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Benard K. Ngeno

Department of Chemistry and Biochemistry,
School of Science, University of Eldoret, P.O
Box 1125-30100, Eldoret, Kenya

Geoffrey K. Maiyoh

Department of Biochemistry and Clinical
Chemistry, School of Medicine, Moi University,
P.O Box 4606-30100, Eldoret, Kenya

Vivian C. Tuei

Department of Biochemistry and Clinical
Chemistry, School of Medicine, Moi University,
P.O Box 4606-30100, Eldoret, Kenya

Correspondence:

Dr. Vivian C. Tuei

Department of Biochemistry and
Clinical Chemistry, School of
Medicine, Moi University, P.O Box
4606-30100, Eldoret, Kenya
Email: vtuei@mu.ac.ke

Antidiabetic properties of *Tarhontanthus camphoratus* in fructose-induced diabetic Wistar rats

Benard K. Ngeno, Geoffrey K. Maiyoh, Vivian C. Tuei

Abstract

Tarhontanthus camphoratus (TC) has been used traditionally to manage diabetes mellitus (DM) in Kenya but its efficacy has not been scientifically evaluated. This study aimed at evaluating the antidiabetic properties of TC crude leaf extract in diet-induced diabetic Wistar rats. DM was induced using high fructose (25% w/v) in drinking water for 12 weeks. Rats were divided into five groups (n=7): Groups I: normal control; II: diabetic untreated; III, IV & V; diabetic treated (21 days) with metformin (100 mg/kg.bw/day), 300 and 600 mg/kg.bw/day of TC extract respectively. Fasting body weights and blood glucose levels were monitored weekly. Oral glucose tolerance test, serum lipid profile, creatinine, urea, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total proteins (TP), C-reactive protein (CRP), albumin (ALB) and triglyceride (TG) mass in skeletal muscle were analysed at end of the study. Qualitative phytochemical analysis was done using standard procedures. Diabetic untreated rats had significantly higher body weights ($p < 0.05$) compared to other groups. There was a significant reduction in fasting blood glucose in TC treatment groups compared to untreated controls. Increased glucose tolerance was observed in treated groups. TC extract significantly improved fructose-induced hypertriglyceridemia compared to DM groups. ALP, ALT, and CRP were significantly lowered while TP and ALB were elevated in the extract treated rats compared with untreated DM rats. DM group also exhibited significantly higher skeletal muscle TG mass when compared to normal control and diabetic treated groups. The phytochemical-rich TC leaf extract therefore possess potential alternative medicine for DM management.

Keywords: *Tarhontanthus camphoratus*, Rat, Diabetes Mellitus, Fructose diet.

INTRODUCTION

Diabetes mellitus (DM) or chronic hyperglycemia is a metabolic disorder that results from abnormalities in carbohydrate, fat and protein metabolism that occurs as a result of impaired insulin secretion, insulin action or both [1]. There are three types of diabetes mellitus namely; type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), and gestational diabetes. Particularly, T2DM is a progressive, life-long disease that results from body's ineffective use of insulin thus makes it difficult to maintain blood glucose levels within target range [2]. Elevated blood glucose levels can cause complications such as blindness, cardiovascular disease, renal failure, peripheral neuropathy, lower-extremity amputations and erectile dysfunction [3]. Consequently, there is a necessity for proper glycemic control among patients with T2DM so as to reduce the risk of diabetes-related microvascular complications. Fortunately, good diabetes care and management can prevent or retard the onset of these complications [4].

Both the prevalence and incidence of T2DM are increasing worldwide, particularly in developing countries, in conjunction with increased obesity rates and westernization of lifestyle and diet [5, 6]. In Kenya, the adult population nationally adjusted prevalence of diabetes mellitus was estimated to be 3.1% in 2019 and is projected to rise to 4.4% in 2035 if mitigations are not put in place to address this rise. More than 15,000 diabetes-related deaths were registered in Kenya in 2021 [7]. This rise in diabetes mellitus is associated with demographic and societal changes such as globalization, urbanization, aging population, adoption of sedentary lifestyles and consumption of unhealthy diets [8]. In fact, the World Health Organization (WHO) has predicted that the major burden of DM will occur in developing countries. Life expectancy may be halved by diabetes mellitus especially in lower middle-income countries (LMICs) where its prevalence is increasing and treatment is often unavailable. It is also projected that diabetes mellitus will be the seventh leading cause of death in the world by 2030 [9].

The attendant economic burden for health care systems is skyrocketing owing to the costs associated with low productivity, treatment and diabetes complications. Furthermore, T2DM remains a leading cause of cardiovascular disorders, blindness, end-stage renal failure, amputations, and hospitalizations. It is also associated with increased risk of cancer, serious psychiatric illness, cognitive decline, chronic liver disease, accelerated arthritis, and other deadly conditions [10]. Therefore, it is crystal clear that effective T2DM management strategies are of great importance.

Management of T1DM requires use of exogenous insulin whereas T2DM requires oral medication but may also need insulin, blood pressure control and foot care at later stage [11]. There are three main classes of oral hypoglycemic drugs; sulphonylureas (e.g., glimepiride), biguanides (e.g., metformin), and dipeptidyl peptidase-4 inhibitors (DPP-4 Inhibitors) e.g., vildagliptin [12]. Other classes are sodium-glucose cotransporters-2 (SGLT2) inhibitors, glucagon-like peptide 1 receptor agonists (GLP-1RA), glinides, thiazolidinediones and α -glucosidase inhibitors [13]. These diabetes drugs have numerous side effects, are often inaccessible or too costly and insulin for example needs a cold chain for preservation.

On the other hand, herbal plants have been used by traditional health practitioners for management of diabetes mellitus. Plants with antidiabetic properties are important for the development of economically viable and effective treatment of the disease. One such plant with promising potential for use in the treatment of diabetes mellitus is *Tarchonanthus camphoratus* (TC). TC is a plant of uplands in Kenya mostly growing in natural environment of altitudes ranging from 1,000 to 3,000 meters above the sea level. It's also widely distributed in a variety of habitats, including thickets of Masai Mara, grassland, forest and semi-desert in Kenya. Traditional health practitioners from the Kipsigis community in Kenya use TC for management of diabetes mellitus [14]. TC is known to contain many compounds including saponins, flavanols and tannins [15] that are potentially hypoglycemic. Previous in vitro studies of aqueous and ethanolic extracts of TC on Chang liver cells and C2C12 muscle cells respectively showed effectiveness in glucose utilization in concentration-independent trends [16]. Despite this good anti-diabetic potential, the anti-diabetic efficacy of TC aqueous extracts has not been shown. This study was thus designed to determine the antidiabetic effects and efficacy of TC crude leaf extracts in fructose-induced diabetic Wistar rats.

MATERIALS AND METHODS

Ethical considerations

The experimental protocols were approved by the Research Ethics Committee of University of Eastern Africa, Baraton (Reference; REC: UEAB/6/3/2017). The research was conducted in compliance with the ARRIVE guidelines 2019 and in accordance with the UK Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments and the National Institutes of Health (NIH) guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

Collection and identification of plant material

Leaves of TC were harvested in December 2021 at Longisa area in Bomet County, Kenya (Latitude/Longitude (Dec): -0.8667° S, 35.3833° E). The leaves were then packed and transported to University of Eldoret. Sample plant material was identified and authenticated by Mr. Dennis Onyango a qualified taxonomist at University of Eldoret and a voucher number MUH/TORC/013/1987 was assigned and the plant specimen kept in the herbarium of Department of Biological Sciences at University of Eldoret. The leaves were then dried at room temperature away from direct sunlight.

Preparation of plant crude leaf extract

The dried leaves were ground into fine powder using an electric miller (Disk Mill FFC-23, China). 400 g of dried powder of TC plant were subjected to maceration in 4 litres of distilled water for 72 hours in a ratio

of 1:10 (w/v). The organic extracts were then filtered using Whatmann No. 1 filter paper and the filtrate was then freeze-dried (LSL Secfroid SR, Model 3021, Switzerland) for 24 hours as described by [17]. The paste obtained was put in a clean glass bottle and stored at -20° C until required for use. The dried extracts were freshly dissolved and made up to the appropriate volume with distilled water just before use on each day of the experiment.

Qualitative phytochemical analysis of leaf extract

Qualitative phytochemical screening of TC leaf extracts was carried out to determine the presence or absence of saponins, tannins, flavonoids, phenolic compounds, alkaloids, cardiac glycosides, terpenoids and steroids using standard procedures previously described by [18].

Experimental animals

Thirty-five, 6- to 8-week-old male Wistar rats (*Rattus norvegicus*) of similar weights (mean weight variation not exceeding ± 10 g) were obtained from the animal house of the Biology Department, Chiromo Campus, University of Nairobi, Kenya. The Wistar rats were transported to University of Eldoret animal facility where they were kept in cages and allowed to acclimatize for 1 week before experimentation. During this acclimatization period, the rats were fed with commercial formulated rat feed (regular rodent chow) obtained from Unga Farmcare, East Africa Limited, Nakuru, Kenya and water ad libitum. Also, the Wistar rats were exposed to environmental temperature ($25 \pm 2^\circ\text{C}$), 40-60% room humidity and natural day and night cycles.

Study diet and induction of type 2 diabetes mellitus

After 2 weeks of acclimatization, all the Wistar rats were labeled then their baseline fasting body weights were taken. Blood glucose levels were measured with a glucometer (On-Call Plus, Acon Laboratories, San Diego USA) using whole blood drawn from the tail vein following 14 hours overnight fast. The rats were then distributed into five groups (n=35) with similar baseline mean blood glucose values; One (1) of this groups were then randomly selected to represent the normal control group (n=7). The remaining (n=28) were randomly distributed to form the experimental groups. The normal group (normal control) was fed with normal rodent chow and allowed free access to drinking water while the experimental group was fed a high fructose diet (HFD) consisting of standard rodent chow supplemented with 25% fructose (w/v) in drinking water for twelve weeks to induce T2DM [19]. At the end of the 12 weeks feeding with HFD, fasting blood glucose and weights were measured.

Experimental design

The minimum number of animals used in the study was determined according to Arifin and Zahiruddin [20].

The animals were placed into five groups (n=7) as follows:

- I) Normal control group: received oral gavage of distilled water at 100 μ L daily.
- II) Diabetic untreated group: fed on high fructose diet (HFD) in drinking water.
- III) Diabetic + 100 mg/kg.bw metformin group: fed HFD and treated with metformin orally (100 mg/kg.bw).
- IV) Diabetic + 300 mg/kg.bw extract group: fed on HFD and treated with TC orally (300 mg/kg.bw)
- V) Diabetic + 600 mg/kg.bw extract: fed on HFD and treated with TC orally (600 mg/kg.bw).

Animal treatment

Diets were provided for 15 weeks (12 weeks of T2DM induction followed by 3 weeks of treatment). During the entire treatment period, the rats were

observed daily for clinical signs of toxicity on behavioral changes, morbidity and mortality. Fructose at 25% (w/v) in drinking water was prepared daily by diluting 25 g of fructose with tap water to make 100 mL fructose solution in bottles; 500 mL of water or fructose solution were provided per cage. Aluminum foil was used to cover the bottles to avoid fructose fermentation. The specified doses of TC aqueous extracts and metformin at 100 mg/kg.bw as described in the experimental design were administered at 0800hrs orally once daily for 21 days post confirmation of diabetes status [21]. Fasting blood sugar and body weights were determined at baseline and weekly. The weekly body weights were used to adjust treatment dosages accordingly to maintain the prescribed doses per body weights as depicted above in the experimental design.

Oral glucose tolerance test (OGTT)

OGTT was performed at the end of the treatment period (day 21) following 14 hours overnight fasting of the rats. 200 grams of glucose was dissolved in warm distilled water to make 1 litre of glucose solution and the rats were given 2 g glucose per kg.bw orally [19]. Then appropriate amount of TC extract (300 mg/kg.bw and 600 mg/kg.bw), oral gavage of 100 µL distilled water to healthy control and standard drug (100 mg/kg.bw) was then administered. The tail of the rats was sterilized using surgical spirit and blood was drawn from the tail vein before (0 min) and after 30, 60, 90, and 120 minutes after administration of glucose solution. Blood glucose levels were measured using glucometer as earlier described and the results were recorded. Glucose tolerance was determined by plotting mean blood glucose values against time for each group.

Animal sacrifice, serum parameters analyses and tissue processing

At the end of the study and a day after performing OGTT, all the rats were fasted overnight (14 hours) and body weights measured then euthanized under mild anesthesia of chloroform. The rats were then mounted on dissection bench and dissected using dissecting kit. Blood was collected through cardiac puncture and put in clot activator vacutainer blood collection tubes. Skeletal muscle was carefully removed from the hind limb, washed with ice cold phosphate buffered saline (PBS), weighed using analytical balance (AUW220 Shimadzu corporation, Tokyo, Japan) and then immediately stored at -20°C for biochemical assays. The relative tissue weight (ROW) was calculated and recorded in proportion to the body weight according to the following equation:

$$\text{Relative organ weight (ROW)} = \frac{\text{Absolute organ weight}}{\text{Body weight of rat}} \times 100$$

On the day of sacrifice, serum was obtained by centrifugation of whole blood collected in plain bottles at 3,000 x g for 20 minutes. Immediately after centrifugation, the serum samples were placed in ice and kept at 4°C. Fasting blood glucose, total cholesterol (T.CHOL), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), and triglycerides (TGs) were analyzed in serum. For renal function test, serum creatinine and urea were analyzed and for liver function indices, serum alkaline phosphatase (ALP), aspartate

aminotransferase (AST) and alanine aminotransferase (ALT), total proteins (TP), C-reactive protein (CRP) and albumin (ALB) were also analyzed. These tests were done using COBAS INTEGRA 400 plus auto-analyzer (Roche Diagnostic, Mannheim, Germany) at Moi Teaching and Referral Hospital laboratory, Eldoret, Kenya following manufacturer's instructions.

Analysis of triglyceride mass in skeletal muscle tissue

Skeletal TG mass was measured as described by [22]. The frozen samples of skeletal muscle were thawed at room temperature for 1 hour until completely thawed. 100 mg of frozen tissue was minced and homogenized in 2 mL of sucrose buffer (0.3 mol/L sucrose, 25 nmol/L 2-mercaptoethanol, and 10 mmol/L EDTA, pH 7.0). 200 µL aliquot of the suspension was transferred into 5 mL glass test tubes. 800 µL of chloroform was then added to each tube, mixed and allowed to stand for 30 min at room temperature. 250 µL of chloroform was added and followed immediately by addition of 250 µL of 0.15M NaCl and allowed to stand for 1 hour at room temperature. The mixture was then centrifuged at 1000rpm for 15 min. The lower organic phase was collected into clean labeled 5 mL tubes. The aqueous phase was then washed with 800 µL of chloroform to recover the lipids and centrifuged as above. The lower organic phase was collected and added to that separated from previous step. The combined organic phases were dried under a vacuum, and the lipids resuspended in 100 µL of 95% ethanol. 20 µL of the suspension were used for TG levels determination with a kit according to the manufacturer's instructions (Triglycerides GPO-PAP Method, Beacon Diagnostics Pvt. Ltd, Navsari, India). The rose-colored dye produced during oxidative condensation of 4-Chlorophenol and 4-Aminophenazone (4 AAP) was measured using spectrophotometer (Wagtech DU® 720, California, USA) at 550 nm. TG measurements were then normalized to the weight of each tissue (milligrams of TG per gram of tissue).

Statistical analysis

The data was entered into Microsoft Office Excel and transferred to R software for statistical analysis. Quantitative data were expressed as mean ± standard error mean (SEM). Statistical data analysis was by Tukey's test and analysis of variance (ANOVA). Values with p < 0.05 were considered to be statistically significant.

RESULTS

Qualitative phytochemical evaluation of TC crude aqueous leaf extract

The qualitative phytochemical analysis of the TC crude aqueous leaf extract revealed the presence of flavonoids, saponins, phenolic compounds, terpenoids, tannins and steroids and absence of alkaloids and cardiac glycosides (Table 1).

Table 1: Phytochemical constituents of aqueous *Tarchonanthus camphoratus* leaf extract.

Phytochemicals	Presence (+) or absence (-)
Saponins	+
Tannins	+
Flavonoids	+
Terpenoids	+
Phenolic compounds	+
Steroids	+
Alkaloids	-
Cardiac glycosides	-

Clinical physical observations and body weight changes of rats on TC leaf extract treatment

The physical observation of diabetic induced animals showed a reduction in physical activity than the normal control. Transient clinical signs that was most pronounced after dosing of rats and that lasted for about 30 minutes included raised fur and rubbing of oral cavity indicating irritation whenever the treatments were administered. Figure 1 shows that body weight gain was similar in all diabetic rats over the 12-week fructose supplementation period ($p > 0.05$ versus initial weight, $n=7$). Over the treatment period the diabetic untreated group had significantly higher body weights compared to all other groups. Rats in the normal control group showed a significant lower mean body weights from day 0 to 21st day compared to all the treatment groups ($p < 0.05$). The mean body weights in all the groups except diabetic + 600 mg/kg extract showed insignificant declining trend (7.8%) and had an overall trend of increase across the study period; however, the diabetic untreated group showed significant ($p < 0.05$) higher mean body weights (9.6% increase) compared to diabetic + 300 mg/kg extract treated and normal control groups (1.07% and 2.5% increases respectively).

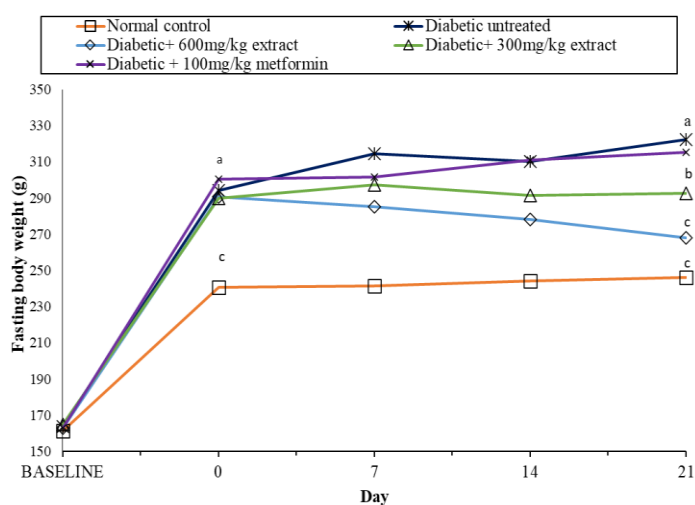


Figure 1: Effects of *T. camphoratus* on fasting body weights

Aqueous leaf extracts at 300 mg/kg.bw and 600 mg/kg.bw on body weights of rats. Values represents the mean \pm SEM; $n=7$. Superscript ^{a, b, c, d} significantly different between the diabetic untreated and the normal control ($p < 0.05$, ANOVA).

Effect of TC leaf extract on fasting blood glucose of rats

Before treatment (day 0), all the rats in the diabetic induced group had significantly higher levels of fasting blood glucose when compared to normal control ($p < 0.05$) as shown in Figure 2. Diabetic untreated group had higher fasting blood glucose levels than normal control rats (6.30 ± 0.28 vs. 3.76 ± 0.28 mmol/L, $p < 0.05$). However, treatment with 300 and 600 mg/kg.bw of TC leaf extract, and 100 mg/kg.bw of metformin for 21 days, resulted in significant reduction in mean fasting blood glucose levels when compared with diabetic untreated group ($p < 0.05$). Metformin 100 mg/kg.bw treatment resulted in maximum reduction of 48.4% in fasting blood glucose, while 600 mg/kg.bw and 300 mg/kg.bw leaf extract treated groups exhibited a fall of 33.9% and 27.3% respectively (Figure 2).

Effect of TC leaf extracts on oral glucose tolerance test (OGTT) in rats

OGTT was performed at day 21 of treatment period. OGTT results (Figure 3) showed that compared to normal control rats, blood glucose levels of diabetic untreated group peaked at the 30th min and had a falling trend after the 60th min after ingestion of high dose of glucose (30 min: 8.67 ± 0.42 mmol/L vs. 7.83 ± 0.42 mmol/L; 120 min: 5.77 ± 0.42 vs. 4.40 ± 0.42 mmol/L, both $p < 0.05$). Blood glucose for diabetic untreated group remained high at 120th min compared to normal control and other treatments and was highly significant ($p < 0.05$). The blood glucose level in the normal control rats rose to the peak at 30th min after glucose load

and decreased to near normal levels at 120th min. Untreated diabetic rats peak increase in blood glucose concentration was observed after 30th min and remained high over the next 90 minutes.

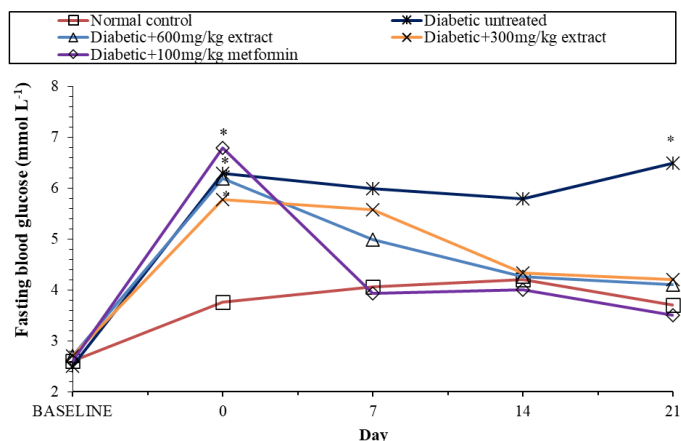


Figure 2: Effects of *T. camphoratus* on fasting blood glucose

Aqueous leaf extracts at 300 mg/kg.bw and 600 mg/kg.bw on fasting blood glucose of rats. Values represents the mean \pm SEM; $n=7$. *Significantly different as compared to normal rats ($p < 0.05$).

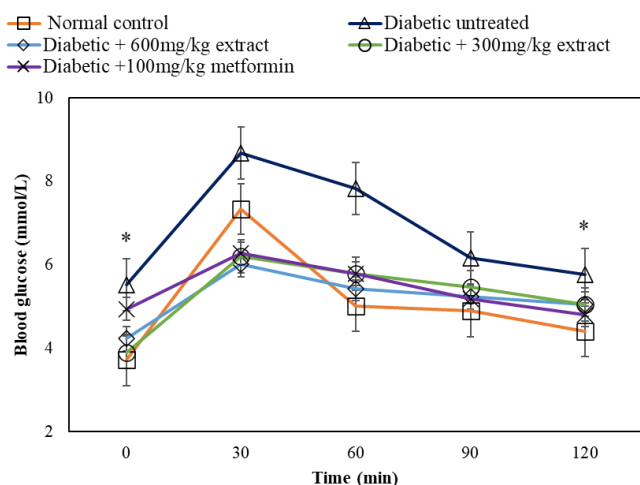


Figure 3: Effects of *T. camphoratus* on glucose tolerance

Aqueous leaf extract on oral glucose tolerance test in rats. Data is expressed as the mean \pm SEM ($n=7$). *Significantly different in diabetic untreated group compared to normal and treated groups ($p < 0.05$).

Effect of TC leaf extract on serum lipid profile and indices of liver and kidney function of rats

As shown in Table 2, diabetic untreated rats exhibited a statistically significant ($p < 0.05$) elevated serum levels of ALT, ALP, and CRP as well as reduced TP and albumin levels when compared with the normal control and all other treatment groups. AST was insignificantly changed in all treatment groups compared to normal control rats. A significant increase in TP levels was recorded in diabetic rats treated with 100 mg/kg.bw metformin compared with normal control rats ($p < 0.05$). The normal control and diabetic rats treated with 600 mg/kg.bw TC extract and 100 mg/kg.bw metformin respectively recorded significant higher albumin levels than diabetic untreated rats. The levels of ALT and ALP were significantly reduced when diabetic rats were treated with 300 and 600 mg/kg.bw of TC extract and 100 mg/kg.bw of metformin as compared with diabetic untreated rats. There was no significant change in all serum kidney function indices (urea and creatinine) examined in all treatment groups when compared with respective normal controls. There were no significant alterations in levels of serum T.CHOL, LDL-C and HDL-C in treatment and control groups. However, there was a significant elevation ($p < 0.05$) in serum triglycerides levels in diabetic untreated rats when compared with normal control, TC 300 mg/kg.bw, TC 600 mg/kg.bw group and 100 mg/kg.bw metformin treated groups. Aqueous extract of TC significantly reduced ($p < 0.05$) the levels of serum

triglycerides in diabetic groups to near normalcy comparable to the values observed in normal control after 21 days of treatment.

Table 2: Effect of *Tarhonoranthus camphoratus* leaf extracts on serum lipid profile and indices of liver and kidney function of rats.

Parameter	Sex (M-Male)	Normal Control	Diabetic untreated	Diabetic+300mg/kg TC extract	Diabetic +600mg/kg TC extract	Diabetic+100mg/kg metformin
ALT (U/L)	M (n=7)	64±4.53	92.1±4.53*	56.6±5.36 [#]	64.2±6.00 [#]	60±4.90 [#]
ALP (U/L)	M (n=7)	95.9±25.93	206.2±21.24*	106.9±23.20 [#]	121.5±25.91	95.7±21.2 [#]
AST (U/L)	M (n=7)	128±15.92	147±15.90	112±18.80	124±21.00	115±17.20
CRP (mg/L)	M (n=7)	0.12±0.05	0.40±0.05*	0.60±0.06*	0.60±0.06*	0.60±0.05*
TP (g/L)	M (n=7)	65.4±0.87	65.9±0.87	67.2±1.03	68.8±1.15	69.2±0.87*
Albumin (g/L)	M (n=7)	37.7±1.02	33.3±1.02*	37.5±1.2	39.6±1.35 [#]	40.1±1.10 [#]
Creatinine (µmol/L)	M (n=7)	35.2±1.71	36.3±1.71	30.4±2.03	29.2±2.27	33.2±1.85
Urea (mmol/L)	M (n=7)	5.25±0.58	7.45±0.58	5.2±0.58	5.08±0.58	5.78±0.58
LDL-C (mmol/L)	M (n=7)	0.26±0.03	0.30±0.03	0.29±0.03	0.26±0.04	0.28±0.03
HDL-C (mmol/L)	M (n=7)	0.77±0.05	0.75±0.04	0.79±0.05	0.83±0.05	0.91±0.05
TG (mmol/L)	M (n=7)	0.55±0.04	0.82±0.04*	0.51±0.04 [#]	0.49±0.04 [#]	0.44±0.04 [#]
T.CHOL (mmol/L)	M (n=7)	1.13±0.06	1.33±0.06	1.29±0.06	1.22±0.07	1.16±0.06

Values represent mean ± SEM; n=7. *Significant difference compared to normal control, [#]Significant difference compared with diabetic untreated group ($p < 0.05$, ANOVA). ALP (alkaline phosphatase), ALT (alanine aminotransferase), AST (aspartate aminotransferase), CRP (C-reactive protein), TP (total protein), LDL-C (low density lipoprotein cholesterol), HDL-C (high density lipoprotein cholesterol), TGs (triglycerides), T.CHOL (total cholesterol) and TC (*Tarhonoranthus camphoratus*).

Table 3: Effect of *Tarhonoranthus camphoratus* leaf extract on relative weights and triglyceride mass of skeletal muscle of rats.

Treatment group	Sex (M-male)	TGM (mg/g)	RTW (%)
Normal control	M (n=7)	1.69±0.15	0.46±0.01
Diabetic untreated	M (n=7)	3.37±0.15*	0.60±0.09
Diabetic + 300 mg/kg TC	M (n=7)	2.35±0.20 [#]	0.45±0.01
Diabetic + 600 mg/kg TC	M (n=7)	2.22±0.18 [#]	0.39±0.01
Diabetic + metformin (100 mg/kg)	M (n=7)	2.12±0.16 [#]	0.49±0.00

Values are expressed as mean ± SEM. M, male; TGM, triglyceride mass (mg/g); RTW, relative tissue weight; TC, *Tarhonoranthus camphoratus*. *Significant difference compared to normal control, [#] Significant difference compared to diabetic untreated group ($p < 0.05$).

Effect of TC leaf extract on relative weights and triglyceride mass (TGM) of skeletal muscle of rats

Diabetic untreated group exhibited a significant ($p < 0.05$) increase in TGM compared to normal control and treated groups (Table 3). For relative skeletal muscle weight, we found insignificant difference among treated groups although diabetic untreated group had increased skeletal tissue weight compared with normal control group (Table 3).

DISCUSSION

Medicinal plants have been explored for their potential in management and prevention of DM. The present study sought to determine the phytochemical components and antidiabetic properties of *Tarhonoranthus camphoratus* (TC) leaf extract. Results revealed the presence of saponins, flavanoids, terpenoids, phenolic compounds and steroids, and absence of alkaloids and cardiac glycosides. Different approaches have been used to induce diabetes in experimental animals. In this study, Diabetes was induced using 25% (w/v) fructose in drinking water for 12 weeks.

In this study, significant weight gain was observed in diabetic untreated rats and this was attributed to increased energy intake due to fructose supplementation. A fructose-rich diet increases abdominal adipocyte

mass, impairs insulin sensitivity and may also suppress food intake [23]. Similar results were observed by [24] who demonstrated that high fructose diet promotes weight gain in rodents through the positive energy balance associated with high concentration of free fatty acids which diminish insulin sensitivity and thus increase blood glucose level [25]. Increase in mean body weights was observed in all the groups except diabetic + 600 mg/kg.bw TC extract across the study period; however, diabetic untreated group showed significantly higher body weights (9.6% increase) compared to diabetic + 300 mg/kg.bw TC and normal control groups (1.07% and 2.5% increase respectively). Declining trend in body weights of rats observed in diabetic + 600 mg/kg.bw TC-treated rats could be attributed to extract's effects of increasing glucose utilization hence saving the body fat and muscle protein which contrarily are utilized in diabetic rats [26]. Diabetic rats treated with metformin and TC extract did not undergo extreme body weight changes compared to untreated rats. TC extract at 600 mg/kg.bw was more effective in improving body weights compared to 300 mg/kg.bw TC and metformin (100 mg/kg.bw).

TC extract at 300 and 600 mg/kg.bw significantly reduced fasting blood glucose levels in a dose dependent manner (27.3% and 33.9% respectively) and metformin treated group resulted in maximum reduction (48.4%). The hypoglycemic effect of TC aqueous extract might be due to the present phytochemical compounds [27]. For instance,

flavonoids have been shown to have insulin-like effects, cause lipogenesis and glucose transport in adipocytes thus reduction of blood glucose while terpenoids reduces diastolic blood pressure and lower blood glucose [28]. Based on our findings, we hypothesize that the reduction of blood sugar levels either relates to effects of TC extract on the activity of pancreatic β cells to produce insulin or prevention of absorption of glucose from the intestine or may enhance the glucose uptake in peripheral tissues, and/or increase hepatic glycogenesis. These actions may be responsible for amelioration and/or management of DM and its complications [29].

Oral Glucose Tolerance Test (OGTT) measures the body ability to utilize glucose as the main source of energy. OGTT was performed to assess the efficacy of antidiabetic effects of TC extract. Increased fasting glycemia due to fructose supplementation is associated with reduced glucose tolerance. During glucose tolerance testing, diabetic untreated group had higher plasma glucose levels compared to other groups. This could be attributed to establishment of insulin resistance. Previous findings by [30] revealed that fructose feeding leads to glucose intolerance. Glucose lowering effects of TC extract treated groups were comparable to those of metformin at 100 mg/kg.bw. This could be indicative that TC extracts promotes glucose tolerance by either stimulation or regeneration of pancreatic β cells.

On the other hand, there were no significant alterations in levels of serum T.CHOL, LDL-C and HDL-C in the treatment and control groups suggesting that TC leaf extract did not affect these parameters. However, there was a significant elevation in serum levels of TG in diabetic untreated rats compared with normal control and treated groups. Dyslipidemia is a common characteristic observed in T2DM marked by serum hypertriglyceridemia, increased LDL-C and reduced levels of HDL-C and is also directly linked to insulin resistance [31]. Results from this study, therefore suggests fructose successfully induced hypertriglyceridemia through either liver injury marked with an increase in lipogenesis or impaired carbohydrate metabolism that resulted in insulin resistance and augmented TG serum levels [32]. TC leaf extract reduced serum TG levels in diabetic treated groups to near normalcy comparable to the values observed in normal control group. This finding can be attributed to extract's prevention effects of triglycerides elevation [16].

Skeletal muscle is a major site of insulin-mediated glucose disposal and is closely related to insulin resistance. Increased fructose intake impairs glucose utilization, normal lipid and carbohydrate metabolism leading to non-enzymatic glycation reaction resulting to enhanced accumulation of advanced glycation end products in skeletal muscle hence impaired glucose homeostasis [33]. Significant increase in TG flow to skeletal muscle was observed in diabetic untreated rats and a significant increase in relative skeletal muscle weight compared to normal control. These findings suggest that high-fructose diet even without polyphagia is able to increase lipid flow to skeletal muscle and mitochondrial energetic efficiency, with two hypothesized deleterious effects: (i) energy sparing that contributes to the early onset of obesity and (ii) reduced oxidation of fatty acids and lipid accumulation in skeletal muscle, which could generate insulin resistance [34]. The TC extract treated groups exhibited significant decrease in relative skeletal muscle weight compared with the diabetic untreated rats; this might have been brought about by the bioactive components present in TC leaf extracts that prevents adiposity and alleviate insulin resistance. These findings are in agreement with [35] who reported decreased tissue weights observed with increasing amounts of tannins, phenols and flavonoids. In our study, the lowest TG mass level and tissue weight gain was observed in diabetic rats receiving 600 mg/kg TC extract.

Liver dysfunction cause changes in its biochemical enzyme indices that determined the extent of hepatocyte injury. DM is one of the metabolic syndromes associated with elevation of these liver marker enzymes [36]. Diabetic untreated rats exhibited significantly elevated serum levels of ALP, ALT and CRP and reduction in total proteins and albumin levels; that could be as a result of damaged hepatocytes due to fructose-induced hyperglycemia [37]. CRP is thought to be a useful marker during an acute inflammatory assault. Increased CRP levels can be used to assess liver

damage brought on by chronic inflammation [38]. There was significant reduction of ALT, ALP levels in TC extract and metformin treated rats compared to diabetic untreated rats. This may be an indication of nontoxic nature and protective action of the extract in reversing liver damage due to DM. Significant increase in TP levels was recorded in the diabetic rats treated with 100 mg/kg.bw metformin compared with normal control rats. The normal control, TC treated rats and metformin groups recorded significantly higher albumin levels than diabetic untreated rats. Therefore, TC extract administration to the rats restored the albumin and TP levels to normalcy. This further confirmed bestowment of protection of the liver of diabetic rats by TC bioactive components. On the other hand, renal function was not affected as evident by insignificant change of serum creatinine and urea. Further studies on detailed mechanistic anti-diabetic properties of the crude leaf extract such as on gene expression studies and in vivo anti-oxidant properties can provide more information as this was not explored in the scope of this study.

CONCLUSION

In conclusion, *Tarconanthus camphoratus* (TC) aqueous leaf extract is rich in phytochemicals that proved to possess health benefits, including a role in the treatment of hyperlipidemia and diabetes mellitus management in diabetic rats. The 3-week oral administration of TC leaf extract in diabetic rats exhibited significant amelioration of body weights and blood glucose levels. TC leaf extracts promoted glucose tolerance and also the results revealed significantly reduced serum ALT, ALP, CRP and triglycerides levels in diabetic rats and this may be indicative of the nontoxic nature of the extract hence hepatoprotective properties and improvement in the imbalance in lipid metabolism experienced during diabetes mellitus. Further, in this diabetic rat model, we provided evidence that the 15-week high-fructose diet supplementation and 300 mg/kg.bw and 600 mg/kg.bw TC extract treatment did not compromise the renal functions. TC crude leaf extract therefore possess potential alternative medicine for diabetes mellitus management and treatment.

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Declaration of competing interest

The authors declare no conflict of interests.

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