

2020-12-31

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<https://doi.org/10.9734/ijtdh/2020/v41i2230408>

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Hypoglycemic Effects of *Cymbopogon citratus* Ethanol Leaves' Extract and Its Fractions in Alloxan-Induced Diabetic Mice

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Authors' contributions

This work was carried out in collaboration among all authors. Author JT designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors HDM and ES managed the analyses of the study. Author ES managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJTDH/2020/v41i2230408

Editor(s):

(1) Dr. Zhiheng Zhou, Guangzhou University, China, Harvard Medical School, USA.

Reviewers:

(1) Adedayo Michael Oyetayo, Rufus Giwa Polytechnic, Nigeria.

(2) Jhon Carlos Castaño-Osorio, Universidad del Quindío, Colombia.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/63880>

Original Research Article

Received 10 October 2020

Accepted 18 December 2020

Published 31 December 2020

ABSTRACT

Varieties of plants, including *Cymbopogon citratus*, are traditionally used in controlling hyperglycemia by either stimulating insulin secretion, inhibition α -Glucosidase or α -amylase activity. This study evaluated hypoglycemic effects of *Cymbopogon citratus* ethanol leaves' extract and its fractions in alloxan-induced diabetic mice. *Cymbopogon citratus* leaves were shade dried, grinded into fine powder and then extracted by cold maceration using ethanol. Fractionation was done by VLC using dichloromethane, ethyl acetate and ethanol. OGTT was performed for both crude extract and fractions. Diabetes was induced in mice by intraperitoneal injection of freshly prepared alloxan monohydrate (170 mg/kilogram body weight). The mice were treated with ethyl acetate fraction once daily at 400 mg/kilogram body weight dose for the period of 20 days. FBG and weight were then recorded in days 1, 5, 10, 15 and 20 after six hours of fasting. Safety of crude water extract and ethyl acetate fractions were evaluated in mice by using Lorke's method, followed

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by 5 days observation for their mortality and behavioral changes. Comparisons of results among groups were analyzed using One-way ANOVA. The difference between the means of the two population groups (each against negative control) was considered significant at $p < 0.05$. Results were expressed as mean \pm SD. Both crude and ethyl acetate fractions from *C. citratus* showed significant hypoglycemic activity. Moreover, higher hypoglycemic activity was shown by ethyl acetate fraction ($p = 0.004$). No mortality was observed at 5000 mg/kilogram body weight dose but sleeping and tremor were observed at a 1000 -5000 mg/kilogram body weight dose. Good hypoglycemic and safety results from ethyl acetate fraction highly suggest that *Cymbopogon citratus* extracts are effective against insulin-dependent hyperglycemia, which may be contributed by the action of screened alkaloids, saponins, anthraquinone, phenol and tannins. Isolation and testing of the active ingredients from the *C. citratus* extract are thus warranted for use in developing pharmaceutical anti-hyperglycemic drugs from this herbal plant.

Keywords: *Cymbopogon citratus*; hyperglycemia; diabetic mice; oral glucose tolerance test; bioactive compounds.

1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder of compound etiology delineated by prolonged hyperglycemia that is concomitant with absolute or relative inadequacy in insulin secretion or function [1]. It is the third and fastest growing disease in the world, subsequent to cardiovascular and oncological disorders according to World Health Organization [2]. Based the global estimate on diabetes prevalence, there were 371 million people with diabetes worldwide in 2012 and it is projected that the number will rise to 552 million people by 2030 [3,4]. Furthermore, Tanzania National Survey in 2012 projected an increase in diabetic population from 3.6% to 9.1% of the total adult population from 1980 to 2014 [5].

According to the American Diabetes Association (2014), the overwhelming majority of diabetes cases falls under two broad etiopathogenetic categories namely [6]: Type 1 diabetes (insulin-dependent diabetes or juvenile-onset diabetes) which is caused by an absolute deficiency of insulin secretion resulting from a cellular-mediated autoimmune destruction of pancreatic β -cells and Type II diabetes (non-insulin-dependent or adult-onset diabetes) which is characterized by insulin resistance which then causes insulin deficiency [7] and accounts for 80-90% diabetes cases [8]. The risk factors for type II diabetes (T2D) are unhealthy diet, tobacco use, excessive alcohol intake, overweight, obesity and physical inactivity [9]. Oral consumption of plant-based diets as well as the use of chemical and biochemical agents are currently used to control hyperglycemia [10]. Despite the current interventions the number of people with diabetes has been

observed to increase rapidly worldwide, hence making it a global health concern [11].

Cymbopogon citratus is an herbal plant within the Poaceae family commonly called lemon grass [12]. Lemon grass is utilized traditionally in Tanzania for reducing medical conditions like hyperlipidemia, hypercholesteremia and hyperglycemia which might lead to metabolic disorders like obesity and diabetes [13]. The anti-hyperglycemic effects of *C. citratus* is due to high amount of alkaloids, saponins, tannins, anthraquinones, steroids, phenols and flavonoids phytochemicals; each of which may have protective and therapeutic effect in controlling hyperglycemia [12]. In line with Ademuyiwa et. al, oral dosing of crude ethanolic and water extract at a dose of 200 mg/Kilogrambody weightin 30 days cause significance reduction in blood glucose in albino rats [14]. However, other previous studies reported daily oral dosing of 125–500 mg/Kilogrambody weightof fresh leaf aqueous extract of *C. citratus* in 28-42 days reduced hyperglycemia [15,16].

Ethno medical information has shown that theconsumption of *Cymbopogon citratus* leaves may be used in controlling diabetes in human [13,17,18]. However, scientific studies regarding its use, bioactive compounds in fractions and safety have not well documented. Moreover, the efficacy of *Cymbopogon citratus* formulation in treating diabetes is not well known. Therefore, this calls for scientific studies to validate the active fractions and phytochemicals claimed to be present in *C. citratus* plants, which may then be used to develop hypoglycemic drugs. The proposed study focused on evaluating the efficacy, phytochemical screening and safety of *C. citratus* fractionated extracts in controlling hyperglycemia in diabetic mice. The

aim was to establish baseline bioactive compounds from *C. citratus* for drug development and effective ways of using the studied plant.

2. METHODS

2.1 Materials, Chemicals and Reagents

Cymbopogon citratus leaves were collected from cultivated farms at Mnazi village in Korogwe District in Tanga Region in the North Eastern highlands of Tanzania by the permission of farmers. The plant was identified with voucher No. JT/01 by Emmanuel Mboya (Field Botanist/Herbarium Technician) at Tropical Pesticides Research Institute (TPRI) Arusha-Tanzania. The plant leaves were shade dried for three weeks then grinded into fine powder. The following materials and equipment were used in this study; glucometer and testing strips (GlucoPlus Inc., Canada), 1 ml syringes, gloves, masks, aluminum foil, normal saline, cotton wools, percolator, distilled water, CMC, 1% Tween 80, Methylated spirits, Ethyl acetate, Dichloromethane, Chlorpropamide tablets (Dibonis®, Cosmos Ltd, Nairobi, Kenya), 95% Ethanol (Tanzania Oxygen Ltd), Sodium pentobarbitone and Hydrochloric Acid (MERCK KILOGRAMaA group, Darmstadt, Germany).

2.2 Extraction and Fractionation of Lemon Grass (*Cymbopogon citratus*) Extract

Cymbopogon citratus powder (1700 g weight) was dissolved in 7000 ml of 95% ethanol in a percolator for 24 hours and thereafter filtered using cotton wool followed by Wolfman filter paper No.1. 50°C temperature was used to dry the extracts in a rotary evaporator to reduce phytochemical decomposition then allowed to air dry for 36 hours. Fractionation was done by using VLC, where by 30g of unextracted ethanol extract was adsorbed with 40g of silica gel then crammed in a column. Solvents were poured from DCM, EtOAc and EtOH, and then eluted by using a vacuum pump.

2.3 Extraction of *C. citratus* Water Extract

100 g of *C. citratus* powder was poured in 1000 ml of boiled water, mixed well then cooled. The filtrate was filtered with cotton wool then Watman paper No.1 followed by freeze drying to obtain dry sample [15].

2.4 Animal Selection and Identification

Albino mice were obtained from Muhimbili School of Health and Allied Science (MUHAS) at Pharmacognosy Department. Male and female albino mice of 8 to 12 weeks old weigh 20-32 gramms were selected in study. They were fed with food pellets and water and then exposed to light for 12 hours a day and 12hours of darkness at a night. Acclimization was done for 4 days before the experiment for all mice, then randomly allocated as experimental and control groups. Identification of mice was done using permanent colored dye for each animal in different body parts for each dosage.

2.5 Oral Glucose Tolerance Test (OGTT)

Forty (40) identified mice were fasted from food and allowed access to water for 24hours and grouped in eight groups with five (5) mice each as indicated below:

- Group 1 and 2: Negative controls – mice were administered with CMC and 1% Tween 80.
- Group 3 and 4: mice were administered with water and ethanol crude extract at 400 mg/kilogram body weight dose.
- Group 5, 6 and 7: DCM, EtOAc and EtOH fractions were administered in mice at the constant ratio dose of 400mg/kilogram body weight [19].
- Group 8: Positive control - mice were administered with chlorpropamide 400 mg/kilogram body weight.

Prior to experiment, Fasting Blood Glucose (FBG) for each mouse was measured and recorded as t=0. An oral glucose loading dose of 1g/kilogram body weight was given by gavage to each mouse 30 minutes later. Hyperglycemic level for each mouse at each dose was then taken at 0.5, 1, 2- and 3-hours intervals after glucose loading dose by using glucometer [20,21].

2.6 Induction of Diabetes in Mice

Before diabetic induction, 30 mice were fasted for 24 hours, their weight and FBG were measured and recorded as t=0. The mice were injected with a single intraperitoneal administration of freshly prepared alloxan monohydrate in normal saline at 170 mg/kilogram body weight dose then provided

with food 30 minutes later. Diabetic symptoms were thereafter monitored within 72 hours after alloxan injection [22]. FBG test was done after six hours mice fasting where those mice with FBG greater/ equal to 11.1mmol/L were considered diabetic. Twenty (20) mice turned to be diabetic.

2.7 Extract Administration and Hypoglycemic Activity in Mice

Diabetic mice were orally administered with ethyl acetate fraction 72 hours after alloxan induction at 400mg/kilogram body weight daily. The study was done for 20 days whereby FBG and weight were measured on days 1, 5, 10, 15 and 20 of treatment. Treatments were carried out as follows;

- Group 1: Negative control – mice were administered with 1% Tween 80.
- Group 2: Mice were administered with EtOAc fraction at a constant ratio of 400mg/kilogram body weight dose.
- Group 3: Positive control - mice were administered with 400 mg/kilogram body weight chlorpropamide

The use of mice followed internationally accepted principles for European Union (EU) directive 2010/63/EU on the use of animals for research [23]. After the study, the experimented mice were euthanized by intraperitoneal dosing of 200 mg/Kilogram body weight of 18% Sodium pentobarbitone [24] in accordance with AVMA guideline for the euthanasia of animals 2020 edition [25], then disposed at Muhimbili National Hospital incinerator.

2.8 Phytochemical Screening of Ethyl Acetate Fraction

Qualitative tests for determining secondary metabolites present in the ethyl acetate fraction were performed as follows:

a) Test for alkaloids: Wagner's test: 10 mg of extract was taken, then few drops of Wagner's reagent were added and the formation of a reddish-brown precipitate indicated the presence of alkaloids.

b) Test for Flavanoids: Lead acetate test: 10 mg of extract was taken and few drops of 10% lead acetate solution were added. The

appearance of the yellow color precipitate indicated the presence of flavonoids.

c) Test for Phenols and Tannins: Lead acetate test: 10 mg of extract was taken and 0.5 ml of 1% lead acetate solution was added and the formation of precipitate indicated the presence of tannins and phenolic compounds.

d) Test for steroids and sterols: Salkowski's test: 5 mg of extract was dissolved in 2 ml of chloroform and an equal volume of concentrated sulphuric acid was added along the sides of the test tube. The upper layer turned red and the lower layer turned yellow with green fluorescence, indicating the presence of steroids and sterol compound in the extract.

e) Test for Carbohydrates: Benedict's test: 5mls of Benedict's solution was added to 0.5 mg of extract and boiled in a water bath. The appearance of red or yellow or green precipitates indicated the presence of reducing sugars.

f) Test for Saponins: Honey comb test: 0.5 mg of extract was taken in a test tube and few drops of 5% sodium bicarbonate solution were added. The mixture was shaken vigorously and kept for 3 minutes. The formation of honey comb-like froth indicated the presence of saponins.

2.9 Acute Toxicity Test

Lorke's method (1983) was used to test plant toxicity, in which nine mice were grouped into three groups then administered with different doses (10, 100 and 1000 mg/kilogram) of ethyl acetate fraction per group. The mice were then kept under observation for 24 hours to observe their behavior and mortality. No mortality in phase one was observed in mice. Three mice were grouped in three (3) groups of one (1) mouse each in phase two. The mice were administered with ethyl acetate fraction at 1600, 2900 and 5000 mg/kilogram body weight doses [26]. The mice were then placed under observation for 5 days to observe their behavior and mortality.

2.10 Data Analysis

Results were expressed as mean \pm SD, comparison among groups was analyzed using One-way ANOVA. At significance level $p < 0.05$, the construct between the means of the two population groups against negative control was considered significant.

3. RESULTS

3.1 Hypoglycemic Test (OGTT)

The mean blood glucose in experimental mice varied with time. After the glucose loading dose at 30 minutes, mean glucose level increased generally for some tested groups except for ethyl acetate fraction and standard drug. All extracts showed a subsequent lessen in mean blood glucose load in respect to their negative control after 1 hour of glucose load. During the 2nd and 3rd hour after glucose load, the mean hyperglycemic level for all groups dropped at a reasonably steady rate as shown in Table 1. Moreover, ethyl acetate fraction showed significant hypoglycemic activity compared to negative control ($p = 0.004$ at 95% confidence level). The comparison was done due to solubility possibilities in which crude H₂O, crude ethanol and ethanol fraction were compared with CMC whereby DCM fraction and EA were compared with 1%T80.

3.2 Diabetes Induction Test

Diabetes induction test was performed following a modification of the study that was done by Szkudelski in 2001. Day 0 was the day before alloxan induction where FBG was measured followed by 178mg/kilogram body weight alloxan induction. Elevation of FBG was observed between 48-72 hours after alloxan induction, approximately three to four times for normal mice. Increased rate of urination (polyuria), water intake due to thirsty (polydipsia), high rate of polyphagia and body weakness were observed in the experimental mice induced with alloxan.

The mean FBG level for 20 days of treatment is presented in Table 2. The mean glucose level for chlorpropamide (positive control) and ethyl acetate fraction decreased from day 5 contrary to 1%Teen 80 (negative control) in which FBG increased. Decreased mean glucose level was observed in days 10, 15 and 20 for positive control and the ethyl acetate fraction. However, the mean glucose level increased in negative control as shown in Fig. 1. Moreover, the mean body weight decreased in diabetic mice during the experiment as shown in Table 3. Unremitting decline in mean body weight was also observed for the ethyl acetate fraction from day 1 to day 20 but increased body weight was observed in the positive control group in the first 5 days

and was associated with an irregular decrease in mean body weight in the experimental mice.

3.3 Phytochemical Screening of *C. citratus* Ethyl Acetate Fraction

The ethyl acetate fraction contained alkaloids, phenols and tannins, saponin and anthraquinones on preliminary phytochemical screening, as revealed in Table 4.

3.4 Acute Toxicity Test

No mortality was observed in mice in all two experimental phases. However, some behavioral changes were observed among mice in the first hour of the experiment as shown in Table 4. Tremors and excessive sleep were observed in mice treated with both crude water extracts and ethyl acetate fraction at a dose above 1000 mg/kilogram body weight as shown in Table 5.

4. DISCUSSION

Almost 1200 species of indigenous herb plants are traditionally used in hyperglycemic managements worldwide [27]. *Cymbopogon citratus* plants has hypoglycemic property in customary and hyperglycemic mice [28,29,15]. Hypoglycemic activity of lemon grass is due to interaction of various bioactive chemical compounds (secondary metabolites) or several compounds in isolation [30]. Oral glucose loading is a physiological induction of diabetes mellitus by fleetingly increasing the blood glucose level of experimental animal without damaging pancreas for diagnosis of impaired glucose tolerance, diabetes mellitus and gestational diabetes [31]. The results in Table 1 showed that, administration of both crude extracts and fractions from *C. citratus* at 400 mg/kilogram body weight resulted into steady decrease in blood glucose level within 90 minutes of treatment. Moreover, the findings indicated that the ethyl acetate fraction had a significant of $p = 0.004$ reduction of blood glucose where hyperglycemic activity increased as polarity extraction decreased. Variation in hypoglycemic activity of *C. citratus* extracts may be due to presence of bioactive compounds which may have antagonistic effects or synergetic effects, which may occur between compounds present in extracts or fractions [14,17,15]. Furthermore, a dosage variation of different medicinal plants has been reported to influence hypoglycemia [18,32].

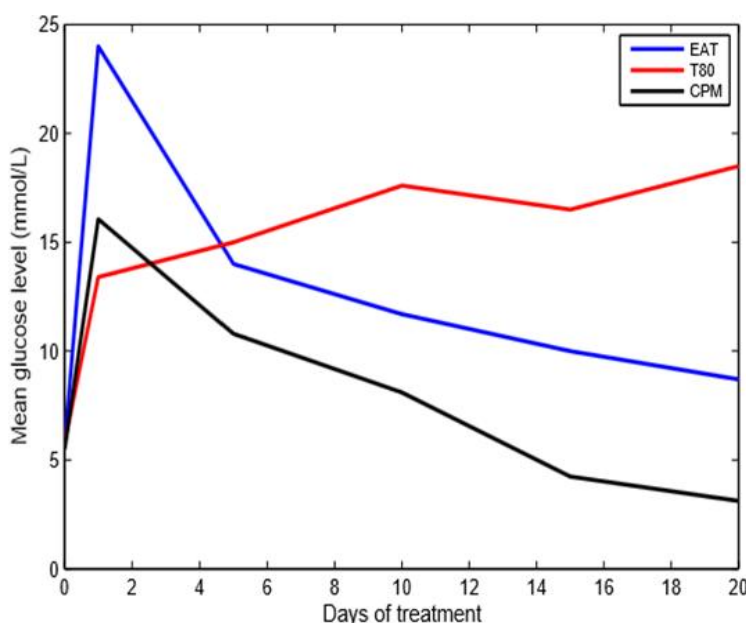


Fig. 1. Effects of ethyl acetate fraction on blood glucose level of alloxan induced diabetic mice

NB: EA-FR- Ethyl acetate fraction, T80- Tween80 and CPM- chlorpropamide

Alloxan induces diabetes by selectively inhibiting glucose-induced insulin secretion through specific inhibition of glucokinase (a glucose sensor for beta cells). It causes a state of insulin-dependent diabetes through its ability to induce ROS formation resulting in selective necrosis of beta cells [33]. alloxan has diabetic action when administered intravenously, intraperitoneally or subcutaneously. However, the dosage administration depends on animal species, route of administration and nutritional status of an animal. This study shows that administration of 170mg/kilogram body weight of alloxan was sufficient to induce diabetes in the albino mice used in the study. The intraperitoneal dose below 150 mg/kilogram body weight may be insufficient for inducing diabetes in mice and rats [22]. The mean FBG for 20 days of ethyl acetate fraction treatment showed a statistically significant reduction in blood glucose as indicated in Table 2. Increased blood glucose was observed in albino mice treated with 1% T80, since it was used as a negative control for hypoglycemic action comparison. Decreased blood glucose was also associated with a steady decrease in body weight for the experimental mice and the positive control group. However, a discontinuous weight elevation was observed in mice treated with 1% T80, which might be contributed by the high rate of water and food while remaining untreated. Observed hypoglycemic activity of *C.*

citratus from ethyl acetate fraction may be contributed by bioactive compounds present in the fraction [34,35]. *C. citratus* contains alkaloids, saponins, anthraquinone, phenols and tannins present in the extract/fraction [16,36,37]. There are two anthraquinones, chrysophanol-8-*O*- β -d-glucopyranoside and chrysophanol, that were isolated from *Korean rhubarb* rhizome and found to enhance glucose transport in myotubes with moderate cytotoxicity. The chrysophanol-8-*O*- β -d-glucopyranoside compound was found to significantly enhance insulin-stimulated glucose transport by insulin receptor activation. Moreover, Chrysophanol influenced insulin-responsive glucose transport by increasing GLUT4 mRNA expression [37]. Another anthraquinone, Sennidin A, was found to induce the translocation of GLUT4 in rat adipocytes. On the other hand, saponins may help reduce cholesterol levels, strengthen the immune system, treat diabetes, and inhibit tumor growth. They also improve lipid metabolism and may help prevent and treat obesity, all of which are common in type 2 diabetes. The actions of the two secondary metabolites (saponins and anthraquinones) found in this study may also have been responsible for the observed antihypertensive activity in the treated albino mice in this study [38]. Further study on these compounds is also warranted.

Table 1. Mean FBG (mmol/l) for OGTT

Time (hrs)	Mean FBG (mmol/l) \pm S.D n=5							
	Controls		Crude Extracts and fractions at 400mg/kilogram body weight					
	CMC	1%T80	Positive	H ₂ O crude	Ethanol crude	Ethanol fraction	DCM fraction	EA fraction
0	3.16 \pm 0.86	7.24 \pm 2.45	5.82 \pm 0.40	2.96 \pm 0.57	2.9 \pm 0.59	3.4 \pm 0.80	7.14 \pm 1.80	8.44 \pm 1.43
0.5	3.52 \pm 1.03	9.50 \pm 2.11	3.22 \pm 0.35	5.1 \pm 1.35	4.66 \pm 1.46	4.82 \pm 2.45	7.9 \pm 3.08	7.68 \pm 2.14
1	3.22 \pm 0.67	5.46 \pm 0.61	4.34 \pm 1.11	4.3 \pm 1.15	3.68 \pm 0.79	3.7 \pm 1.06	7.14 \pm 2.88	5.68 \pm 1.51
2	3.1 \pm 0.62	4.86 \pm 0.51	3.14 \pm 0.47	4.22 \pm 1.07	3.46 \pm 0.56	3.28 \pm 0.78	4.8 \pm 0.51	4.28 \pm 0.86
3	3.22 \pm 1.00	3.94 \pm 0.38	3.16 \pm 0.60	3.78 \pm 0.50	3.46 \pm 0.60	3.16 \pm 0.91	4.02 \pm 0.50	3.62 \pm 0.69

Table 2. Mean FBG (mmol/l) for alloxan induced diabetes mice

Time (Days)	Mean FBG (mmol/l) \pm S.D n=5				
	Controls		Fraction		
	Positive		1%T80		Ethyl acetate
0	5.52	\pm 0.82	5.82	\pm 0.35	5.72 \pm 0.70
1	16.06	\pm 5.87	13.35	\pm 3.29	24.8 \pm 9.43
5	10.8	\pm 6.08	15.03	\pm 5.65	14.86 \pm 10.47
10	8.1	\pm 3.70	17.55	\pm 6.90	11.72 \pm 6.39
15	6.24	\pm 1.67	16.45	\pm 5.16	10.25 \pm 5.10
20	4.65 \pm 1.02		18.45	\pm 5.92	8.725 \pm 1.83

Table 3. Mean body weights of alloxan treated mice

Time (days)	Mean body weights \pm S.D (g), n = 5		
	Control		Fraction
	Positive	1%T80	Ethyl acetate
0	25.2 \pm 5.45	25.67 \pm 1.03	28 \pm 3.94
1	25.4 \pm 5.32	25.33 \pm 1.63	28 \pm 3.94
5	27 \pm 6.04	26.17 \pm 2.56	26.2 \pm 3.56
10	26.4 \pm 7.16	25.67 \pm 1.75	24.8 \pm 3.11
15	23.8 \pm 5.98	24 \pm 2.5	23.25 \pm 2.83
20	21.4 \pm 3.22	23.83 \pm 2.48	22 \pm 2.83

Table 4. Phytochemical screening of *C.citratus* ethyl acetate fraction

Plant phytochemical	Ethyl acetate fraction	Test name
Alkaloids	+	Wagner's test
Flavonoids	—	Lead Acetate test
Phenol and Tannin	+	Lead Acetate test
Steroids	—	Salkowski's test
Carbohydrates	—	Benedicts test
Saponins	+	Honey Comb test
Glycosides	—	Glycosides test
Protein	—	Biuret test
Anthraquinone	+	Borntragers test

NB: + indicates the present of tested phytochemical and - indicates the absence of tested phytochemical

Table 5. Mice behavioral changes

Behaviors	Phase 1 (mg/kilogram body weight)						Phase 2 (mg/kilogram body weight)					
	Water crude			Ethyl acetate fraction			Water crude			Ethyl acetate fraction		
	10	100	1000	10	100	1000	1600	2900	5000	1600	2900	5000
Tremors	*	*	✓	*	*	✓	✓	✓	✓	✓	✓	✓
Convulsions	*	*	*	*	*	*	*	*	*	*	*	*
Salvation	*	*	*	*	*	*	*	*	*	*	*	*
Diarrhea	*	*	*	*	*	*	*	*	*	*	*	*
Lethargy	*	*	*	*	*	*	*	*	*	*	*	*
Sleep	*	*	✓	*	*	✓	✓	✓	✓	✓	✓	✓
Respiratory changes	*	*	*	*	*	*	*	*	*	*	*	*
Mortality	*	*	*	*	*	*	*	*	*	*	*	*

NB: * - indicates the absence of indicated behavior at a specific dose

Although most herbal products are regarded as safe without strictly prescribed doses, some medicinal plants are reported to have side effects to both human and other animals [39]. The toxicity results from this study showed that *C. citratus* water extract and ethyl acetate fraction were non-toxic since no mortality was observed at a dose up to 5000 mg/kilogram body weight following 5 days after single oral plant extract administration. However, mild toxicological signs were observed in the form of active movement of the mice in 10 minutes of extract administration, followed by tremors and deep sleeping at the dose above 1000 mg/kilogram body weight. These results are in accord with findings from study by [16], where hepatotoxic and nephrotoxic effects were also reported in mice treated with fluid extracts of *C. citrates* [40]. These findings

suggest that *C. citratus* use should not be taken at very high doses (above 1000 mg/kilogram body weight) when used as a hypoglycemic agent to ensure personal safety.

5. CONCLUSION

Considering the significant hypoglycemic activity and body weight reduction by ethyl acetate fraction demonstrated in this study, *Cymbopogon citratus* extracts are possibly effective against insulin-dependent hyperglycemia. Further study on active compounds (possibly anthraquinones and saponins) from studied *C. citratus* extract is therefore warranted. Finally, detailed clinical trials on human subjects should also be done, including a prior sub-acute toxicity tests to verify their safety in organs.

CONSENT AND ETHICAL APPROVAL

KNCHREC issued the study ethical clearance with reference no. KNCHREC 00034. Informed consent for laboratory work and in vivo study was obtained from Muhimbili School of Health and Allied Science (MUHAS) at the Pharmacognosy Department.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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