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AQUEOUS *OCIMUM GRATISSIMUM* LEAVES EXTRACT ALTERS THE HEPATIC AND RENAL FUNCTION BIOMARKERS OF ALLOXAN-TREATED WISTAR RATS

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ABSTRACT

Ocimum gratissimum, also called scent leaf, is widely used as a culinary herb and in traditional medicine practice in Nigeria. This study was done to assess the effect of aqueous *Ocimum gratissimum* leaves extract (AOLE) on some hepatic and renal function biomarkers of male Wistar rats treated with Alloxan (a hyperglycaemic inducer). The rats were divided into three groups of 5 rats in each group. Group 1 served as the Normal Control, Group 2 received 100 mg/kg body weight (bwt) of freshly prepared Alloxan (in saline water) and Group 3 received the Alloxan + 200 mg/kg bwt AOLE. The freshly prepared Alloxan was administered to Groups 2 and 3 by intraperitoneal injection and hyperglycaemia established by measuring their blood glucose. AOLE was subsequently administered to the rats in Group 3, by oral gavage, daily for 7 days. At the end of 7 days all the rats were assessed, sacrificed and their blood collected for analysis of serum Alanine aminotransferase (ALT), Alkaline phosphatase **(**ALP), Aspartate aminotransferase **(**AST), Urea, Creatinine, Sodium, Potassium and Chloride content using standard Randox kit methods. The results showed that treatment with Alloxan increased ALT, AST and ALP activity significantly ($p<0.05$) compared with the untreated control. However, administration of the AOLE significantly ($p<0.05$) decreased ALT, AST and ALP activity to level comparable with the untreated control. Treatment with Alloxan also caused significant ($p<0.05$) increase in serum urea and creatinine levels as well as significant ($p<0.05$) decrease in serum sodium. However, there was significant ($p<0.05$) increase in serum urea and sodium levels, and significant ($p<0.05$) decrease in creatinine and chloride levels of the Alloxan + AOLE treated rats compared to those treated with Alloxan

only. These results suggest that the AOLE exhibit hepatoprotective potential against an Alloxan-induced hepatotoxicity while its effect on renal function biomarkers was nephrotoxic.

KEYWORDS: *Ocimum gratissimum,* Hepatic function, Renal function, Alloxan, Wistar Rats.

INTRODUCTION

The use of herbs for medicinal purposes is gaining global acceptability as a greater percentage of the world's population, especially in rural communities; rely on botanical preparations to meet their health needs. Many countries have established regulatory and monitoring agencies responsible for addressing the concerns about toxicity and dosage of herbal medicine [1].

Ocimum gratissimum, commonly known as scent leaf, is a well-known plant used in folk medicine for curing headache, fever, diarrhoea and pneumonia. It is an aromatic herb that has been introduced extensively across tropical and subtropical regions of the world. It is also a food spice which has been recommended for the treatment of various diseases [2].

Afolabi et al. [3] revealed that the leaves of *Ocimum gratissimum* contain tannins, steroids, terpenoids, flavonoids and cardiac glycosides. Their findings suggested that the rich variety of phytochemicals, with good antioxidant activity, in *Ocimum gratissimum* may be responsible for its popular and constant use in traditional medicine. Effraim et al.[4] also revealed that despite the popularity of the plant as a condiment and herbal medicine, the extract has been observed to possess possible hepatoprotective activity in rabbits, and this is attributed to the presence of flavonoids and saponins (terpene glycosides) in the plant.

Diabetes mellitus is a metabolic disease characterized by chronic hyperglycaemia resulting from defect in insulin secretion, insulin action, or both [5]. The classification of diabetes mellitus is important and has implications for treatment strategies. This is not an easy task and many patients do not easily fit into a single class especially younger adults [6]. Also, it is estimated that about 10% of those initially classified may require revision [7].

Mahmud et al. [8] reported that aqueous leaves extract of *Ocimum gratissimum* possesses anti-hyperglycemic effects and is relatively safe for use in the treatment of diabetics. This study therefore assessed the alterations in some hepatic enzymes and renal function biomarkers in Alloxan-induced male Wistar rats following treatment with aqueous *Ocimum gratissimum* leaves extract.

MATERIALS AND METHODS

Sample Collection

Ocimum gratissimum leaves (scent leaf) were obtained from a farm at Eneka in Obio-Akpor local Government Area, Rivers State, Nigeria and brought to the Biochemistry laboratory of Rivers State University, Port Harcourt, Nigeria. The leaves were identified at the Department of Plant Science and Biotechnology, Rivers State University and subsequently allowed to dry at room temperature.

Adult male albino rats weighing between 115 – 130g were obtained from the animal house of the Department of Clinical Pharmacology, College of Medical Science, Rivers State University and transferred to the animal house of the Department of Biochemistry, Faculty of Science, Rivers State University, Port Harcourt, Nigeria.

Sample Preparation and Preservation

Plant Extraction

The *Ocimum gratissimum* leaves were dried at room temperature and pulverized into powder using electric blender. 200g of the powder was weighed, mixed with 1 liter of distilled water and macerated for 48 hours in a wide-mouth glass bottle (Balmer bottle) with screw cap. The macerate was double filtered using cheese cloth and Whatman No. 1 filter paper to obtain a clear filtrate which was transferred to an amber glass container and stored in the refrigerator prior to the time of administration. The preservation was done to maintain stability of the sample without significant alteration of their biological properties and loss or reduction of target analytes.

Experimental Animals

Fifteen adult male Wistar rats, weighing between 115 - 130g, were randomly selected and acclimatized for one week prior to the commencement of treatment. The animals were housed in separate cages and maintained under standard laboratory conditions. They were provided with standard rodent feed and water *ad libitum*

Drug Used

Alloxan, obtained from the chemical store of the Department of Biochemistry, Rivers State University, Port Harcourt, was used to induce hyperglycaemia in the Wistar rats. The Alloxan drug was prepared by dissolving 2.0g of alloxan monohydrate powder in 100 ml of 0.9% w/v saline water. The drug was prepared immediately before injection. 100mg/kg body weight (bwt) was administered to the rat, by intraperitoneal injection, to induce hyperglycaemia. The

blood glucose level of the rats was tested to confirm hyperglycaemic condition has been induced.

Blood sample collection

Blood sample was obtained by cardiac puncture using syringe and needle. The blood was collected and transferred into a lithium heparin tube. The tube was immediately inverted 8-10 times to mix and ensure adequate anticoagulation of the sample. The sample was immediately sent to the laboratory and centrifuged to obtain the plasma.

Experimental Design

The rats were divided into 3 groups with 5 rats in each group.

Group	Treatment
1 (Control)	Rats received normal feed and water *ad libitum* and were not administered either alloxan drug or *Ocimum gratissimum* leaves extract
2 (Alloxan)	Rats received normal feed and water *ad libitum* + with 100mg/kg alloxan drug without treatment with the *Ocimum gratisimum* leaves extract
3 (Extract)	Rats received normal feed and water *ad libitum* + 100mg/kg bwt alloxan drug + 200mg/kg bwt *Ocimum gratissimum* leaves extract

Biochemical Analysis

Biochemical analysis for hepatic function enzymes (ALT, AST, ALP) and renal function biomarkers (Urea, Creatinine, Potassium, Sodium, Chloride) were conducted using Randox Laboratories United Kingdom reagent kits.

Statistical Analysis

Statistical analysis was done using SPSS software. Data were analyzed using one-way analysis of variants (ANOVA) and Duncan's posthoc test for comparison. Results were expressed as Mean ± Standard Deviation and values were regarded as statistically significant at $p < 0.05$.

RESULTS

Results of serum Alanine aminotranferase (ALT), Aspartate amino transferase (AST) and Alkaline phosphatase (ALP) activity in male Wistar rats treated with aqueous *Ocimum gratissimum* leaves extract are presented in Table 1. Treatment with alloxan (Group 2) significantly ($p < 0.05$) increased ALT, AST and ALP compared to Control (Group 1). Treatment with the extract (Group 3) significantly ($p < 0.05$) decreased ALT, AST and ALP

when compared to the Alloxan-treated group (Group 2). ALT and ALP were reduced to levels equivalent to Control (Group 1).

Table 1: Hepatic function enzyme biomarkers of Wistar rats treated with *Ocimum gratissimum* Leaves extract.

GROUP	ALT (U/L)	AST (U/L)	ALP (U/L)
1 (Control)	$25.367^{a} \pm 0.351$	$85.867^{a} \pm 1.380$	$130.200^{a} \pm 0.625$
2 (Alloxan)	$98.700^{b} \pm 4.912$	$160.200^{b} \pm 2.740$	$163.500^{b} \pm 1.054$
3 (Extract)	$30.000^{ac} \pm 0.954$	$101.200^{c} \pm 3.208$	$128.533^{ac} \pm 1.747$

Values are Mean ± Standard Deviation. Values with the different superscript are statistically significant at $p< 0.05$. Values with the same superscript are not statistically significant at $p <0.05$

Results of renal function biomarkers of male albino rats treated with aqueous *Ocimum gratissimum* leaves extract are presented in Table 2. Treatment with alloxan increased blood urea and creatinine levels and decreased blood sodium level significantly ($p < 0.05$) compared to Control. Treatment with the extract increased blood urea and sodium level and decreased blood creatinine and chloride levels significantly ($p < 0.05$) compared to the alloxan-only treated group. Alterations in blood potassium levels were minimal among the treated groups investigated.

Table 2: Renal function biomarkers of Wistar rats treated with aqueous *Ocimum gratissimum* leaves extract.

GROUP	UREA (µmol/L)	CREATININE (µmol/L)	POTASSIUM (mmol/L)	SODIUM (mmol/L)	CHLORIDE (mmol/L)
1	$6.067^{a} \pm 0.153$	$90.867^{a} \pm 1.069$	$4.533^{a} \pm 0.116$	$133.330^{a} \pm 1.528$	$104.67^{a} \pm 1.528$
2	$9.133^{b} \pm 0.058$	$104.133^{b} \pm 3.803$	$4.733^{ab} \pm 0.058$	$121.670^{b} \pm 1.528$	$104.00^{a} \pm 3.000$
3	$9.733^{c} \pm 0.058$	$95.100^{ac} \pm 2.750$	$4.867^{bc} \pm 0.058$	$136.67^{ac} \pm 1.526$	$98.000^{b} \pm 2.000$

Values are Mean ± Standard Deviation. Values with the different superscript are statistically significant at $p <0.05$. Values with the same superscript are not statistically significant at $p <0.05$

DISCUSSION

From the findings of this study it can be deduced that the administration of aqueous *Ocimum gratissimum* leaves extract (AOLE) has more ameliorative effects on the liver than the kidney

of alloxan-treated Wistar rats. Alloxan treatment increased the ALT, AST and ALP values of the Groups 2 rats compared to control (Group 1). This shows that the induced hyperglycaemia may lead to an increase in hepatic enzyme activities in blood which is an indication of possible liver damage. However, administration of the AOLE reduced the ALT and ALP activity of the treated rats (Group 3) to levels not significantly different ($p > 0.05$) from the untreated control (Group 1). This may be attributed to the unique composition of bioactive phytochemicals in the *Ocimum gratissimum* leaves which helps to ameliorate the harmful hepatic effect leading to the reduced ALT and ALP values in blood. This effect which can be exploited for therapeutic purposes is consistent with the report of [8] that aqueous leaves extract of *Ocimum gratissimum* possesses anti-hyperglycemic effects and is relatively safe for use in the treatment of diabetics.

The AST, ALT and ALP results also aligns with the earlier findings of [4] who reported possible hepatoprotective activity of *Ocimum gratissimum* leaf extract in rabbits because of the presence of flavonoids and saponins.

There were alterations in renal function parameters of the treated rats compared to the control. When alloxan was administered to the rats in Group 2, it resulted in an increase in blood urea when compared to those of the control group not administered alloxan. This shows that the hyperglycaemia induced by alloxan could impair renal function leading to accumulation of urea in blood. There was also a significant ($p < 0.05$) increase in blood urea when AOLE was administered to the rats in Group 3 compared to Group 2 and the control (Group 1) indicating that AOLE exacerbated that toxic effect.

Blood creatinine concentration was significantly ($p < 0.05$) increased on administration of alloxan to rats in Group 2 compared to the control group but there was reduction of the creatinine level following administration of the extract to levels comparable with control (Group 1). This shows that the AOLE has ameliorative effect on the creatinine concentration in the hyperglycaemic induced rats.

There was no statistical significant difference in the value of potassium between those rats that were administered with alloxan (Group 2) from those not induced with alloxan (Group 1), showing that the induced hyperglycaemia had no significant effect on the potassium level. But there was a significant ($p < 0.05$) rise in potassium value of rats administered AOLE (Group 3) compared to those of control (Group 1). This means AOLE may impair renal function leading to accumulation of blood potassium in the hyperglycaemic induced rats.

Blood sodium reduced significantly in rats administered alloxan (Group 2) as compared to control without alloxan (Group 1) indicating that the induced hyperglycaemic condition led to

increased sodium loss in the rats. However, administration of AOLE increased the sodium concentration significantly ($p < 0.05$) to levels comparable with control (Table 2). This shows that the AOLE may exert renal effects that could lead to accumulation of sodium in the blood of the hyperglycaemic induced rats.

There was no significant difference between the levels of chloride in Group 2 as compared to the control group, but there was a significant decrease in the value of chloride between the control group and Group 3. This implies that the induced hyperglycaemic condition did not significantly alter the blood chloride level but AOLE reduced the level of chloride ions in the blood significantly ($p< 0.05$). The significant ($p< 0.05$) increases in blood urea, potassium and sodium levels as well as significant ($p< 0.05$) decrease in blood chloride levels following administration of the aqueous *Ocimum gratissimum* leaves extract suggest potential nephrotoxic effect that may require monitoring if the extract is used for therapeutic purposes.

CONCLUSION

Oral administration of aqueous *Ocimum gratissimum* leaves extract on alloxan-treated male Wistar rats significantly reduced the elevated hepatic enzyme biomarkers in blood, and altered renal function biomarkers like blood urea, potassium, sodium and chloride. These results suggest that the extract may have potential hepatoprotective and nephrotoxic effects.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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