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Effect of Ethanol Extract of *Cymbopogon citratus* on Blood Sugar and Haematological Indices in Alloxan-Induced Diabetic Wistar Rats

[1]Ebenezer Morayo Ale, [1]Ojochenemi Ejeh Yakubu, [1]Emochone Roy Yohanna, [1]Steve Osagie Asuelimen, [1]Victoria Ifeoluwa Ayo, [1]Mgbede Joy Timothy, [2]Chinedu Christain Iheanacho, [1]Bilyaminu Habibu, [1]David Chinonso Anih and [1]Sovy Makong Andrew

[1]Department of Biochemistry, Faculty of Pure and Applied Science, Federal University Wukari, Wukari 670102, Taraba, Nigeria

[2]Department of Microbiology, Faculty of Pure and Applied Science, Federal University Wukari, Wukari 670102, Taraba, Nigeria

ABSTRACT

Background and Objective: *Cymbopogon citratus* is a famous medicinal plant with reported pharmacological actions. Herein, the effect of *Cymbopogon citratus* ethanolic extract on the blood sugar and haematological indices of alloxan-induced diabetic rats was investigated. **Materials and Methods:** Fifteen rats were distributed into Five groups and treated intraperitoneal for 14 days. Group I: Normal control (untreated), Group II: Negative control (untreated diabetic rats), Group III: Positive control (5 mg kg^{-1} glibenclamide, standard drug-treated rats), Group IV: 100 mg kg^{-1} extract-treated diabetic rats and Group V: 200 mg kg^{-1} extract-treated diabetic rats. Blood sugar and body weights were measured, rats were euthanized and blood was collected for hematological analysis. **Results:** The administration of alloxan-monohydrate provoked a marked elevation in the rats' blood sugar and a drastic fall in body weights. However, oral ethanol extract of *Cymbopogon citratus* administration led to a profound reduction ($p<0.05$) in blood glucose levels and a significant ($p<0.05$) increase in weight in a concentration-dependent manner. In addition, results revealed a marked ($p<0.05$) reduction in the hematological indices such as Total White Blood Cell (TWBC), neutrophil, lymphocytes and Red Blood Cell (RBC), but extract administration restored normalcy comparable to that of the standard drug. **Conclusion:** *Cymbopogon citratus* ameliorated the anomaly elicited by alloxan on the blood sugar and hematological parameters in rats. Consequently, it is rational to suggest that the extract of *C. citratus* could be an ideal candidate for the design of a potent hypoglycemic drug and formulation of the immune booster.

KEYWORDS

Cymbopogon citratus, diabetes, hematological indices, glibenclamide, hyperglycemic, alloxan, albino rats

INTRODUCTION

Diabetes mellitus (DM) is a metabolic or endocrine disorder delineated by prolonged hyperglycaemia that is concomitant with absolute or relative inadequacy in insulin secretion or function. Over 100 million of the global population (6% population) are reported to be affected by *Diabetes mellitus*[1]. It is found to

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damage many body systems over time, particularly blood vessels, kidneys, nerves, eyes and heart[2]. It is an expensive and severe disorder associated with alteration in lipid, protein and carbohydrate metabolism and is among the four leading deadly diseases in developed countries[2,3] and reported to be an epidemic in several developing countries it affects approximately 25% of the population[4]. Despite the current interventions, its rampage has been observed to increase rapidly worldwide, hence making it a global health concern[5].

Plants are very important sources of drugs used for centuries in the treatment of various microbial infections[6]. Since the use of synthetic drugs is often accompanied by several disadvantages such as microbial resistance, side effects and high cost[7], the use of natural products from plants as a safe alternative with better effectiveness is necessitated[8]. Reports from traditional usage have demonstrated that numerous plants elicit antimicrobial actions[9]. Worth mentioning is the fact that since the advent of medicine, natural products of plant origin have served as a tremendous source of therapy for mankind[6]. For several decades, plants' phytochemicals have served as a pivot for pharmaceutical discovery[10]. The efficacy of plants' bioactive compounds in medicine and agriculture has attracted the interest of scientists[8].

Cymbopogon citratus (lemon grass) is a plant with abundant phytochemicals and constitutes a great source of herbal remedies for different ailments and diseases. An intriguing study revealed that the plant possesses antioxidant, anti-hypertensive, anti-fungal, anti-cancer, anti-nociceptive, anti-mutagenic and anxiolytic properties[11]. However, there is a dearth of information as regards the hypoglycaemic property. This work, therefore, sought to evaluate the effect of ethanol extract from leaves of *Cymbopogon citratus* on blood sugar and haematological parameters in alloxan-induced diabetic Wistar rats.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Biochemistry, Federal University Wukari, Taraba State, Nigeria between February and June, 2021.

Collection of *Cymbopogon citratus* sample: The sample of *Cymbopogon citratus* leaf used in this study was obtained from the Wapan-Nghaku area in Wukari town, Taraba State, Nigeria in February, 2021. The leaves were dried under shade and ground with the aid of a mechanical blender. The powdered leaf sample (150 g) was soaked in 600 mL of absolute ethanol with seldom shaking for 48 hrs and was filtered with a clean white handkerchief thereafter. With the aid of a water bath, the filtrate was concentrated. The concentrated extract was then transferred into a sterilized bottle and then refrigerated until it was ready for use.

Experimental animals: Fifteen Wistar rats were employed in the study. The rats weighed between 150-230 g. The rats were raised in the Departmental animal house. The rats were provided with steady feed and water throughout the experiment. The animals were handled by standard protocols approved by the Faculty of Pure and Applied Sciences.

Ethical consideration: This study was carried out according to standard guidelines of the Committee on Care and Use of Experimental Animal Resources of Faculty of Pure and Applied Sciences, Federal University Wukari, Nigeria with the approval number, FUW/FPAS/21/010.

Induction of diabetes: The animals were induced diabetes intraperitoneally by injecting 140 mg kg^{-1} b.wt., alloxan monohydrate, after dissolution of alloxan monohydrate crystals in normal saline[12]. Blood was collected from each rat's vein by orbital puncture of the tail vein. The blood glucose was taken before the induction of alloxan monohydrate and after induction, the fasting glucose level was checked 3 days after induction to detect those that are hyperglycaemic. Animals with a blood glucose of

200 mg dL^{-1} upward were considered markedly hyperglycaemic and used for the study. The blood glucose level was determined during the period of treatment within seven days' intervals using an Accu-Check Active Glucometer (Roche Diabetes Care GmbH, Mannheim, Germany).

Experimental design: Animals were distributed into five groups of three each receiving treatment as follows:

- **Group I:** Non-alloxan monohydrate-induced rats (normal control)
- **Group II:** Alloxan monohydrate-induced untreated diabetic rats (negative control)
- **Group III:** Alloxan monohydrate-induced diabetic rats treated with 5 mg kg^{-1} glibenclamide, standard drug (positive control)
- **Group IV:** Alloxan monohydrate-induced diabetic 100 mg kg^{-1} extract-treated diabetic rats
- **Group V:** Alloxan monohydrate-induced diabetic 200 mg kg^{-1} extract-treated diabetic rats

Estimation of body weight: The rats' weights were taken before induction of diabetes using an electronic weighing balance (FEJ-3000B, Leo Scales Industries, Madras Sea, India). The weight of all the rats was also taken after diabetes induction and subsequently at seven-day's intervals.

Fasting blood sugar measurement: The rats' fasting blood sugar was measured using an Accu-Check Active Glucometer (Roche Diabetes Care GmbH, Mannheim, Germany) by tail vein puncturing and the concentration of blood sugar reported in mg dL^{-1} was obtained by dropping them on the strip and inserting the strip in the glucometer thereafter on the intervals of 0, 3, 7 and 14 days, while the feed was withdrawn from the rats a night before checking the blood glucose to obtain fasting blood glucose.

Collection of blood sample: Animals were anesthetized with ether and blood was collected by jugular puncture in anticoagulant tubes and stored in a refrigerator for use.

Haematological analysis: Hematological analysis was carried out using a haematology-analyzer (Mindray Auto-analyzer, BC-5200, Nanshan, Shenzhen, China) in accordance with manufacturer's guidelines. The parameters measured include total white blood cells (TWBC), neutrophils, lymphocytes, eosinophils, monocytes, red blood cells (RBC) and packed cell volume (PCV).

Statistical analysis: Comparisons using SPSS (version 21). Values were recorded as mean±standard deviation and were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Effect of *Cymbopogon citratus* on haematological indices: The effect of *Cymbopogon citratus* on white blood cells. White Blood Cells (TWBC) were observed to be significantly ($p<0.05$) low in group II when compared with the control upon alloxan treatment as shown in Table 1. However, extract treatment markedly ($p<0.05$) elevated the level of TWBC in groups IV and V in a dose-dependent manner and this effect was comparable to that of the standard drug. Similar results were observed in the levels of Neutrophils and Lymphocytes as the extract caused a significant ($p<0.05$) rise in their levels in groups IV and V when compared with the alloxan-treated group. On the contrary, no significant ($p<0.05$) change was observed in the monocytes and eosinophils counts between the extract-treated groups and the other groups.

The result of red blood cells is presented in Table 2. Administration of alloxan led to a profound ($p<0.05$) reduction in the PCV and RBC in group II upon administration of alloxan. However, these

Table 1: Effect of *Cymbopogon citratus* on white blood cell parameters

Treatment group	TWBC (×10^9/L)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Monocytes (%)
Control	15.50±04.90^{ab}	64.00±01.00^{b}	29.00±01.00^{b}	1.50±0.50^{a}	5.50±0.50^{a}
Alloxan	05.90±05.11^{a}	26.00±22.61^{a}	19.00±16.46^{a}	1.33±0.58^{a}	5.33±2.52^{a}
Alloxan+glibenclamide	16.33±09.25^{ab}	67.00± 07.21^{b}	27.00±03.61^{b}	2.67±2.31^{a}	3.33±1.53^{a}
Alloxan+100 mg extract	18.05±10.19^{ab}	68.75±03.20^{b}	26.75±03.30^{b}	1.75±1.71^{a}	3.50±3.00^{a}
Alloxan+200 mg extract	21.13±02.50^{b}	70.33±09.61^{b}	25.67±08.62^{b}	1.67±1.15^{a}	2.55±0.58^{a}

Each value represents mean±SD, n = 3, mean values with different superscripts are significantly different (p<0.05) across the column and TWBC: White blood cells

Table 2: Effect of *Cymbopogon citratus* on packed cell PCV and RBC

Treatment group	PCV (%)	RBC (10^{12}/L)
Control	47.50±07.50^{a}	4.75±0.75^{a}
Alloxan	31.33±14.01^{b}	2.37±2.03^{b}
Alloxan+glibenclamide	45.33±4.51^{a}	4.40±1.25^{a}
Alloxan+100 mg extract	40.00±04.36^{ac}	3.80±0.82^{a}
Alloxan+200 mg extract	44.50±04.43^{a}	4.63±0.13^{a}

Each value represents mean±SD, n = 3, mean values with different superscripts are significantly different (p<0.05) across the column, RBC: Red blood cells and PCV: Packed cell volume

Table 3: Effect of *Cymbopogon citratus* on blood glucose levels (mg dL^{-1}) in alloxan-induced diabetic rats

Treatment group	Day 0	Day 1	Day 7	Day 14
Control	089.33±12.74^{a}	102.67±09.500^{a}	105.00±011.00^{a}	061.33±05.030^{a}
Alloxan	103.00±05.00^{a}	416.33±224.98^{b}	479.33±228.23^{c}	335.00±201.81^{d}
Alloxan+glibenclamide	97.33±24.58^{ab}	555.00±090.09^{b}	451.67±047.81^{bc}	180.67±038.14^{d}
Alloxan+100 mg extract	82.75±09.84^{a}	532.50±153.84^{b}	370.75±097.23^{bc}	122.00±026.18^{a}
Alloxan+200 mg extract	122.33±14.36^{a}	513.67±048.79^{b}	247.33±054.93^{c}	93.67±011.15^{a}

Each value represents mean±SD, n = 3 and mean values with different superscripts are significantly different (p<0.05) across the row

Table 4: Weight changes (g) of Wistar rats observed during the fasting blood sugar analysis

Treatment group	Day 0	Day 1	Day 7	Day 14
Control	187.00±18.33^{bc}	181.33±28.01^{bc}	194.33±20.59^{c}	199.33±019.55^{a}
Alloxan	206.33±14.74^{c}	209.33±17.56^{c}	217.33±21.38^{c}	132.33±121.22^{a}
Alloxan+glibenclamide	187.33±24.03^{bc}	183.67±21.46^{c}	190.00± 23.64^{c}	181.00±06.24^{a}
Alloxan+100 mg extract	163.75±21.53^{a}	150.75±20.81^{a}	154.50±23.91^{a}	156.25±25.86^{a}
Alloxan+200 mg extract	164.00±04.00^{a}	171.67±05.51^{a}	183.67±04.51^{a}	191.00±08.89^{a}

Each value represents mean±SD, n = 3 and mean values with different superscripts are significantly different (p<0.05) across the row

parameters were significantly (p<0.05) increased in group IV and V following treatment with extract of *Cymbopogon citratus* when compared with group II treated with alloxan and this effect was comparable to that of group III which received glibenclamide.

Effect of *Cymbopogon citratus* on blood sugar levels in alloxan-induced diabetic rats: There was a marked (p<0.05) increase in the fasting blood sugar in groups II (103.00 to 416.33 mg dL^{-1}), III (97.33 to 555.00 mg dL^{-1}), IV (82.75 to 532.50 mg dL^{-1}) and V (122.33 to 513.67 mg dL^{-1}) after alloxan treatment. Administration of *Cymbopogon citratus* leaf extract elicited a marked (p<0.05) reduction in blood sugar in group V (513.67 to 247.33 mg dL^{-1}) on the seventh day when compared with group II (416.33 to 479.33 mg dL^{-1}), III (555.00 to 451.67 mg dL^{-1}) and IV (532.50 to 370.75 mg dL^{-1}) and on the fourteenth day of administration, a profound (p<0.05) reduction in blood sugar was recorded in group IV (370.75 to 122.00 mg dL^{-1}) and V (247.33 to 93.67 mg dL^{-1}) and the efficacy was higher than that of the standard drug (451.67 to 180.67 mg dL^{-1}) as shown in Table 3.

Effect of *Cymbopogon citratus* on body weight of diabetic rats: The alloxan group with a mean weight of 206.33 g on day 0 had their weight reduced to 132.33 g on day 14. However, the alloxan+200 mg extract group with a mean weight of 164 g on day 0 experienced a weight increase to 191.00 g. Moreover, no significant weight decrease was observed in group IV when compared with the normal control as shown in Table 4.

Diabetes is a metabolic disorder characterized by undermined glucose metabolism with subsequent impairment in the intermediary metabolism of carbohydrates and lipids. It is categorized among the deadly diseases with the highest rate of mortality[13]. Severe side effects are often associated with synthetic hypoglycemic drugs such as biguanides and sulphonylureas used in the treatment of diabetes. As a result, herbal therapy and medicinal plants have attracted interest all over the world as an alternative source of therapy for diabetes owing to the fact that they offer effective treatment with little or no side effects[14,15].

Cymbopogon citratus is widely consumed across the globe, because of its numerous benefits including nutritional, medicinal and cosmetic purposes[16]. Several bioactive constituents such as flavonoids, saponins, phenols, tannins, anthraquinone, deoxysugars, essential oils and alkaloids have been reported to be present in the plant[15]. Numerous secondary metabolites have also been linked to its pharmacological actions such as anti-carcinogenic[17], cardioprotective[18], antitussive, antiseptic and anti-rheumatic activities. It is also being used to prevent aggregation of platelets[19] and treat gastrointestinal disturbances, dyslipidemia[20], malaria[21], fever, flu and pneumonia[22].

In the present study, 140 mg kg^{-1} of alloxan was administered to all animals except animals in group 1 (control), the administration of alloxan significantly increased the animals' blood sugar levels (Table 3). The initial blood glucose levels of all the animals were below 130 mg dL^{-1} but the induction of alloxan led to an elevation in their blood glucose levels to over 400 mg dL^{-1} making them diabetic or hyperglycaemic. In group II (negative control), the induction of alloxan led to an increase in the blood glucose levels from 103 to 416 mg dL^{-1} on the first day, which increased to 479 mg dL^{-1} on the seventh day and reduced to 335 mg dL^{-1} on the fourteenth day probably due to starvation or an underlying disease condition. This might indicate that alloxan monohydrate gradually destroys the pancreatic β-cells of the islets of Langerhans, preventing the secretion of insulin. The rats in groups III, IV and V were administered 5 mg kg^{-1} of glibenclamide, 100 mg kg^{-1} *Cymbopogon citratus* leaf extract and 200 mg kg^{-1} *Cymbopogon citratus* leaf extract, respectively. The fasting blood sugar of group I (control) rats underwent a non-significant ($p<0.05$) increase from day 1 to day 7 but a decrease was observed on day 14. The sugar level of group III animals increased greatly after the induction of alloxan on day 1, but glibenclamide caused a marked ($p<0.05$) decrease in blood sugar level from 555.00 to 180.67 mg dL^{-1} on day 14. In groups IV and V, a significant ($p<0.05$) increase in blood glucose levels was recorded after the induction of diabetes from 82.75 and 122.33 mg dL^{-1}, respectively to 532.50 and 513.67 mg dL^{-1}, respectively, but treatment with 100 and 200 mg kg^{-1} of the ethanol leaf extract of *Cymbopogon citratus*, respectively, profoundly ($p<0.05$) reduced the blood sugar level (122.00 and 93.67 mg dL^{-1}, respectively) after 14 days (Table 3).

The diabetic rats administered with 5 mg kg^{-1} b.wt., glibenclamide showed a marked ($p<0.05$) fall in sugar level from 555.00 to 180.67 mg dL^{-1} (Table 3). It is therefore worth mentioning that 200 mg kg^{-1} ethanol extract of *Cymbopogon citratus* elicited more hypoglycemic action when compared with 100 mg kg^{-1} extract and the standard drug and this suggests the plant could be an alternative source of conventional antidiabetic drugs. Apparently, *Cymbopogon citratus* could be able to alleviate complications in diabetic patients[23].

The effects of *Cymbopogon citratus* on the body weights of diabetic rats are presented in Table 4. In the normal control, the rats' weight decreased slightly on day 1 (from 187.00 to 181.33 g), but a gradual increase in weight was recorded from day 7 to day 14 (199.33 g). After the induction of 140 mg dL^{-1} of alloxan, the weight (206.33 g) of the rats in the alloxan group also increased slightly on the first and seventh days but significantly ($p<0.05$) decreased to 132.33 g on day 14. A non-significant ($p<0.05$) weight decrease (from 163.75 to 156.25 g) was observed in group IV (alloxan+100 mg kg^{-1} extract) animal, while 200 mg kg^{-1} extract rather led to a marked ($p<0.05$) increase in weight of group V from 164.00 g recorded

on day 0 to 191.00 on day 14. The positive weight change ($p<0.05$) observed in the extract-treated diabetic rats when compared with the standard and the normal control suggests that *Cymbopogon citratus* might be a suitable candidate in the management of lipid metabolic dysfunction, a side effect often experienced when sulphonylureas are used in the treatment of diabetes[15].

The hematological assessment provides biochemical information about the blood component, the immune and the reticuloendothelial system. Furthermore, the hematological indices, majorly, the WBCs, RBCs and PCV of albino rats were investigated before and after administering *Cymbopogon citratus* to the diabetic rats. The induction of alloxan led to a marked ($p<0.05$) decrease in the TWBC in the alloxan-treated or diabetic group (group II) in comparison with the control, but extract treatment ameliorated the damage by significantly ($p<0.05$) increased the TWBC level in group IV and V in a dose-dependent manner. Some substances that found their way into the bloodstream triggered the generation of WBCs, thus playing a defensive role against any of the substances that might likely be toxic to the immune system. The high concentration of WBCs in rats administered with the extract is indicative of the efficacy of the immune system functionality in defense against toxins. The number of WBCs that are neutrophils reduced significantly ($p<0.05$) in the diabetic rats (group II) when compared with the remaining groups (Table 1), indicating an immune system that may be under attack by foreign substances, since neutrophils are responsible for the first line of defense issued by an immune system. On the contrary, administration of extract profoundly ($p<0.05$) raised the neutrophil level. In group II, there was a non-significant ($p<0.05$) decrease in Lymphocyte levels, but markedly ($p<0.05$) increased upon extract administration in groups IV and V (Table 1). High counts of lymphocytes are seen during infection, after exercise and with stress while abnormally low counts may be seen if there is suppression of the immune system. In all cases, the extract elicited a strong and comparable effect to the standard drug (glibenclamide). Group V displayed an infinitesimal increase in eosinophil level in comparison to group I, while monocytes showed a non-significant ($p<0.05$) decrease in group V when compared with group I (Table 1).

The red blood cells (RBCs) facilitate oxygen transport as well as carbon (iv) oxide. A reduction in RBC level could imply a inadequate amount of oxygen transported to tissues and can lead to hypoxia and organ failure[24]. Table 2 reveals that alloxan administration led to a significant ($p<0.05$) reduction in PCV and RBC in group II. Conversely, *Cymbopogon citratus* extract treatment markedly ($p<0.05$) increased the PCV and RBC in groups IV and V when compared to group II and this effect was comparable to that of group III which received glibenclamide (Table 2).

Worth mentioning is the fact that the phytochemical composition of medicinal plants and herbs is what greatly determines their physiological and pharmacolocological effects[16]. Elegant research demonstrated that *Cymbopogon citratus* contains phenolic and flavonoids which include luteolin, apiginin, quercetin and kaempferol and essential oils which include geraniol, geranyl acetate, terpinolene and myrecene. It has also been documented that *Cymbopogon citratus* contains alkaloids, tannins, steroids and anthraquinones[25] and each and every phytochemical possesses one or more therapeutic properties. Interestingly, studies revealed that lemon grass extracts possess hypoglycemic, hypolipidaemic and antioxidant potential in hyperlipidaemic and hyperglycaemic rats[26]. From the foregoing, considering the fact that the phytoconstituents of the plant like flavonoids, alkaloids, carotenoids, terpenoids and glycosides are allegedly anti-diabetic[25], it is rational to link the anti-diabetic property of this plant to these resident bioactive constituents. This study, therefore, reveals that *Cymbopogon citratus* leaf extract could be a potent antihyperglycemic or antidiabetic agent.

Cymbopogon citratus leaf extract could be suggested as an effective candidate for diabetes management and should therefore be considered in the design of a potent hypoglycemic drug as well as an immune booster. However, further research needs to be carried out to identify and extract the active hypoglycaemic compounds in *Cymbopogon citratus*.

CONCLUSION

This study reveals that *Cymbopogon citratus* leaf extract possessed hypoglycaemic activity comparable to glibenclamide (standard drug). It also elicited a modulatory effect on the hematological parameters of diabetic rats. This is consequent to its efficacy in blood sugar reduction in extract-treated diabetic rats. The results herein, therefore, necessitate clinical trials of *Cymbopogon citratus*.

SIGNIFICANCE STATEMENT

Considering the increased menace and mortality caused by diabetes, there is a need to embark on this project work to provide a lasting solution to this health issue. Also, consequent to the reported side effects and other disadvantages of antidiabetic conventional drugs, efforts to find cheaper and safe sources of therapy using herbal extract necessitated this research to determine and evaluate the hypoglycaemic efficacy of ethanol extract of leaf of *Cymbopogon citratus* in Wistar rats. Results revealed that extract possesses a strong hypoglycemic effect and ameliorated hematological indices in alloxan-induced diabetic rats.

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