Curve				
	UREA	5.6	+	1.78		mmol/L		_	1	4				
	ALT	105.7	t	031		U/L			3.000				-	
ice	AA Na	1	†	135	140	mmol/L			2.625				-	
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Program Input	Name: G15 Gender: Diagnosis: Date Date No. Patient Name Print status	
	Position No. 5 Department: OPD  Sample Type: serum Cu21-10-19 Position No. 5 Department: OPD Sample Type: serum Cu21-10-19 Cu21-10-1	
Item Setting	Age: 0 Y 0 M Bed No.: Doctor. V ID: 3 G12 unprint	
	A G13 unprint Name Result Note RefValue Unit Symbol A 5 G14 unprint	
	ALB 35.3 † 3.5–5.5 mg/dL 6 G15 unprint	
Reagent	ALP         161         1         45–135         U/L         7         G21         unprint           AST         210.2         1         0–31         U/L         8         G2         unprint	
	CHO 2.07 1 2.34-5.2 mmoVL E 9 G23 unprint	
Data Processing	CREA 28.0 1 97–177 umo/L 11 G25 unprint v	
	DBIL         0.66         07         umol/L         In the second	
La la	K 6.02 † 3.5-5.1 mm0/L LDLC 0.00 0-335 mm0/L	
Maintenance	TBL         3         2-20.5         umo/L           TG         0.50         1         0.7-1.7         mmo/L         2.05	
User Setting	APTT TT B Manually © Current V Picture Mode 1875	
	FiB Add automatic items All In Order	
- <u>88</u>	HBAAD HBeAg - Add Calculated Items © Range 0750	
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			37. 0*						Currer	n:2.1.0.5A nt User:1000 10/19/2021 4	1:36 PM	
Program Input	Print Curves QC Res Patient Information Name: G15 Position No. 5 Age: 0 Y 0 M	Gender: Department: OPD Bed No.:	Diagnosis:     Sample Type:     Doctor:	serum -	Date 2021-10-19 ID:	<b></b>	Sample N No. 1 2 3 4	Patient Name mercy cherono G11 G12 G13	Print status unprint unprint unprint unprint	E		
Reagent Data Processing Maintenance	Name         Rest           CL         1239           CREA         280           DBIL         086           HDL-C         0.00           TBIL         3           TG         0.50           UREA         7.8           ALT         117.1           Na         16	tt Note ↑ ↓ ↓ ↑ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓	07 0.77-225 3.5-5.1 03.35 220.5 0.7-1.7 1.7-8.3 031	Unit mmol/L umol/L umol/L mmol/L mmol/L umol/L umol/L U/L U/L	Symbol		5 6 7 8 9 10 11 	G14 G21 G21 G22 G23 G24 G25 Curve	unprint unprint unprint unprint unprint unprint			
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Print Curves Patient Informati Name: G21 Position No.:[6 Age: 0 Y	On Gender: Department: OPT	Diagnosis:	Test Results Correction Date 2021-10-19 ID: ID:	•	Sample No.           No.         Patient Name         Print status         *           1         mercy cherono         unprint         *           2         Gf1         unprint         *
A ALP AAP AST CKHO CL CREA DBIL DBLC TBIL TG FIB HBAG HBAG HBAG HBAG HBAG HBAG HBAG HBA		46-135 0-31 234-52 96-105 97-177 0-7 0-7 0-7 0-7 0-7 0-7 0-7 0-7 0-7 0		P	4 G13 unpint 5 G14 unpint 6 G15 unpint 8 G22 unpint 9 G23 unpint 10 G24 unpint 11 G25 unpint 10 G24 unpint 11 G25 unpint 12 G25 unpint 12 G25 unpint 12 G25 unpint 12 G25 unpint 13 G25 unpint 14 G15 Unpint 15 G16 Unpint 16 G16 Unpint 17 G21 Unpint 18 G16 Unpi

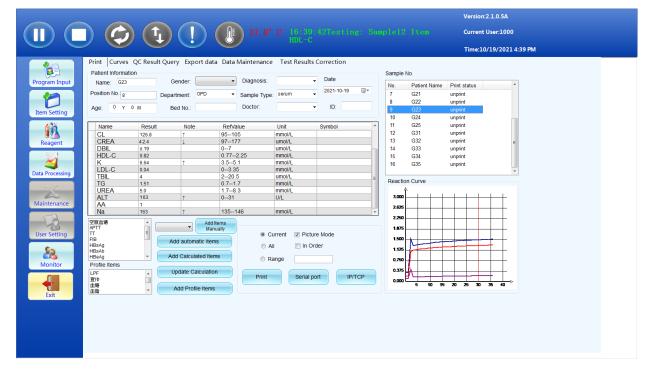
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- 193	Name Result CL 128.7	↑ 95				6 G15	unprint		
Reagent	CREA 44.6 DBIL 0.69	↓ 97 07	umol/L	L		7 G21 8 G22	unprint unprint		
	HDL-C 0.68 K 7.24	↑ 3.5		L		9 G23 10 G24	unprint		
Data Processing	LDL-C 0.06 TBIL 3	03	).5 umol/L	L		11 G25 Reaction Curve	unprint •		
	TG 1.15 UREA 4.6	0.7	8.3 mmol/			<u> </u>			
Maintenance	ALT 133.8 AA 1	↑ 03				2.625			
	Na 165 空龍血糖	↑  135- Add Items	-146 mmol/	1.	~	2.250			
User Setting	APTT TT E	Manually	Ourrent	Picture Mode		1.875			
6.	FIB HBsAg HBsAb	Add automatic items	IIA 🔘	🔲 In Order		1.500			
Monitor	HBeAg +	Add Calculated Items	Range			0.750			
	LPF	Update Calculation	Print	Serial port	IP/TCP	0.375			
Exit	血糖 血脂 *	Add Profile Items				5 10 15	20 25 30 35 40 >		
EXIT									

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	Print Curves QC Res	ult Query Export data Dat	a Maintenance	Test Results (	Correction							
	Patient Information		Diagonaia		Date		Sample N	lo.				
Program Input	Name: G22 Position No.;7	Gender:	<ul> <li>Diagnosis:</li> </ul>		2021-10-19		No.		Print status	*		
			<ul> <li>Sample Type:</li> <li>Doctor:</li> </ul>	serum -	ID:		1 2	mercy cherono G11	unprint			
Item Setting	Age: O Y O M	Bed No.:	Doctor.		ID.		3	G12 G13	unprint unprint			
	Name Resu		Value	Unit	Symbol	-	5	G14 G15	unprint			
Reagent	ALB 33.3 ALP 388	↑ 3.5- ↑ 45	135	mg/dL U/L			7	G21	unprint unprint			
- E2	AST 226.0 CHO 3.71	↑ 03 2.34		U/L mmol/L		_	8	G22 G23	unprint unprint			
4	CL 127.7 CREA 41.7	↑ 95 ↓ 97		mmol/L umol/L			10 11	G24	unprint			
Data Processing	DBIL 1.81 HDL-C 0.82	07		umol/L mmol/L			Reaction	G25 n Curve	unprint			
	K 7.11 LDL-C 0.05	↑ 3.5- 03	-5.1	mmol/L mmol/L			3.000 Î			1 1		
Maintenance	TBIL 5	1 22	0.5	umol/L			2.625					
	TG 1.15	0.7-	-1.7	mmol/L		*	2.250					
User Setting	空旗血糖 APTT TT E	Manually	O CI	irrent 🔽 Picture	Mode		1.875	1				
	FIB HBsAg	Add automatic items	⊚ Al				1.500	M		=		
- 23	HBsAb HBeAg ~	Add Calculated Items	© Ra	ange			0.750					
Monitor	Profile Items	Update Calculation					0.375	$\land$				
	LPF A 宣体 D 由语	Add Profile Items	Print	Serial po	t IP/1	TCP	0.000	5 10 15	20 25 30	35 40 >		
Exit	<u>血脂 </u>	Add Profile items										

3	Patient Informatio	n		a Data Maintenance				Sample N	0.		
Input	Name: G22	Ger		Diagnosis:	-	2021-10-19		No.	Patient Name	Print status	·
F	Position No. 7	Departm	nent: OPD	<ul> <li>Sample Type:</li> </ul>	serum -	2021-10-19		1	mercy cherono	unprint	
	Age: 0 Y 0	M Bed	No.:	Doctor:	-	ID:		2	G11 G12	unprint unprint	
ing								4	G13	unprint	i
	Name	Result	Note	RefValue	Unit	Symbol	^	5	G14	unprint	
	CL	127.7 41.7	1		mmol/L		_	6	G15 G21	unprint	
nt	DBIL	41.7	1		umol/L umol/L			8	G21 G22	unprint	
	HDL-C	0.82			mmol/L			9	G23	unprint	
	К	7.11	1		mmol/L			10	G24	unprint	
ssing	LDL-C TBIL	0.05			mmol/L umol/L			11	G25	unprint	-
	TG	1.15	1		mmol/L		=	Reaction	Curve		
	UREA	5.1		1.78.3	mmol/L			Ŷ			
	ALT		1	031	U/L			3.000			<u> </u>
ince	AA Na	1 163	†	135146	mmol/L			2.625			
	と版血糖				mmore			2.250			
) A	PTT	<u> </u>		Items				1.875			
ing T	T IB			• Cu	rrent 🛛 🗹 Pictur	e Mode		1.500			
н	IBsAg	Add	automatic iten	ns 💿 All	🔲 In Ord	ler			A		<del>_</del>
	IBsAb IBeAg	T Add	Calculated Iter	ns				1.125			
	Profile Items		ouronatou nor	lis 💿 Ra	nge			0.750			
	.PF		tate Calculatio	n				0.375			
별	登(体 血糖			Print	Serial po		TCP	0.000	5 10 15	20 25 30	35 40 >
	11.18° 11.18°	- Ad	Id Profile Items	;					5 10 15	20 20 30	35 10
	in Alle	- Ad	Id Profile Items								

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	Print Curves QC Resu Patient Information	ult Query Export data [	Data Maintenance	Test Results (	Correction		Sample N					
Program Input	Name: G23	Gender:	Diagnosis:	-	Date 2021-10-19		No.	Patient Name	Print status	*		
	Position No.: 8 Age: 0 Y 0 M	Department: OPD Bed No.:	<ul> <li>Sample Type:</li> <li>Doctor:</li> </ul>	serum -			7 8	G21 G22 G23	unprint unprint			
Item Setting			RefValue	Unit	Symbol		9 10 11	G23 G24 G25	unprint unprint unprint			
Reagent	ALB 35.7 ALP 383	↑ 3 ↑ 4	.55.5 I5135	mg/dL U/L	Symbol	-1	12 13	G31 G32	unprint unprint	E		
	AST 237.0 CHO 3.38 CL 126.8	2	.345.2	U/L mmol/L mmol/L			14 15 16	G33 G34 G35	unprint unprint			
Data Processing	CREA 42.4 DBIL 0.19	↓ 9 0	)7177 )7	umol/L umol/L			Reaction		unprint	-		
X	HDL-C 0.82 K 6.64 LDL-C 0.04	1 3 0	.55.1 I3.35	mmol/L mmol/L mmol/L			3.000			++		
Maintenance	TBIL 4 TG 1.51	0	).71.7	umol/L mmol/L		-	2.625 2.250					
User Setting	空腹血糖 APTT TT FIB	Add Item Manually	1	rrent 👿 Picture	e Mode		1.875			++		
- 88	HBsAg HBsAb HBeAg T	Add automatic items Add Calculated items	O AI	📄 In Ord	ler		1.125	<b>}</b> ===				
Monitor	Profile Items	Update Calculation	© Ra				0.750					
Exit	宣体 血糖 血脂 ▼	Add Profile Items	Print	Serial po		rcp	0.000 E	5 10 15	20 25 30	35 40 →		
Exit												



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Program Input	Print       Curves       QC Result Query       Export data       Data         Patient Information <ul> <li>Name:</li> <li>624</li> <li>Gender:</li> <li>Diagnosis:</li> <li>Date</li> <li>2021-10-19</li> <li>mercy cherono</li> <li>uprint</li> </ul> Name:     Openartment:     OpD     Sample No.	
Item Setting	Age:         0         Y         0         M         Bed No.:         Doctor:         ID:         2         G11         unprint           4         G13         unprint         #         G13         unprint         #	
Reagent	Name         Result         Note         RefValue         Unit         Symbol         2         5         G14         unprint           ALB         38.9         1         36.9.5.5         moldL         6         G15         unprint           ALP         391         1         45135         U/L         7         G21         printed           AST         283.2         1         031         U/L         8         G22         unprint	
Data Processing	CL         119         7         05-105         mmol/L         10         024         uppint           CREA         46.5         J         97-177         umol/L         11         625         uppint           DBL         0.75         0-7         umol/L         Reaction Curve         *           HDLC         0.82         0.77-2.55         mmol/L         Reaction Curve         *	
Maintenance	K         7.19         7         3.5–5.1         mmol/L           LDLC         0.08         0         -3.35         mmol/L           TG         1.82         1         0.7–1.7         mmol/L         -           UREA         4.6         1.7–0.3         mmol/L         -         -           28/Ib.06         Addlems         -         -         -         -	
User Setting	APTT TT I I I I I I I I I I I I I I I I I	
Monitor	Heak-g        •       •       Aoo Calculateo items         •       Range       •       ·       ·       ·       ·       ·	
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			$\mathbf{\overline{\mathbf{V}}}$								Time:10/19/2021 5:22 PM
	Print Curves QC R Patient Information	Result Query	Export data	Data Mainten	ance Test Re				Sample N	lo.	
Program Input	Name: G24	Ger	nder:	<ul> <li>Diagnos</li> </ul>	sis:	- Dat			No.	Patient Name	Print status
	Position No.	Departm	nent: OPD	<ul> <li>Sample</li> </ul>	Type: serum	- 202	1-10-19		7	G21	printed
Item Setting	Age: 0 Y 0 M	Bed	No.:	Doctor:		▼ 10	<b>D</b> :		8 9	G22 G23	unprint unprint
									10	G24 G25	unprint
- 193	Name Re CHO 3.9	Result	Note	RefValue 2.345.2	Unit mmol/L	Symb	ol		12	G25 G31	unprint
Reagent		91	t	2.345.2 95105	mmol/L				13	G32	unprint =
Reagent	CREA 46		i	97177	umol/L				14	G33	unprint
	DBIL 0.7			07	umol/L				15	G34	unprint
	HDL-C 0.8			0.772.25	mmol/L				16	G35	unprint
ata Processing	K 7.1 LDL-C 0.0		Ť	3.55.1	mmol/L			_			-
	LDL-C 0.0 TG 1.8		•	03.35 0.71.7	mmol/L mmol/L			=	Reaction	n Curve	
	UREA 4.6		1	1.78.3	mmol/L				4		
4		57.1	t	031	U/L			_	3.000		
Aaintenance	AA 1								2.625		
~	Na 16	18	1	135146	mmol/L			-			
User Setting	APTT TT FIB HBsAg HBsAg HBsAg HBsAg HBsAg HBsAg Profile Items LPF 童体 血糖	Add	Add I Mani automatic item Calculated Item date Calculation d Profile Items	ially s is	All     Range	Picture Mode In Order erial port	IP/T(	CP	2,250 1,875 1,500 1,125 0,750 0,375 0,000	5 10 15	

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Program Input Program Input Item Setting Reagent Data Processing Data Processing User Setting User Setting	Print         Curves         QC Resident           Patient Information         Name:         625           Position No.         10         Age:         0         Y         0         M           Age:         0         Y         0         M         M         Marce         CL         0         Alb         349         CL         0         ALP         391         AST         2220         CL         0253         CL         0253         CL         0263         CL         0253         CL         0263         CL         0253         CL         0263         CL         0253         CL         0263         CL         0253         CL         0264         010         010         044         010         044         010         045         010         010         044         010	Bed No.: 1 45- 1 45- 1 03 2 3 3 1 95- 1 97- 07	Value     Soft     Sample Type     Doctor:     Value     Soft     Sof	Unit mg/dL U/L U/L U/L U/L mmo/L umol/L	Date 2021-10-19 ID: Symbol	7 C 8 C 9 C 10 C 12 C 13 C 14 C 15 C 16 C Reaction C 2280 1475 2280 1475 2280 1475 200 15 C 16 C 16 C 16 C 16 C 16 C 16 C 17 C 18 C 19 C 19 C 19 C 19 C 10 C	321 322 323 324 331 332 333 334 335	Print status printed unprint unprint unprint unprint unprint unprint unprint 20 25 30			

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n Input	Print Curves Patient Informat Name: G25 Position No. 10 Age: 0 Y	ion	Gender:	ata Data Maintenance Diagnosis: Sample Type Doctor:	serum	Date		Sample N No. 7 8 9	D. Patient Name G21 G22 G23	Print status printed unprint unprint	· · · ·		
etting gent cessing	Name CL CREA DBIL HDL-C K LDL-C TBIL TG UREA ALT AA	Result 125.3 45.1 0.24 0.73 5.33 0.08 6 1.66 4.9 144.7 1	Note	RefValue 95105 97177 0-7 0.77225 3.55.1 03.35 220.5 0.71.7 1.78.3 031	Unit mmol/L umol/L mmol/L mmol/L mmol/L umol/L mmol/L U/L	Symbol	*	10 12 13 14 15 16 Reaction	G24 G25 G31 G32 G33 G34 G35	unprint unprint unprint unprint unprint unprint			
etting F itor F	Na 21 gambie 1977 TT 18 HBsAg HBsAb HBsAg Profile Items LPF Èrit mile mile			ems O A tems O R tion Print		der	ГСР	2.625 2.250 1.875 1.500 1.125 0.750 0.375 0.000	5 10 15	20 26 30	35 40		

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		-	Query Export data	Data Maintenance	Test Results (	Correction							
Program Input	Patient Informatio Name: G31	n	Gender:	<ul> <li>Diagnosis:</li> </ul>	-	Date		Sample I					
	Position No. 11		Department: OPD	<ul> <li>Sample Type:</li> </ul>	serum 👻	2021-10-19		No. 7	Patient Name G21	Print status printed	^		
1	Age: 0 Y C		Bed No.:	Doctor:		ID:		8	G22	unprint			
Item Setting	Age. VI C	m	Bed NO	Doctor.		10.		9 10	G23 G24	unprint unprint	_		
	Name	Result	Note			Symbol	-	11 12	G25 G31	unprint unprint			
Reagent	ALB ALP	34.6 306	↑ ↑	45135	mg/dL U/L			13	G32	unprint	=		
	AST CHO	158.3 1.39	î		U/L mmol/L		_	14 15	G33 G34	unprint unprint			
- <b>4</b>	CL CREA	122.5	†	95105	mmol/L umol/L		=	16	G35	unprint			
Data Processing	DBIL	1.61		07	umol/L			Reactio	n Cunve		*		
×	HDL-C K	0.71 6.42	Ť.	3.55.1	mmol/L mmol/L			î	in ourve				
Maintenance	LDL-C TBIL	0.00			mmol/L umol/L			3.000					
	TG	0.6			mmol/L		-	2 2 2 5 0					
	空腹血糖 APTT	Î	Add Ite     Manu	ally				1.875					
User Setting	TT FIB		Add automatic items		irrent V Picture			1.500					
- 28-	HBsAg HBsAb HBeAg	- (	Add Calculated Items	● AI		er		1.125	FT-		+		
Monitor	Profile Items			© Ra	inge			0.750					
	LPF 宣体		Update Calculation	Print	Serial po	t IP/1	CP	0.375	$\wedge \rightarrow$		$\pm$		
Exit	血糖 血脂	-	Add Profile Items						5 10 15	20 25 30	35 40		
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		Print Curves	QC Resu	t Query Exp	ort data Data	Maintenance	Test f	Results Co	orrection							
		Patient Informatio	n								Sample	No.				
Prog	ram Input	Name: G31		Gender:		<ul> <li>Diagnosis:</li> </ul>		-	Date		No.	Patient Name	Print status	*		
		Position No. 11		Department:	OPD ·	Sample Type:	serum	-	2021-10-19		7	G21	printed			
		Age: 0 Y C	) M	Bed No.:		Doctor:		•	ID:		8	G22	unprint			
Iter	n Setting	Age.		bea No							9	G23 G24	unprint unprint			
	(A)	Name	Result	Not	e Ref	/alue	Unit		Symbol	*	11	G25	unprint			
		CL	122.5	t	95		mmol/L				12	G31 G32	unprint			
R	eagent	CREA DBIL	36.0	1	97 <sup>-</sup> 07		umol/L umol/L				13	G32 G33	unprint unprint	E		
		HDL-C	0.71	Ļ	0.77	-2.25	mmol/L				15	G34	unprint			
	4	K	6.42	1	3.5		mmol/L				16	G35	unprint			
Data	Processing	LDL-C TBIL	0.00		03.		mmol/L umol/L							-		
	1	TG	0.6		0.7	1.7	mmol/L				Reaction	n Curve				
	X	UREA	5.5		1.7		mmol/L				· · · · · ·					
	ntenance	ALT AA	79.6	1	03		U/L			_	3.000					
		Na	162	1	135-	-146	mmol/L				2.625					
		空腹血糖	A (		Add Items						2.250					
	1	APTT TT	-	•	Manually						1.875					
Use	er Setting	FIB		Add autor	matic items			Picture	Mode		1.500					
	6-	HBsAg HBsAb		, 100 0010		© AI		In Orde	er		1.125	A	━╆╼┿╼┽			
	8	HBeAg	-	Add Calcu	lated Items	© R	inge				0.750	<b>r</b>				
N	Ionitor	Profile Items									0.375					
	<b>(111)</b>	LPF 宣体	Â	Update 0	Calculation	Print		Serial port		P/TCP	0.000					
	+	血糖	-	Add Pro	file Items						0.000	5 10 15	20 25 30	35 40		
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Patient Information gram Input     Patient Information     Diagnosis:     Date     Sample No.       Position No.     12     Department:     OPD     Sample Type:     serum     2021-10-19     T     3     G21     print status     7     G21     printed 8     G22     G22     grant data	
Age: 0 Y 0 M Bed No.: Doctor:   ID: 9 G23 unprint 10 G24 unprint	
Name         Result         Note         ReValue         Unit         Symbol         11         G25         uppint           ALB         356         7         35-55         mpdit.         12         G31         unpint           ALP         282         1         45-135         U/L         13         G32         unpint           CHO         2.82         2.24-52         mmo/L         14         G33         unpint	
DB/L         1.45         07         umol/L           HDL_C         0.67         1         0.77-2.25         mmol/L           K         6.87         1         3.5-5.1         mmol/L           LDL_C         0.00         03.35         mmol/L           TBlL         3         2-20.5         umol/L           TG         0.79         0.7-1.7         mmol/L	
UREA     4.1     1.7-8.3     mmo/L     -       VERA     4.1     1.7-8.3     mmo/L     -       VERA     Add lams	
HBaAg • Add Calculated Items Profile Items Update Calculation Frit tit tit tit tit tit tit tit	

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Print       Curves       QC Res         Program Input       Patient Information         Name:       G32         Position No:       12         Age:       9 0 M         Name:       G32         Position No:       12         Age:       9 0 M         Name:       G32         Position No:       12         Age:       9 0 M         Name:       G32         Position No:       12         Age:       0 Y 0 M         Name:       G32         Position No:       12         Age:       0 Y 0 M         Name:       G32         Position No:       12         Age:       0 Y 0 M         Name:       G32         Name:       G32         Position No:       12         Age:       0 Y 0 M         Name:       G32         Position No:       12         Age:       0 Y 0 M         Name:       0 Y 0 M	↑ 0-31 234-52 ↓ 07-177 ↓ 07-75 ↑ 35-51 0-335 2-205 0.7-17 ↑ 0-31 ↑ 0-31 ↑ 135-146 Add automatic items	IS: Date ype: serum  Quality Date Unit  Quality Date Unit  Quality Date Unit  Quality Date Unit  Quality Date Unit  Quality Date Qualit	Sample No.

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			Time:10/20/2021 11:48 AM
Print Curves QC Resu Program Input Program Input Position No.1 Age: 0 Y 0 M	It Query Export data Data Maintenance Gender: Diagnosis: Department: OPD Sample Type: Bed No. Doctor:	Date	Sample No. No. Patient Name Print status 1 G33 unprint 2 G34 unprint 3 G35 unprint
Image: Constraint of the section of	1         3.5-5.5           1         45-135           1         031           1         2.34-5.2           1         97-177           07         0.77-2.25           1         3.5-5.1           03.35         1           2-20.5         1           1.7-8.3         Additems           Manually         Manually		4 G41 urprint 5 G42 urprint 7 G44 urprint 8 G45 urprint 9 G51 urprint 10 G52 urprint 10 G52 urprint 10 G53 urprint 10

			<b>37. 0°</b>						2.1.0.5A User:1000 0/20/2021 11	-49 AM	
Program Input	Print Curves QC Rest Patient Information Name: G33 Position No.[1 Age: 0 Y 0 M	It Query Export data Gender: Department: Bed No.:	Data Maintenance  Diagnosis:  Sample Type: Doctor:	<b></b>	Date 2021-10-20	 Sample No.	lo. Patient Name G33 G34 G35 G41	Print status unprint unprint unprint unprint	E		
Reagent Data Processing Maintenance	Name         Resu           CHO         1.76           CREA         4.9.9           DBIL         1.75           HDL-C         0.37           K         5.45           LDL-C         0.00           TBIL         4           TG         0.75           UREA         4.8           ALT         55.5           AA         1		97177 D7 3.5-5.1 D3.35 220.5 3.71.7 1.78.3	Unit mmol/L umol/L umol/L mmol/L mmol/L umol/L mmol/L mmol/L U/L	Symbol	5 6 7 8 9 10 11 Reaction 3.000 2.625	G42 G43 G44 G45 G51 G52 G53 O Curve	unprint unprint unprint unprint unprint unprint	++		
User Setting Monitor	Na 137 Statution APTT TT TBA0 HBA0 HBA0 HBA0 Profile Items LOF St4 DIR LIF A Bt8 DIR A A A A A A A A A A A A A	Add tem Add automatic items Add Cakulated items Update Calculation Add Profile items	ıs	🗐 In Orde	er	 2255 2250 1875 1500 1125 0.750 0.375 0.000	5 10 15	20 25 30	35 40		

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	Print Curves QC Res	ult Query Export data	Data Maintenance	Test Results (	Correction							
Program Input	Patient Information Name: G34	Gender:	Diagnosis:	-	Date		Sample N					
	Position No.	Department:	<ul> <li>Sample Type:</li> </ul>	serum 👻	2021-10-20		No. 1	Patient Name G33	Print status unprint	Â		
2	Age: 0 Y 0 M	Bed No.:	Doctor:	-	ID:		2	G34 G35	unprint unprint	н		
Item Setting							4	G41 G42	unprint			
- 193	ALB 33.6	1	3.55.5	Unit mg/dL	Symbol		6	G43	unprint			
Reagent	ALP 319 AST 163.6	↑ ↑	031	U/L U/L			7 8	G44 G45	unprint unprint			
	CHO 2.38 CREA 39.0	Ļ	97177	mmol/L umol/L		=	9 10	G51 G52	unprint unprint			
Data Processing	DBIL 1.48 HDL-C 0.62	1	0.772.25	umol/L mmol/L			11	G53	unprint	-		
	K 6.35 LDL-C 0.00	†	03.35	mmol/L mmol/L			Reaction	i Curve				
Maintenance	TBIL 4 TG 0.98		0.71.7	umol/L mmol/L			3.000					
R	UREA 3.4			mmol/L		*	2.250					
User Setting	空腹血糖 APTT TT =	Add II Manu	ally	rrent 🔽 Picture	e Mode		1.875					
	FIB HBsAg HBsAb	Add automatic item	s 💿 All	🔄 In Ord	ler		1.500					
Monitor	HBeAg •	Add Calculated Item	s 💿 Ra	nge			0.750	n –				
	LPF at at a second seco	Update Calculation	Print	Serial po	rt IP/TC	P	0.375		_			
	□1+ 血糖 血脂 -	Add Profile Items					0.000 L	5 10 15	20 25 30	35 40		
Exit												

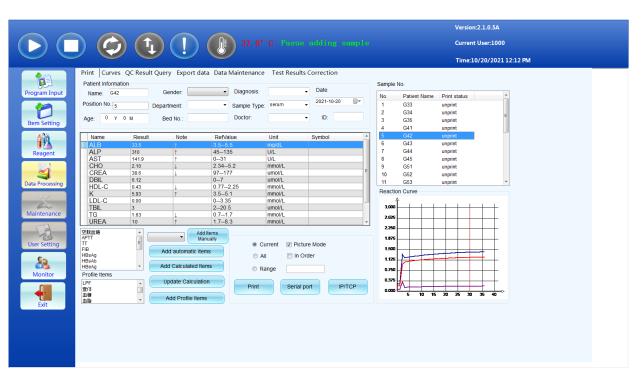


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	Print Curves QC Result Query Export data Data Maintenance Test Results Correction	
Program Input		
	Position No 3 Department: Sample Type: serum 2021-10-20 V 1 G33 unprint 2 G34 unprint 2 G34 unprint 2	
Item Setting	Age: 0 Y 0 M Bed No.: Doctor: VID: 13 C35 unprint 4 G41 unprint	
- <u>19</u>	Name         Result         Note         RefValue         Unit         Symbol         5         G42         unprint           ALB         32.0         1         35–5.5         moldit         6         G43         unprint           ALB         32.0         1         35–5.5         moldit         7         G44         unprint	
Reagent	ALP         311         ↑         45135         UL         7         G44         unprint           AST         155.2         ↑         031         U/L         8         G45         unprint           CHO         2.29         2.34-5.2         mm0/L         9         G51         unprint	
Sata Processing	CREA         40.9         1         97177         umol/L         E         10         G52         unprint           DBIL         1.24         07         umol/L         E         11         G53         unprint	
	HDL-C         0.74         1         0.77-225         mmo/L         Reaction Curve           K         7.12         ↑         3.5-5.1         mmo/L         Reaction Curve           LDL-C         0.00         0-3.35         mmo/L         ↑	
Maintenance	TBIL         4         220.5         umol/L         3000         4         4           TG         1.26         0.7-1.7         mmol/L         26%         1         1         1	
	UREA         4.2         1.7-8.3         mmol/L         *           2½/Lth 16         Add Henns         Add Henns         1.875         1.875	
User Setting	FIB Add automatic items © Current © Picture Mode 150	
- 28	HBsAg HBsAb HBsAb HBsAb HBsAb	
Monitor	Profile Items Update Calculation Print Serial port IP/TCP 0.376	
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	Time:10/20/2021 12:11 PM	
	Print Curves QC Result Query Export data Data Maintenance Test Results Correction Patient Information Sample No.	
Program Input	Name:     G41     Gender:     Diagnosis:     Date       Position No. 4     Department:        • Sample Type: serum       • 2021-10-20       •        •        1       • G33       unprint	
Item Setting	Age:         0         Y         0         M         Bed No.:         Doctor:         ID:         3         G35         unprint         #           4         G41         unprint         #	
Reagent	Name         Result         Note         RetValue         Unit         Symbol         *           I ALB         310         1         39–55         mg/dL         5         G42         unprint           ALP         307         1         45–155         U/L         6         G43         unprint           AST         127.7         1         0–31         U/L         6         G44         unprint           CHO         279         1         234–52         mmo/UL         6         G43         unprint           DBL         0.96         0–7177         umo/L         10         652         unprint           HDL-CO         074         1         0.77–225         mmo/L         7         Reaction Curve           K         6.55         1         3.5–5.1         mmo/L         7         Reaction Curve	
Maintenance User Setting	TBIL     2     1     2-20.5     umol/L       TG     1.63     0.7-1.7     mmol/L     265     0       UREA     9     1     1.7-8.3     mmol/L     265     0       Statistic     Manualy     Current V Picture Mode     1.97     1.97     1.97       File     Add automatic items     All     in Order     1.90     0	
Monitor Exit	Haab Heab	

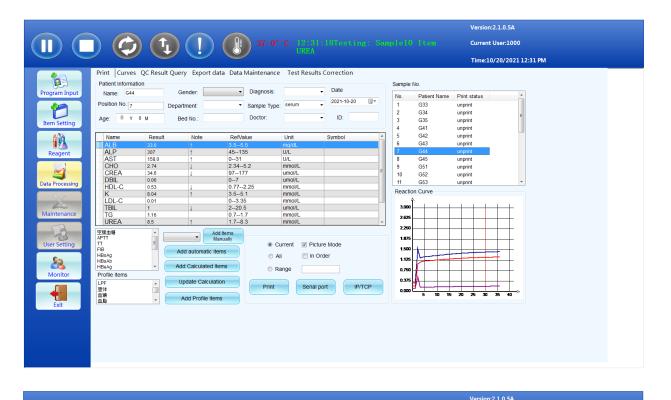
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Patie	ent Information		ta Data Maintenance		orrection Date		Sample No.				
	ime: G41	Gender:	<ul> <li>Diagnosis:</li> </ul>	-	2021-10-20			ent Name Print status		<b>^</b>	
Positi	tion No. 4	Department:	<ul> <li>Sample Type:</li> </ul>	serum 👻	2021-10-20		1 G33	1 C C C C C C C C C C C C C C C C C C C			
Age:	0 Y 0 M	Bed No.:	Doctor:	-	ID:		2 G34 3 G35	unprint unprint	1	E	
em Setting							4 G41	unprint			
	lame Result	Note	RefValue		Symbol	^	5 G42				
	HO 2.79	1		mmol/L			6 G43 7 G44	unprint			
	REA 34.3 BIL 5.95	1		umol/L umol/L		-	8 G45	unprint unprint			
HE HE	DL-C 0.44	1		mmol/L		_	9 G51	unprint			
K		1		mmol/L			10 G52	unprint			
	DL-C 0.07			mmol/L			11 G53	unprint		-	
		1		umol/L		1	Reaction Curv				
TO	G 1.63 REA 9	1		mmol/L mmol/L			A				
		1		U/L		_	3.000				
laintenance A			0.01	0/2			2.625				
Na		1	135146	mmol/L		-					
Ser Setting 和TT FIB HBsAg HBsAg HBsAg HBsAg	g b	Add	Items nually   Cu ms  Al ms  Ra on  Print	rrent 🗹 Picture	er	TCP	2,250 1,875 1,500 1,125 0,790 0,375 0,000 5	10 15 20 25	30 35 40		



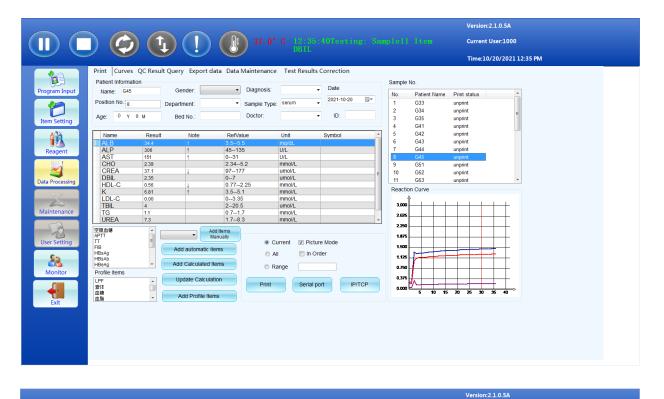


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							Time:10/20/	/2021 12:13 PM	
Program Input	Print Curves QC Re- Patient Information Name: G43 Position No. 6 Age: 0 Y 0 M	Gender.	Data Maintenance  Dlagnosis:  Sample Type: Doctor:	Date		Sample No. No. Patient Na 1 G33 2 G34 3 G35 4 G41	ime Print status unprint unprint unprint unprint	A B	
Reagent Data Processing Waintenance User Setting Monitor	Name         Res           ALB         340           ALP         285           AST         142           CHO         273           CRCA         377           DBL         172           HDL-C         070           K         6.38           LDL-C         010           TG         1.2           UREA         9.8           STREA         9.8           Profile tems         -           Profile tems         -           UFF         .           Stf         30%	1 1 1 1 1 1 1 1	3.6-5.6         r           4.5-135         L           031         L           2.34-5.2         r           97-177         L           07.7         L           0.7-2.25         r           0.7-2.25         r           033.6         C           0.7-1.7         r           1.7-8.3         r           attribute         @ Curr           attribute         All	Unit Symbol modul Unit Symbol Mult Unit Unit Unit Unit Unit Unit Unit Uni	IP/TCP	5 G42 7 G44 8 G45 9 G51 10 G52 11 G53 Reaction Curve 2,055 2,055 1,05 2,055 1,05 2,055 1,05 2,055 1,05 2,055 1,05 2,055 1,05 2,055 1,05 2,055 1,055 2,055 2,055 1,055 2,055 1,055 2,055 1,055 2,055 1,055 2,055	unprint un	4	





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Print Curves Q0 Patient Information Name: G44	C Result Query Export da	ta Data Maintenance ▼ Diagnosis:	e Test Results Correction	Sample No. No. Patient Name Print status
Position No. 7 Item Setting	Department: M Bed No.:	Sample Type:     Doctor:	: serum ▼ 2021-10-20 ■▼ ▼ ID:	1 G33 unprint 2 G34 unprint 3 G35 unprint 4 G41 unprint
Reagent     CREA       Data Processing     DBIL       HDL-C     K       LDL-C     TBIL       TG     UREA       ALT     ALT		2-20.5 0.71.7 1.7-8.3 031 135146 3ftems ms 0 CL 0 CL 0 CL 0 CL 0 CL 0 CL 0 CL 0 CL		Reaction Curve



	Print Curves			<b>37. 0°</b> Ta Data Maintenance	UREA	1:01Teating: Sa	Time:10/20/2021 12:36 PM
Program Input	Patient Informati Name: G45 Position No. 8 Age: 0 Y	on G	ender: tment: ed No.:	Diagnosis:     Sample Type     Doctor:	serum	Date     2021-10-20     ID:	Sample No.           No.         Patient Name         Print status           1         G33         unprint           2         G34         unprint           3         G35         unprint           4         G41         unprint
Reagent Data Processing Waintenance User Setting Monitor	Name CHO CREA DBIL HDL-C K LDL-C TG UREA ALT AA Na TIB HBAA HBAA HBAA PTT TIB HBAA HBAA PTT TIB HBAA PTT TIB HBAA PTT TIB HBAA HBAA HBAA DBIL HBAA HBAA HBAA HBAA HBAA HBAA HBAA HBA			ms © A ms © R on Print	li 📃 in C		5 642 outprint 6 643 unprint 7 644 unprint 9 651 unprint 10 652 unprint 11 653 unprint 12 62 unprint 13 651 unprint 14 653 unprint 14 00 15

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Winterance       Print       Curves       QC Result Query       Exponential         Program Inpit       Patent Information       Department:       Data         Postion No:       Department:       Sample Type:       num       2021-10-20         Age:       Y       M       Bed No:       Doctor:       up       Doctor:       Doct	$\square$	<b>7 (c) (c) 37.0° C 12:240</b> -23Tost 6 rugt Stupple 12: Filent Current User:1000
Patient information       Patient information         Name:       G51       Gender:       Datagnosis:       Date         Version No.       G       Department:       Sample Type:       serum       Date         Version No.       G       Department:       Sample Type:       serum       Date       Date         Version No.       G       Department:       Sample Type:       serum       Date       Date         Version No.       G       Department:       Sample Type:       serum       Date       Date <thd< th=""><th></th><th></th></thd<>		
Program Input       Name: 651       Gender:       Diagnosis:       Date         Position No.       Department:       Sample Type:       202110-20       1         Age:       V       0.11       Bed No.:       Doctor:       ub:         Age:       V       0.11       Bed No.:       Doctor:       ub:         Name:       Result       Note       RefValue       Unit       Symbol         All:       2025       1       3.5-5.5       maiduit       maiduit       3       635       uppint       1       633       uppint       1       643       uppint       5       642       uppint       5       643       uppint       1       634       uppint       5       643       uppint       1       634       uppint       5       643       uppint       1       634       uppint       1       1       634       uppint       1       1       634       uppint       1       1       1       1       1       1       1       1       1       1       1       1       1 <th></th> <th>Print Curves QC Result Query Export data Data Maintenance Test Results Correction</th>		Print Curves QC Result Query Export data Data Maintenance Test Results Correction
Name       Result       Note       Sample Type.       serum       2021-10-20         Item Setting       Po       Patenthame       Print       Sample Type.       serum       2021-10-20         Item Setting       Po       Po       Department       Sample Type.       serum       2021-10-20         Item Setting       Po       Po       Bed No:       Doctor       ID       ID         Item Setting       Name       Result       Note       Refvalue       Unit       Symbol         Item Setting       Data Processing       Name       Result       Octo       Octo       Octo       Octo       Octo         Waittenance       Waittenance		
Position Rol 9       Department       Sample Type:       Setting         Age:       0       Y       0       M       Bed No:       Doctor:       ID         Age:       0       Y       0       M       Bed No:       Doctor:       ID         Age:       0       Y       0       M       Bed No:       Doctor:       ID         Age:       0       Y       0       M       Bed No:       Doctor:       ID         Age:       0       Y       0       M       Bed No:       Doctor:       ID         Name       Result       Note       RefValue       Unit       Symbol       Symbol       Symbol         All       3       332       1       43-452       mmol/L       Symbol       Set 43       upprint         Age:       0       1       0.77-225       mmol/L       ID       Set 33       upprint         HDL-C       0.04       1       0.77-225       mmol/L       ID       Set 33       upprint         IDL-C       0.01       0       0       0       Set 33       upprint         IDL-C       0.01       0       0       ID       ID       ID <td>Program Input</td> <td>Name: Of Control . No. Patient Name Print status</td>	Program Input	Name: Of Control . No. Patient Name Print status
Age:       0 Y 0 M       Bed No:       Doctor.       JD.         Age:       0 Y 0 M       Bed No:       Doctor.       JD.         Age:       0 Y 0 M       Bed No:       Doctor.       JD.         Age:       0 Y 0 M       Bed No:       Doctor.       JD.         Age:       0 Y 0 M       Bed No:       Doctor.       JD.         All:       3 3 33       uppint       641       uppint         ALP       332 1       45-55       mmol/L       544         All:       0 77-225       mmol/L       10 352       uppint         Data Processing       DBlil       312       0-7-7       umol/L         HDL-C       0 01       0-33 5       mmol/L       0         HDL-C       0 01       0-35 5       mmol/L       0         HDL-C       0 01       0-7.17       mmol/L       0         HDL-G       0 92       1 0.7-17       mmol/L       0         UREA       9.2       1 0.7-17       mmol/L       0         UREA       9.2       1 0.7-17       mmol/L       0         UREA       9.2       1 0.7-17       mmol/L       0         UREA       9.2<		Position No. 9 Department:   Sample Type: Serum  Gal  Gal  Gal  unprint
Waintenace       Name       Result       Note       Refvalue       Unit       Symbol       5       6.42       uppint       6         ALIS       325       1       35-65       moilu       1<	Item Setting	Age: 0 Y 0 M Bed No.: Doctor: V ID: 3 G35 unprint
Reagent       ALP       332       1       45-135       UL         AST       216.8       1       0-31       UL       1         CHO       192       1       234-52       mmol/L       1         CHC       192       1       07-177       umol/L       10       652       uppint         DBIL       312       0-7       umol/L       10       652       uppint       1         HDL-C       0.94       1       0.77-2.25       mmol/L       10       653       uppint         IDL-C       0.94       1       0.7-1.7       mmol/L       10       653       uppint         IDL-C       0.91       0-7       3.5       mmol/L       1       10       653       uppint         IDL-C       0.91       0.7-1.7       mmol/L       1       10       2.35       1       1.32       1.32       1.32       1.32       1.32       1.32       1.32       1.32       1.32       1.32       1.32       1.32       1.32       1.32       1.32       1.32       1.32       1.32       1.33       1.33       1.33       1.33       1.33       1.33       1.33       1.33       1.33       1.	ADA	
AST       218.8       1       031       UL         Octor       1.92       1       2.34.5.2       mmol/L       0         Octor       1.92       1       2.34.5.2       mmol/L       0         Data Processing       Maintenance       0       0.77       umol/L       0         Maintenance       10       0.52       uppint       0       0.52       uppint         User Setting       10       0.77       0.71-1.7       mmol/L       0       0.52       uppint       0         Weissetting       10       0.97       1       0.7-1.7       0 <td></td> <td>ALB 32.5 † 3.5–5.5 mg/dL 6 G43 unprint</td>		ALB 32.5 † 3.5–5.5 mg/dL 6 G43 unprint
CREA       38.0       1       97-977       unovi/L       1         Data Processing       CREA       38.0       1       97-977       unovi/L       1         Maintenance       DIL       3.12       0.77-225       mmol/L       1       653       unprint       Reaction Curve         Maintenance       DIL       5       2-20.5       mmol/L       1       10       0.433       10       0.10 <td>Reagent</td> <td>AST 216.8 ↑ 0–31 U/L 8 645 unprint</td>	Reagent	AST 216.8 ↑ 0–31 U/L 8 645 unprint
Data Processing       DBIL       3.12       0-7       umo/u         Maintenance       IDLC       0.94       1       0.77-22.55       umo/u         Maintenance       IDLC       0.01       0-3.35       mmo/u       umo/u         Viser Setting       IDLC       0.01       1       0.7-1.7       umo/u       umo/u         Viser Setting       IDLC       0.97       1       0.7-1.7       mmo/u       umo/u       umo/		CREA 38.0 ↓ 97177 umol/L 10 G52 upprint
K       7.69       1       3.5-5.1       monUl         Maintenance       IDL-C       0.01       3.2-5.5       immol/L         IBL       5       2-20.5       immol/L       immol/L         INDEX       0.97       0.7-1.7       immol/L       immol/L         Uxer Setting       Image       Add litems       immol/L       immol/L         Viser Setting       Add automatic items       Add automatic items       immol/L       immol/L         Monitor       Profile items       Update Calculation       Print       Serial port       IP/TCP         Image       Add Profile items       Print       Serial port       IP/TCP       image       image	Data Processing	DBIL         3.12         0-7         umol/L         11         G53         unprint         *           HDL         0.077-225         mmol/L         11         G53         unprint         *
Maintenance       TBIL       5       2-20.5       ummol/L         UREA       9.2       1       0.7-17       mmol/L         User Setting       2285.01%       Add automatic items       2.20       1.7-8.3       mmol/L         VBEA       9.2       1       1.7-8.3       mmol/L       2.25       2.20       1.7-8.3         Waintenance       Print       Manualty <ul> <li>Current</li> <li>Picture Mode</li> <li>All</li> <li>In Order</li> <li>Bit</li> <li>Update Calculation</li> <li>Print</li> <li>Senial port</li> <li>IP/TCP</li> </ul> 100       100       100           With         Add Profile Items         Print         Senial port         IP/TCP         100         105         100		K 7.69 † 3.55.1 mmol/L Reaction Curve
UREA       9.2       1.7-8.3       mmol/L         User Setting       User Setting       Add automatic items       Add automatic items         User Setting       Add automatic items       Add automatic items       0         Monitor       Profile items       Add Calculated items       0         Profile items       Update Calculation       Print       Serial port       IP/TCP         Bit       Add Profile items       Add Profile items       0       IP/TCP		TBIL 5 2-20.5 umo/L 3.000
User Setting       PTT       Add tems         Ward Add tems       Add automatic items       In Order         Add automatic items       Add cakulated items       Add cakulated items         Monitor       Profile items       Update Cakulation         Print       Serial port       IP/TCP	Wantenance	UREA 9.2 † 1.76.3 mmol/L v 2007
Fig     Add automatic items       Monitor     Profile items       Upf     Update Calculation       Print     Serial port       IP/TCP     10 f5 20 25 30 35 40		型接触 Addiama
Add Calculated Items     Add Calculated Items       Upf     Update Calculation       Print     Serial port       IP/TCP     5 10 15 20 25 30 35 40	User Setting	FIB Add automatic floms
Monitor     Profile Items       Update Calculation     Print       Bit     Add Profile Items	<b>\$</b> 3	HBsAp HBsAb
UP         Opdate calculation         Print         Serial port         IP/TCP         0.000		Profile Items
		Upf Update Calculation Print Serial port IP/TCP
	Exit	



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$(\mathbf{n})$	<b>I</b> ( <b>c</b> ) ( <b>1</b> ) ( <b>l</b> ) 37.0° C <u>12.16.27</u> (testing) LDL C	g: Skung to 18 Thom Current User:1000
		Time:10/20/2021 12:46 PM
	Print Curves QC Result Query Export data Data Maintenance Test Results Correction Patient Information	Sample No.
Program Input	Name: G52 Gender: Diagnosis: Date	No Patient Name Print status
	Position No. 10 Department:  Sample Type: serum  2021-10-20	8 G45 unprint
Item Setting	Age: 0 Y 0 M Bed No.: Doctor: V ID:	10 G52 unprint
	Name Result Note RefValue Unit Symbol	11 G53 unprint 12 G54 unprint 13 G55 unprint
Reagent	ALB         33.2         ↑         3.55.5         mg/dL           ALP         317         ↑         45135         U/L	14 G61 unprint
	AST         185         ↑         031         U/L           CHO         2.06         ↓         2.345.2         mmol/L	15 G62 unprint 16 G63 unprint
3	CREA         37.7         ↓         97177         umo//L           DBIL         1.05         07         umo//L	17 G64 unprint 18 G65 unprint
Data Processing	HDL-C 0.8 ↓ 0.772.25 mmol/L K 7.63 ↑ 3.55.1 mmol/L	Reaction Curve
X	LDL-C 0.00 03.35 mmol/L TBIL 5 220.5 umol/L	3,000
Maintenance	TG 0.75 0.7−1.7 mm0/L UREA 10.2 ↑ 1.7−8.3 mm0/L	2.625
		2250
User Setting	Add lens Add lens FiB	1,875
- 88	HBsAg All In Order	1.125
Monitor	HBeAg   Add Calculated Items  Range  Range	0.750
	LPF Update Calculation Print Serial port IP/TC	CP 0.375
-	血液 人名 Profile Items	0.000 2 5 10 15 20 25 30 35 40
Exit		

		ult Query Export data D	5	C 12:46: TB11.		ng: Sa			Curren	n:2.1.0.5A t User:1000 10/20/2021 :	L2:46 PM	
Program Input	Patient Information Name: G52 Position No. 10	Gender: Department:	Diagnosis:     Sample Type	serum +	2021-10-20		Sample No. 8 9	No. Patient Name G45 G51	Print status unprint unprint	^		
Item Setting	Age: <sup>0</sup> Y 0 M Name Resu CHO 2.06	↓ 2.	Doctor: efValue 345.2	Unit mmol/L	ID:	-	10 11 12 13	G52 G53 G54 G55	unprint unprint unprint unprint	I		
Reagent	CREA         37.7           DBIL         1.05           HDL-C         0.8           K         7.63           LDL-C         0.00	0- ↓ 0. ↑ 3. 0-	772.25 55.1 -3.35	umol/L umol/L mmol/L mmol/L mmol/L			14 15 16 17 18	G61 G62 G63 G64 G65	unprint unprint unprint unprint unprint			
Maintenance	TBIL         5           TG         0.75           UREA         10.2           ALT         137.3           AA         1	0. ↑ 1. ↑ 0-	-20.5 71.7 78.3 -31	umol/L mmol/L U/L		E	- Reaction 3.000 2.625					
User Setting	Na 186 空館曲緒 APTT TT FIB HBsAg	Add automatic items		urrent V Pictur		•	2.250 1.875 1.500		_			
Monitor	HBsAb HBeAg Profile Items	Add Calculated Items Update Calculation	© R			Р/ТСР	1.125 0.750 0.375 0.000		20 25 30	 35 40		
Exit	血糖 一面脂 一面脂 一面脂 一面脂 一面脂 一面脂 一面脂 一面脂 一面脂 一面脂	Add Profile Items						9 10 19	20 25 30	32 40		

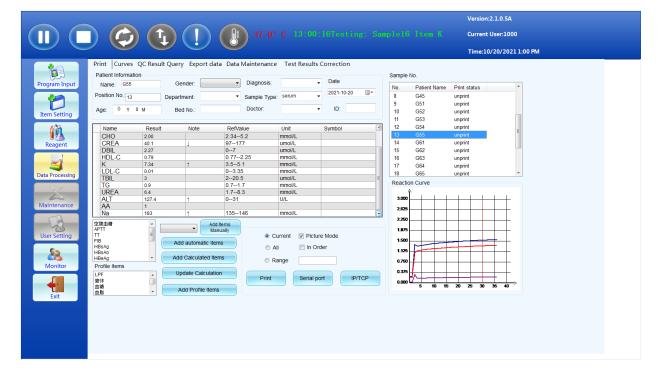
$\sim$								Version:2	2.1.0.5A		
			37.0°C					Current U	Jser:1000		
								Time:10/	/20/2021 12:50 PM	4	
	Print Curves QC Resu	ılt Query Export data Data	Maintenance Test	Results Correct	ion						
	Patient Information					Sample N	lo.				
Program Input	Name: G53	Gender:	Diagnosis:	<ul> <li>Date</li> <li>2021-</li> </ul>	10-20	No.	Patient Name	Print status	<b>^</b>		
	Position No.: 11	Department:	Sample Type: serum	▼ 2021*	10-20 @*	8	G45 G51	unprint unprint			
Item Setting	Age: <sup>0</sup> Y 0 M	Bed No.:	Doctor:	• ID:		10	G52	unprint			
408	Name Resul	t Note RefVa	lue Unit	Symbol	*	11 12	G53 G54	unprint unprint			
<u> (13</u>	ALB 36.5	↑ 3.55.	5 mg/dL			13 14	G55 G61	unprint	E		
Reagent	ALP 327 AST 195.2	↑ 031	U/L			15	G62	unprint			
	CHO 2.06 CREA 39.4	2.34				16 17	G63 G64	unprint unprint			
Data Processing	DBIL 3.20 HDL-C 0.81	07	umol/L 2.25 mmol/L			18	G65	unprint	-		
	K 6.18 LDL-C 0.00	↑ 3.55 03.3	1 mmol/L			Reaction	n Curve				
Maintenance	TBIL 6	220.	5 umol/L			3.000			++		
	TG 0.89 UREA 9.0	0.71 ↑ 1.7-8				2.625			++		
	空腹血糖 APTT TT	Add Items Manually				2.250					
User Setting	TT E	Add automatic items	Ourrent	Picture Mode		1.500					
- 22	HBsAg HBsAb		© All	📃 In Order		1.125			+		
Monitor	HBeAg	Add Calculated Items	Range			0.750	$\left  \right $		++		
	LPF 查体	Update Calculation	Print	Serial port	IP/TCP	0.375			+-+		
<b>+</b>	□1年 血糖 血脂 下	Add Profile Items				0.000 E	5 10 15	20 25 30	35 40 >		
Exit											
	-										



				03Testing: S	amp l	el5 Item	Version:2.1.0.5A Current User:1000 Time:10/20/2021 12:	55 PM
Patient Information Name: G54 Position No. 12 Age: 0 y 0	Gender: Department: M Bed No.:	t data Data Maintenance Diagnosis: Sample Type: Doctor:	serum •	Date 2021-10-20	N 8 9 1	G45 G51 G52 G53	Print status unprint unprint unprint unprint	
Name         ALP         Second         BAR         HBAQ         HBAQ         HBAQ         HBAQ         HBAQ         HBAQ         HBAQ         BBR         BBR         BBR         BBR         BBR         BBR         BBR         BBR	Result         Note           35.7         1           339         1           339         1           229.5         1           203         358           1.17         0           0.67         1           7.18         1           0.00         6           0.05         1           9.1         1           Add automat         Add Calculate           Update Calc         Add Profile	45-135 031 2,34-5,2 97-177 0-77 0,77-2,25 3,5-5,1 0-3,35 2,-20,5 0,7-1,7 1,7-8,3 Add Items Wanualty ic Items c Items C Cu Band Ch Band Ch Ch Band Ch Band Ch Ch Band Ch Band Ch Ch Band Ch Ch Ch Ch Ch Ch Ch Ch Ch Ch Ch Ch Ch C	mg/dL           U/L           umol/L           umol/L           umol/L           mmol/L           mmol/L	Mode	1 1 1 1 1 1 1 1 1 1 1 1 1	3 G55 4 G61 5 G62 6 G63 7 G64 8 G65 8 G65 8 G65 9 G65 9 G65 9 G64 9 G65 9 G64 9 G65 9 G65 9 G65 9 G65 9 G65 9 G65 9 G65 9 G65 9 G62 9 G65 9 G62 9 G62	unprint unprint unprint unprint unprint unprint unprint unprint unprint unprint unprint unprint	

Patient information         Sample No.           Name:         054         Gender:         Diagnosis:         -         Date         8         Geta         8         645         unprint         9         054         0         645         unprint         9         054         0         654         unprint         9         0510         No.         Patient Name         Print status         *         10         052         10         10         052         unprint         10         052         unprint         10         052         unprint         12         054         unprint         12         054         unprint         13         053         unprint         13         055         unprint         13         055         unprint         14         661         unprint         15         0562         unprint         15         054         unprint         15         053         unprint         15         0562         unprint         15         0563 <th></th> <th>unprint unprint unprint</th> <th>No.         Patient Name           8         G45           9         G51</th> <th></th> <th>2021-10-20</th> <th></th> <th></th> <th>Gender:</th> <th>54</th> <th></th>		unprint unprint unprint	No.         Patient Name           8         G45           9         G51		2021-10-20			Gender:	54	
Position No.         12         Department: <ul> <li>Sample Type:</li> <li>Berum</li> <li>2021-10-20</li> <li>9                 <li>G61</li> <li>unprint</li> <li>9                 <li>G61</li> <li>unprint</li> <li>10</li> <li>G62</li> <li>unprint</li> <li>11</li> <li>G63</li> <li>unprint</li> <li>12</li> <li>G64</li> <li>unprint</li> <li>13</li> <li>G65</li> <li>unprint</li> <li>13</li> <li>G65</li> <li>unprint</li> <li>14</li> <li>G61</li> <li>unprint</li> <li>15</li> <li>G62</li> <li>unprint</li> <li>16</li> <li>G63</li> <li>unprint</li> <li>16</li> <li>G63</li> <li>unprint</li> <li>17</li> <li>G63</li> <li>unprint</li> <li>18</li> <li>G61</li> <li>unprint</li> <li>19</li> <li>G62</li> <li>unprint</li> <li>19</li> <li>G62</li> <li>unprint</li> <li>19</li> <li>G62</li> <li>unprint</li> <li>19</li> <li>G62</li> <li>unprint</li> <li>19</li> <li>G63</li> <li>unprint</li> <li>19</li> <li>G63</li> <li>unprint</li></li></li></ul>		unprint unprint unprint	8 G45 9 G51		▼ 2021-10-20	serum •				
Age:         V         0         Marrie         Doctor:         ID:         9         651         unprint           Name         Result         Note         RefValue         Unit         Symbol         10         652         unprint           Name         Result         Note         RefValue         Unit         Symbol         11         GS3         unprint           CREA         35.8         1         97-177         umol/L         13         655         unprint         14         661         unprint         15         G62         unprint         16         663         unprint         16         16         663         unprint         16 <t< td=""><td>1</td><td>unprint</td><td></td><td></td><td></td><td></td><td></td><td>Department:</td><td>12</td><td>Position No. 12</td></t<>	1	unprint						Department:	12	Position No. 12
Name         Result         Note         Refvalue         Unit         Symbol         11         GS3         unprint           11         CHO         2.03         2.34-5.2         mmol/L         13         GS5         unprint           CREA         35.8         1         97-177         umol/L         14         G61         unprint           DBIL         1.17         0-77         umol/L         15         G62         unprint           HDL-C         0.67         1         0.77-2.25         mmol/L         16         G63         unprint					<ul> <li>ID:</li> </ul>					
Name         Result         Note         RefValue         Unit         Symbol         12         054         upport           CHO         2.03         2.34–5.2         mmol/L         13         055         uppint           CREA         35.8         1         97–177         umol/L         14         C61         uppint           DBIL         1.17         0–77         umol/L         15         G62         uppint           HDL-C         0.677         1         0.77–2.25         mmol/L         16         G63         uppint	E				• ID.		Doctor	Bed No.:	YUM	Age: • •
CHO         2.03         2.345.2         mmo//L         13         G55         unprint           CREA         35.8         1         97-177         umo//L         14         G61         unprint           DBIL         11.7         0-7         umo//L         15         G62         unprint           HDL-C         0.67         1         0.77225         mmo//L         16         G63         unprint		unprint		*	Symbol	Unit	RefValue	Note	Result	Name
DBIL         1.17         07         umol/L         15         G62         unprint           HDL-C         0.67         1         0.77-2.25         mmol/L         16         G63         unprint						mmol/L	2.345.2			
HDL-C 0.67 1 0.77-2.25 mmol/L 16 G63 unprint								Ļ		
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LDL-C 0.00 03.35 mmol/L 18 G65 uppoint	-						03.35		0.00	
19 TBIL 6 2-20.5 umol/L		unprint		E						
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UREA 9.1 ↑ 1.78.3 mmoVL			3000							
						0/12	001			
Na 186 † 135146 mmol/L				-		mmol/L	135146	t	186	
空班血液 Add Items 2290 2290 2290 2290 2290 2290 2290 229			2.250				Items			空腹血糖
APTT Manually Manually			1.875				nually			
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	37.0° C 12:50:15Testing: Sample16 Ltom Current User:1000
	DB IL Time:10/20/2021 12:59 PM
	Print Curves QC Result Query Export data Data Maintenance Test Results Correction
Program Input	Patient Information Name: 655 Gender: Diagnosis: Date Diagnosis: Date No. Patient Name Print status
1	Position No. 13 Department: Sample Type: serum V 2021-10-20 V 11 G53 unprint
Item Setting	Age: 0 Y 0 M Bed No.: Doctor: VID: 13 G65 unprint 14 G61 unprint
	Name         Result         Note         RefValue         Unit         Symbol         15         G62         unprint           ALB         37.0         t         3.55.5         mg/dL         16         G63         unprint
Reagent	ALP         360         t         45135         U/L         17         G64         unprint         E           AST         233.0         r         031         U/L         18         G65         unprint         E
4	CHO         200         234-52         mmol/L         19         G71         unprint           CREA         40.1         1         97-177         umol/L         20         G72         unprint         21         G73         unprint         -
Data Processing	HDL-C         0.79         0.77-2.25         mmol/L           K         7.34         †         3.5-5.1         mmol/L         Reaction Curve
Maintenance	LDL-C         001         0-335         mmol/L           TBL         3         2-205         umol/L           TG         0.9         0.7-1.7         mmol/L
	UREA         6.4         1.7-5.3         mmo/L         -         2.85         -         -         -         2.87         2.89         -         -         -         2.89         -
User Setting	Statutie     Add lens       April     Add analy       TT     Fig       Add automatic terms     © Current [2] Picture Mode
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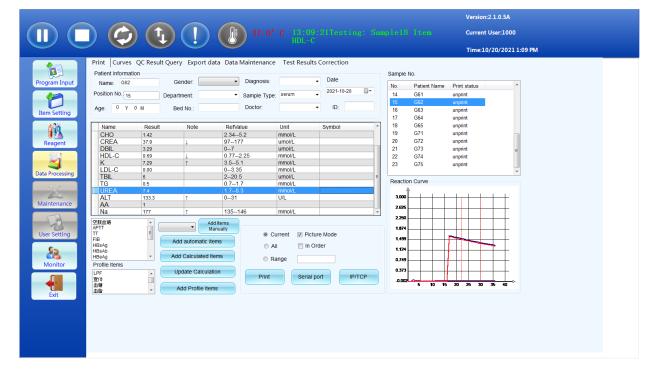


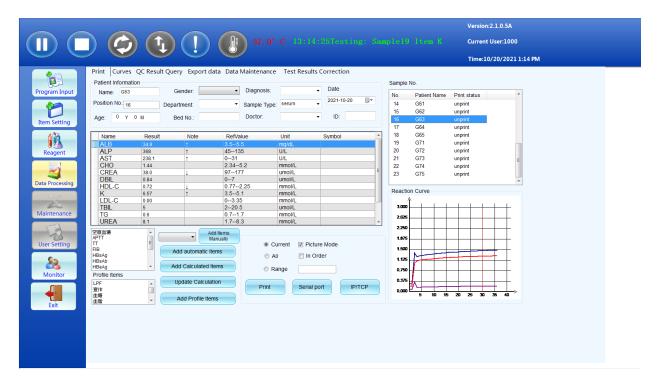
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	Patient Informati		t Query Export data Da	_	Test I		in	Sample I	NO.				
Program Input	Name: G61		Gender:	<ul> <li>Diagnosis:</li> </ul>		<ul> <li>Date</li> <li>2021-10</li> </ul>	-20	No.	Patient Name	Print status	*		
	Position No.: 14		Department:	<ul> <li>Sample Type</li> </ul>	serum		-20 67	8	G45 G51	unprint unprint			
Item Setting	Age: 0 Y	0 M	Bed No.:	Doctor:		<ul> <li>ID:</li> </ul>		10	G52 G53	unprint			
<b>M</b>	Name	Result	Note Re	fValue	Unit	Symbol	^	12	G54	unprint	-		
	ALB ALP	38.2 341		5.5 -135	mg/dL U/L			13 14	G55 G61	unprint unprint	1		
Reagent	AST	217.3	↑ O	31	U/L			15	G62	unprint			
	CHO CREA	1.77		45.2 -177	mmol/L umol/L		_	16 17	G63	unprint			
Data Processing	DBIL	3.66	0	,	umol/L		=	18	G64 G65	unprint unprint	-		
Data Hocessing	HDL-C K	0.50		72.25 -5.1	mmol/L mmol/L			Reactio	n Curve				
	LDL-C	0.00	0	.35	mmol/L			1					
Maintenance	TBIL	6 0.50		-1.7	umol/L mmol/L			3.000					
	UREA	10.6		-8.3	mmol/L			2.625					
	空旗血糖 APTT	Î	Add Items					2.250					
User Setting	TT	=	Manually	• c	urrent	Picture Mode		1.875	1				
	FIB HBsAg		Add automatic items	) o a		🔲 In Order		1.500	M				
- <u>88</u>	HBsAb HBeAg	- (	Add Calculated Items					1.125					
Monitor	Profile Items			⊚ R	ange			0.750					
	LPF 查体	*	Update Calculation	Print		Serial port	IP/TCP	0.375	$\wedge$		<u> </u>		
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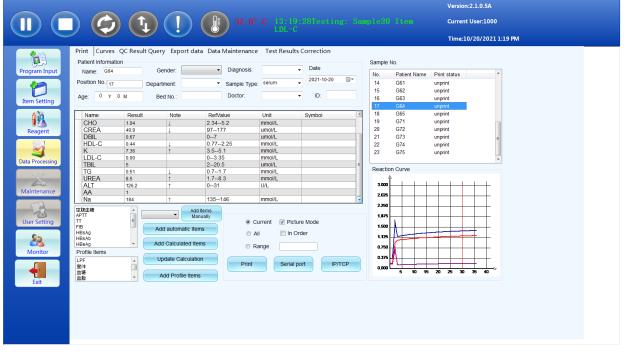
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	Print Curves QC Res	ult Query Export data D	ata Maintenance	Test Results (	Correction							
	Patient Information		_				Sample N	0.				
Program Input	Name: G62	Gender:	<ul> <li>Diagnosis:</li> </ul>	-	Date	_	No.	Patient Name	Print status	*		
	Position No. 15	Department:	<ul> <li>Sample Type</li> </ul>	serum 👻	2021-10-20		14	G61 G62	unprint			
Item Setting	Age: 0 Y 0 M	Bed No.:	Doctor:	-	ID:		15	G62 G63	unprint unprint			
	News	it Note R		11-14	Question		17 18	G64 G65	unprint unprint			
<b>1</b>	ALB 34.5		efValue 55.5	Unit mg/dL	Symbol	- Î	19	G71	unprint			
Reagent	ALP 368 AST 236.0		135 31	U/L U/L			20 21	G72 G73	unprint unprint			
	CHO 1.42	2.	345.2 177	mmol/L			22	G74	unprint			
Data Processing	DBIL 5.29	0-	7	umol/L umol/L		E	23	G75	unprint	-		
Data Processing	HDL-C 0.69 K 7.29		72.25 55.1	mmol/L mmol/L			Reaction	Curve				
X	LDL-C 0.00 TBIL 6		3.35 20.5	mmol/L umol/L		_	3.000					
Maintenance	TG 0.5	0.	/1.7 /8.3	mmol/L mmol/L			2.625					
	UREA 7.4 空腹血糖	Add Items	0.3	mmove		Ŧ	2.250					
User Setting	空腹血糖 APTT TT	Manually	0.0	urrent 📝 Picture	Mode		1.875					
	FIB HBsAg	Add automatic items					1.500	N				
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Monitor	Profile Items	Update Calculation					0.375					
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Exit	血脂 -	Add Profile Items						5 10 15	20 20 30	30 10		











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	Print Curves QC Res	ult Ouery Export data	a Data Maintenance	Test Results (	orrection							
	Patient Information						Sample I	No.				
Program Input	Name: G65	Gender:	<ul> <li>Diagnosis:</li> </ul>	•	Date		No.	Patient Name	Print status	*		
	Position No.: 18	Department:	<ul> <li>Sample Type:</li> </ul>	serum 👻	2021-10-20		14	G61	unprint			
Item Setting	Age: <sup>0</sup> Y 0 M	Bed No.:	Doctor:	-	ID:		15 16	G62 G63	unprint unprint			
							17	G64 G65	unprint			
- (M)	ALB 36.3	ilt Note	RefValue 3.55.5	Unit mg/dL	Symbol	- î	19	G71	unprint unprint			
Reagent	ALP 361	t	45135	U/L			20	G72	unprint			
	AST 286.9 CHO 1.17	1		U/L mmol/L			21 22	G73 G74	unprint unprint	E		
	CREA 30.9	÷ ↓	97177	umol/L		E	22	G74 G75	unprint			
Data Processing	DBIL 0.15 HDL-C 0.68			umol/L mmol/L		_				*		
	K 7.20	÷		mmol/L			Reactio	n Curve				
	LDL-C 0.00			mmol/L			3.000					
Maintenance	TBIL 3 TG 0.4			umol/L mmol/L			2.625					
	UREA 8.5	t		mmol/L		+						
- 23-	空腹血糖 APTT		Items				2.250					
User Setting	TT E	Man	e Cu	rrent 📝 Picture	Mode		1.875	1				
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	LPF	Update Calculatio	n Print	Serial po	rt IP/	тср	0.375			<u>+</u> +		
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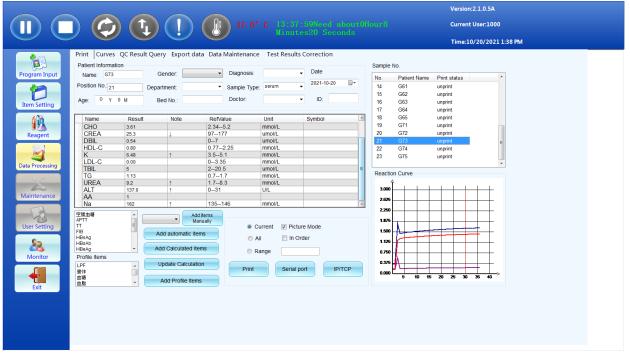




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	Time:10/20/2021 1:32 PM
	Print Curves QC Result Query Export data Data Maintenance Test Results Correction
	Patient Information Sample No.
Program Input	Name: G72 Gender: Diagnosis: Date No. Patient Name Print status
- 19-	Position No. 20 Department: Sample Type: serum V 2021-10-20 V 14 G61 unprint 15 G62 unprint
Item Setting	Age: 0 Y 0 M Bed No.: Doctor. • ID: 16 G63 unprint
	Name         Result         Note         RefNalue         Unit         Symbol         18         G65         unprint
19	ALB 359 1 3.5-5.5 mg/dL 19 G71 unprint
Reagent	ALP         335         1         45135         U/L         20         G72         unprint           AST         236.6         1         031         U/L         21         G73         unprint         =
	CHO         3.40         2.345.2         mmol/L         22         G74         unprint           CREA         37.2         1         97177         umol/L         =         23         G75         unprint
Data Processing	DBIL         0.35         07         umo/L           HDL-C         0.60         1         0.77-2.25         mmo/L
	K 7.21 ↑ 3.55.1 mmol/L Reaction Curve
h	LDL-C         0.00         0-3.35         mmo//L           TBIL         4         2-20.5         umo//L         3.000
Maintenance	TG 173 0.7-17 mm0/L 7255 2255 2255 2255 2255 2255 2255 225
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User Setting	Collent V Pictale Mode
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Program Input Program Input Them Setting	Gender: Gender	a Data Maintenano Diagnosis: Sample Typ Doctor:	2021 10 2		Sample No. No. Patient Name Print status 14 G61 unprint 15 G62 unprint 16 G63 unprint 17 G64 unprint
Reagent       Name       Res         Data Processing       Data Processing       Data Processing         Waintenance       Name       Res         Maintenance       Name       Res         Monitor       Sitt Bits       Name         Piblic       Oside       Name         Version       Sitt Bits       Name         Name       Res       Name         Version       Name       Res         Version       Name       Name         Version       Name       Name         Version       Name       Name         Version       Version       Name         Version       Name       Name         Vers	Add automatic item Add Calculated Item	ns © ms © m Print	Unit Symbol mmol/L umol/L umol/L mmol/L mmol/L mmol/L mmol/L U/L mmol/L U/L U/L Current  ♥ Picture Mode All I In Order Range Serial port	IP/TCP	18       G65       unprint         19       G71       unprint         21       G73       unprint         23       G74       unprint         23       G75       unprint         8eaction Curve       100       100         100       0.000       5       10       15       20       25       30       35       40





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$\square$			37.0°					Current User:	:1000	
				minutes				Time:10/20/2	2021 1:42 PM	
Program Input	Print Curves QC Res Patient information Name: G74 Position No. 22 Age: 0 Y 0 M	ult Query Export data Da Gender.	<ul> <li>Diagnosis:</li> <li>Sample Type:</li> <li>Doctor:</li> </ul>	<b></b>	Date 2021-10-20 ID:	 Sample No. 14 15 16 17	Patient Name G61 G62 G63 G64	Print status unprint unprint unprint unprint	^	
Reagent Data Processing Maintenance User Setting Monitor	Name         Ress           ALB         374           ALP         381           AST         280.5           CHO         363           CREA         315           DBIL         123           HDL-C         0.80           K         6.06           LDL-C         0.04           TG         1.81           UREA         10.5           ZMM#         ^           Profile Items	1 3.5 1 45- 1 0 2.3 1 97- 0 0.7 1 3.5 0 0 1 0.7 1 3.5 0 1 0.7 1 3.5 0 0 1 0.5 0 1 0.5 0 1 0.5 0 0.7 0.7 0.7 0.7 0.7 0.7 0.7 0.7	-135 -1 -5.2 -7.7 -2.25 -5.1 -3.5 0.5 -1.7 -8.3	mg/dL U/L mmo/L mmo/L umo/L mmo/L mmo/L umo	r	18 19 20 21 23 Reaction 2,625 2,250 1,675 1,500 1,125 0,750 0,375 0,000	G65 G71 G72 G73 G74 G75	unprint unpr	v v 40	

Image: Curves QC Result Query Export data Data Maintenance Test Results Correction       Sample No.         Print Curves QC Result Query Export data Data Maintenance Test Results Correction       Sample No.         Program Input       Print Gender:       Diagnosis:       Date         Program Input       Date       Date       Date         Program Input       Patient Information       Sample No.         Rester No.       Patient Name Print status       Official Correction
Print Curves QC Result Query Export data Data Maintenance Test Results Correction Patient Information Name: G74 Gender: Diagnosis: Date Name: G74 Gender: Diagnosis: Date No. Patient Name Print status
Program Input Pr
Position No       22       Department.       Sample Type:       serum       2021-10-20       upint         Age:       Y       0.M       Bed No:       Doctor:       UD:       UD:       UD:         Age:       Y       0.M       Bed No:       Doctor:       UD:

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	Time:10/20/2021 1:46 PM
Program Input	Print     Curves     QC Result Query     Export data     Data Maintenance     Test Results Correction       Patient Information     Sample No.     Sample No.       Name:     G75     Gender:     Diagnosis:     Date
	Position No. 23 Department: Sample Type: serum 2021-10-20 V 14 G61 unprint
Item Setting	Age:         0         Y         0         M         Bed No.:         Doctor:         ID:         15         G62         unprint           16         G63         unprint         17         G64         unprint
693	Name Result Note Refvalue Unit Symbol - 18 G65 unprint
Reagent	ALB         37.4         1         35.5         mg/dL         19         G71         unprint           ALP         387         1         45–135         U/L         20         G72         unprint
Reagent	CHO         3.16         2.34~5.2         mmol/L         21         G73         unprint
	CREA         39.3         1         97-177         umol/L         22         G74         unprint           DBIL         0.35         07         umol/L         =         23         G75         unprint
Data Processing	HDL-C 0.64 1 0.77-225 mmol/L
	LDL-C 0.08 0–3.35 mmol/L Reaction Curve
6	TBIL         5         2-20.5         umol/L           TG         163         0.7-1.7         mmol/L         3000
Maintenance	UREA 11.5 ↑ 1.7-8.3 mmol/L 2676
	ALT 142.0 1 0-31 U/L *
13	ZREADE AddRena Addrena II.876
User Setting	TT E Add automatic items Current @ Picture Mode 1.000
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Patient Informa	-	It Query Export d		viaintenance	rest Kesuit	sconecu	ion		Sample 1	lo			
Name: G75		Gender:	-	Diagnosis:		<ul> <li>Date</li> </ul>			No.	Patient Name	Print status	*	
Position No. 23		Department:	-	Sample Type:	serum	- 2021-1	10-20 🔳	•	14	G61	unprint		
Age: 0 Y	0 M	Bed No.:		Doctor:		• ID:			15	G62	unprint		
Age.		Bourno							16 17	G63 G64	unprint unprint		
Name	Result		RefVa		Unit	Symbol		*	18	G65	unprint		
ALP	287	1	4513		U/L				19	G71	unprint		
CHO	2.16	+	2.345		mmol/L umol/L	_		-11	20 21	G72 G73	unprint unprint		
DBIL	0.35	÷	07		umol/L	-			22	G74	unprint	E	
HDL-C	0.64	1	0.772		mmol/L				23	G75	unprint		
K	6.76	Ť	3.55.	1	mmol/L			-	23	013	unprint	-	
LDL-C	0.00		03.35		mmol/L								
TBIL	5		220.5		umol/L				Reactio	n Curve			
TG	1.03		0.71.		mmol/L				î	·			
UREA	11.5	î	1.78.		mmol/L	_		- 11	3.000				
ALT Na	128.0 179	1	031 1351		U/L mmol/L				2.625				
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## **APPENDIX II: RESEARCH ETHICS COMMITTEE PERMISSION**



OFFICE OF THE DIRECTOR OF GRADUATE STUDIES AND RESEARCH UNIVERSITY OF EASTERN AFRICA, BARATON P.O. BOX 2500-30100, Eldoret, Kenya, East Africa

B1618032020

March 18, 2020

TO: Okuna Damaris Akinyi School of Science Moi University

Dear Damaris,

#### RE: Effects Of *Tithonia diversifoloa* Aqueous Root Extract On Blood Glucose And Serum Lipid Profiles In Western Diet Fed Wistar Albino Rats

This is to inform you that the Research Ethics Committee (REC) of the University of Eastern Africa Baraton has reviewed and approved your above research proposal. Your application approval number is UEAB/REC/16/03/2020. The approval period is 18<sup>th</sup> March, 2020 – 17<sup>th</sup> March, 2021.

This approval is subject to compliance with the following requirements;

- Only approved documents including (informed consents, study instruments, MTA) will be used.
- All changes including (amendments, deviations, and violations) are submitted for review and approval by the Research Ethics Committee (REC) of the University of Eastern Africa Baraton.
- iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to the Research Ethics Committee (REC) of the University of Eastern Africa Baraton within 72 hours of notification.
- Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to the Research Ethics Committee (REC) of the University of Eastern Africa Baraton within 72 hours.
- Clearance for export of biological specimens must be obtained from relevant institutions.
- Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
- Submission of an executive summary report within 90 days upon completion of the study to the Research Ethics Committee (REC) of the University of Eastern Africa Baraton.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) <u>https://oris.nacosti.go.ke</u> and also obtain other clearances needed.

Sincerely yours MAR ZUZ Prof. Jackie K. Obey, PhD Chairperson, Research Ethics Committee A SEVENTH-DAY ADVENTIST INSTITUTION OF WIGHER LEARNING CHARTERED 1991

# APPENDIX III: PERMISSION TO CONDUCT RESEARCH



OFFICE OF DEAN, SCHOOL OF PHARMACY.

P.O. Private Bag – 20157, Kabarak; Tel: 0777223375. Website: www.kabarak.ac.ke. Email: deanpharmacy@kabarak.ac.ke

23rd August 2021

Ms. Damaris Akinyi Department of Medical Biochemistry Moi University P.O. Box 3900, Eldoret – 30100

Dear Ms. Damaris Akinyi,

### SUBJECT: APPROVAL TO CONDUCT YOUR RESEARCH IN SCHOOL OF PHARMACY LABS

I am writing to acknowledge receipt of your request to conduct research titled "Hypoglycemic and Hypolipidemic activities of Aqueous Root Extract of Tithonia Diversifolia (Hemsley) A. Gray and its Biochemical Effects on Liver and Kidney Functions in Western Diet-Fed Wistar Albino Rats" within the School of Pharmacy labs.

We appreciate the thoroughness of your proposal and the efforts you have taken to secure ethical approval from the University of Eastern Africa, Baraton (IREC). The ethical approval number provided (UEAB/REC/16/03/2020) has been duly noted.

After careful consideration, I am pleased to inform you that your request has been approved. You are hereby granted permission to utilize the animal house and Biochemistry lab facilities at our school between the months of September and December 2021 for the aforementioned research project.

To ensure that all activities conducted within our facilities adhere strictly to the ethical protocols outlined in the approved proposal and the accompanying ethical approval letter, you will be assigned an internal supervisor to work with you on this research. Additionally, you are responsible for covering all expenses associated with your research. Report to our office for orientation on September 1<sup>st</sup>, 2021 at 9:00am.

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#### Kabarah University Moral Code

As members of Kabarak University family, we purpose at all times and in all places, to set apart in one's heart, Jesus as Lord. (1 Peter 3:15)



Kabarak University is ISO 9001:2015 Certified



OFFICE OF DEAN, SCHOOL OF PHARMACY.

P.O. Private Bag - 20157, Kabarak; Tel: 0777223375. Website: www.kabarak.ac.ke. Email: deanpharmacy@kabarak.ac.ke

Your commitment to advancing scientific knowledge is commendable, and we are pleased to support your academic pursuits. Should you require any further assistance or have any questions, please do not hesitate to contact us.

Thank you for choosing our institution for your research needs. We look forward to the successful execution of your project.



Dean, School of Pharmacy – Kabarak University. Phone Number: +254733448810 Email: sugetitus@kabarak.ac.ke

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Kabarak University Moral Code

At members of Kabarak, University family, we purpose at all times and in all places, to set apart in one's heart, Jesus as Lord. (1 Peter 3:15)



Kabarak University is ISO 9001:2015 Certified