P-ISSN: 2811-1516
E-ISSN: 2811-1524

AL-HIKMAH JOURNAL OF HEALTH SCIENCES (AJOHS)
Official Publication of Faculty of Health Sciences,
Al-Hikmah University Ilorin, Nigeria
ww.alhikmahuniversity.edu.ng/centralJournal

Original Article

Effect of Aqueous Extract of *Ocimum gratissimum (Scent Leaf)* on Renal Profile of Male Wistar Rats

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ARTICLE INFO

Article history:
Received 29 May 2021
Accepted 10 September 2021
Available online 30 October 2022

Keywords
Ocimum gratissimum
Electrolytes
Urea
Creatinine

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ABSTRACT

Humans have given the leaves of *Ocimum gratissimum* (*O. gratissimum*) a great deal of attention due to their vast medical usefulness, culinary application, and pharmacological effects. As a result, the study evaluated the effect of *O. gratissimum* on renal profile of male Wistar rats. Ten healthy male Wistar rats were randomized into test and control groups comprising of 5 animals each. The test group received 400mg/Kg *O. gratissimum* leaf extract whereas the control group was fed with normal rat diet and water. The rats were euthanized through cervical dislocation at the end of the experiment and blood sample was collected through heart puncture for serum assessment renal profile while the kidney was excised for histological examination. The Results showed a non-significant at $p>0.05$ increase in serum electrolytes and urea while significant at $p<0.05$ decrease in the mean serum creatinine level was observed in the test group compared to the control group. Also, the histological examination of the kidney tissue revealed a mild lymphocytic infiltration in the test group compared to the control group. Conclusively, the study suggested a dose-dependent toxicological effect of *O. gratissimum* on renal profile.

Please cite this article as: Busari, A.O., Salau, F.A., Bashir, S., Bolaji, M., Balogun, M.E., Ibrahim, M., Garuba, W.O., Popoola, N.A., Adunmo, G.O., Lawal, A.Z., Ogunwale, K.T., Adedokun, K.A., Ogundiran, S.M., Ahmad, M.B., Mohammed, I.Y., Akinola, F.F., Adeosun, O.G. (2021). Evaluation of Serum Creatine Kinase and Lactate Dehydrogenase Activities in Hypertensive Patients Attending General Hospital Ilorin, Kwara State, Nigeria, *Al-Hikmah Journal of Health Sciences, 1*(1), 7-13.

P-ISSN: 2811-1516
E-ISSN: 2811-1524

Introduction

O. gratissimum is an herbaceous plant that belongs to the *Labiatae* family from the genius *Ocimum* (Nadkarni, 1999). The plant is indigenous to tropical areas, especially in India and West Africa. It is known by various names in different parts of the world, in the case of Nigeria, the plant is called "effinrin-nla" by the Yoruba-speaking tribe, "Ahuji" by the Igbos, and "Daidoya" by the Hausas (Effaraim *et al*., 2013). *O. gratissimum* is a shrub up to 1.9m in height with stems that are branched. The leaves measure up to 10 x 5 cm, and are ovate to ovate-lanceolate, sub-acuminate to acuminate at apex, cuneate and decurrent at the base with a coarsely crenate, serrate margin, pubescent and dotted on both sides (Lawrence, 2017).

The phytochemical study of the plant shows the presence of several bioactive compounds. The aqueous leaf extract assay shows the presence of steroids, tannins, flavonoids, saponins, terpenoids alkaloids, inulins, phenolic compounds, B-carotene, glycosides (Offiah *et al.,* 1999, Nwanjo *et al.,* 2007) carotenoids, reducing sugars, phlobatannins, anthraquinones and cardiac glycosides (Chetia *et al.,* 2014) with steroidal ring and deoxy–sugar (Akinmoladun *et al.,* 2007). Besides these polyphenols, quinones, coumarins, and catechins (Vilioglu *et al.,* 1998) were also detected in aqueous extract. The methanolic leaf extract shows the presence of flavonoids, alkaloids, tannins, terpenoids, phlobatannins and cardiac glycosides with a steroidal ring (Akinmoladun *et al.,* 2007; Macdonald *et al.,* 2010).

O. gratissimum has been used extensively in the traditional system of medicine in many countries. In the coastal areas of Nigeria, the plant is used in the treatment of epilepsy, high fever gfand diarrhea (Effaraim *et al*., 2013). In the Savannah areas decoctions of the leaves are used to treat mental illness (Akinmoladun *et al*., 2007). *O. gratissimum* is used by the Ibos of Southeastern Nigeria in the management of the baby's cord, to keep the wound surfaces sterile. It is also used in the treatment of fungal infection, fever, cold and catarrh (Ijeh *et al*., 2005). The tribal of Nigeria use the leaf extract in treatment of diarrhoea, while the cold leaf infusions are used for the relief of stomach upset and hemorrhoids (Kabir *et al*., 2005). The plant is commonly used in folk medicine to treat different diseases such as upper respiratory tract infections. There have been speculations that scent leaves may possess hypoglycemic activity in streptozotocin-induced diabetic (Egesie *et al.*,2006).

Although *O. gratissimum* may be useful in culinary dishes and in the treatment of certain ailment, systemic toxicity is also possible and this is dose dependent. Extract of *O. gratissimum* has shown to have sedative activity and to have therapeutic benefit in patients with inflammatory joint disease. However, there is paucity of information concerning the adverse effect of continuous administration of aqueous preparation of *O. gratissimum* on some important organs of the body such as the kidney.

Materials and Methods

Experimental Animals

Healthy male Wistar rats of at least 8weeks old weighing between (140-160) g were used for this study while female Wistar rats were excluded to avoid interference of reproductive hormone while this experiment lasted. The animals were obtained from Animal House of Kwara State University Malete (KWASU) housed in a well-constructed cage and allowed to acclimatize for 14 days. The cage was well ventilated with a controlled environmental condition of 12 h of light/day cycle, the temperature of 21-31ºC, and relative humidity of 45-55%. All animals were made to receive humane care following the principle of laboratory animal care of the National Society of Medical Research (National Institutes of Health Publications no. 80-23, revised 1978) and approved by Kwara State University (KWASU) Ethical review committee on Animal care with reference number KWASU/CRIT/REA/2021/007.

Plant Collection and Identification

Fresh leaves of *O. gratissimum* were obtained from the Ilorin township market in Ilorin, Nigeria. The plant was identified, authenticated, and deposited at the herbarium unit of the Department of Plant Biology, Faculty of Life Sciences, University of Ilorin, Kwara State with the voucher number UILH/001/1356/2021.

Aqueous Extract Preparation

The dried form of the plant was mixed with sterile distilled water in a ratio of 1:10 (100g in 1L solvent), and was stirred and heated for about 4 hours. After cooling, the extract was filtered by using Whatman No.1 filter paper under aseptic conditions into a fresh sterilized glass tube. The final concentration of 1gm/ml was obtained as an aqueous extract which served as the stock solution for dilutions needed during the course of the work.

Phytochemical Analysis of the Extract

A small portion of the dry extract was used for a phytochemical screening test (Harbourne, 1973; Trease and Evans, 1983). Dragenorffs reagents were used to test for alkaloids, ferricholoride for tannins, aluminum chloride for flavonoids, Folin-Ciocalteu reagents and sodium carbonate for phenolics, pyridine

P-ISSN: 2811-1516
E-ISSN: 2811-1524

and sodium nitroprusside reagents for glycoside while Benedict's solution was used to test for saponins.

Experimental Design

The 10 healthy male rats were divided into 2 groups; all the experiments were carried out within two weeks. The different groups were classified as described in the table 1 below:

Table 1: Animal groups with respective treatments

Groups	Species	Gender	Treatment	Total number of animals
Control	Rats	Male	Healthy rats + Rat diet + water	5
Test	Rats	Male	Healthy rats + Rat diet + water + 400 mg/kg of *O. gratissimum extract*	5

All rats had access to food and water throughout the duration of study and were observed daily for toxicity. The administration of 400 mg of the plant extract to the test group was done using oral gavage following OECD's guidelines. At the end of the feeding period, the animals were sacrificed and blood sample was collected for analysis.

Blood Sample Collection and Processing

After two weeks of treatment, the animals were sacrificed through cervical dislocation and about 2-3 mls of blood sample was collected through heart puncture into a plain bottle. After clotting, it was centrifuged at 3000 rpm for 5 minutes and the serum was collected into a plain tube for quantitative estimation of serum electrolytes, urea, and creatinine.,

Protocol for Organs Harvesting

After 4 weeks of the experiment, the animals were sacrificed through cervical dislocation and dissected to excise the kidney. The organs were fixed immediately in 10% formal saline for 48 hours, so as to keep the organs in a life-like manner as possible and to prevent autolysis and putrefaction (Avwioro, 2014).

Laboratory Procedure

Electrolyte Estimation by Ion selective electrode (Tietz, 1983)

Principle

An Ion-Selective Electrode (ISE) makes use of the unique properties of certain membrane material to develop an electrical potential (electromotive force, EMF) for the measurements of ions in solution. The electrode has a selective membrane in contact with both the test solution and an internal filling solution. The internal filling solution contains the test ion at a fixed concentration. Because of the particular nature of the membrane, the test ions will closely associate with the membrane on each side. The membrane EMF is then determined by the difference in concentration of the test ion in the test solution and the internal filling solution.

Urea Estimation by Urease-Berthelot Method (Weatherburn, 1967)

Principle

Urea in serum is hydrolyzed to ammonia in the presence of urease, the ammonia in the presence of phenol and hypochlorite using sodium nitroprusside as catalyst form blue colour of indophenol that is measured at 546 nm.

Calculation

$$\text{Urea Conc.}\,(\text{mmol/L}) = \frac{\text{Abs. of test}}{\text{Abs. of std}} \times \text{Conc. of std}$$

Creatinine Estimation by Jaffe reaction (Jaffe, 1886)

Principle

In an alkaline medium, creatinine from a protein free filtrate, react with picric acid to form an orange-colored complex, the intensity of which is proportional to the concentration of creatinine in the sample measured at wavelength of 520nm.

$$\text{Creatinine Conc.}\,(\mu\text{mol/L}) = \frac{\text{Abs. of test}}{\text{Abs. of std}} \times \text{Conc. of std}$$

Histological Analysis

The histological examination of the kidney tissue was carried out following Harris hematoxylin and eosin staining technique (Avwioro, 2014). The paraffin sections were prepared and stained with haematoxylin and eosin. The thin sections of the kidneys were made into permanent slides and examined under high (400x) resolution microscope with photographic facility and photomicrographs were taken.

Statistical analysis

Statistical package for social science (version 20) was used for the statistical analysis. All measured data were presented in Mean ± standard error of the mean (SEM). Student t- test was used to compare the mean variables of the renal profile parameters between the test and control groups. Level of significance was considered at $p<0.05$.

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Result

Phytochemical Analysis Results

The result of phytochemical analysis of *O. gratissimum* contains tannins, flavonoids, phenolics, pholobatannnis, alkaloids, terpenoids glycoside, steroids and phytic acid in varying concentrations. Table 2 below shows the qualitative and quantitative result obtained from phytochemical results respectively.

Table 2: Phytochemical Analysis of *O. gratissimum*

Phytochemicals	Observation	Quantity (mg/100g)
Flavonoids	+	6.33±0.06
Phenolics	+	13.61±0.60
Tannins	+	6.52±0.12
Saponins	-	5.52±0.30
Phlobatannins	+	
Alkaloids	+	1.62±0.05
Terpenoids	+	2.93±0.43
Glycoside	-	
Phytic acid	+	1.51±0.05
Steroids	+	1.12±0.02
Anthraquinones	-	

*(+) = detected; (–) = Not detected n=3, SEM); (P<0.05)

The table shows the qualitative and quantitative phytochemical results. The quantitative values are expressed as mean ± SEM.

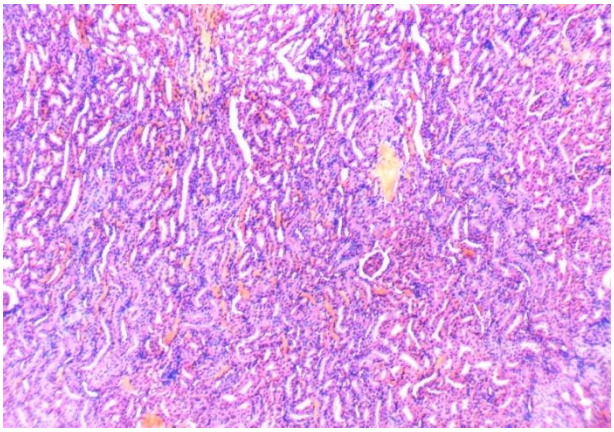
Table 3: Comparison of the Biochemical Renal Profile of the Test and Control groups

Parameters	Test Group (Mean ± SEM)	Control group (Mean ± SEM)	P-value
Sodium (mmol/L)	149.70 ± 7.77	130.30 ± 2.63	0.695
Potassium (mmo/L)	6.81 ± 1.18	4.11 ± 0.61	1.000
Bicarbonate (mmol/L)	25.15 ± 0.75	22.50 ± 0.85	1.000
Chloride (mmol/L)	115.75 ± 6.07	99.00 ± 3.65	0.865
Urea (mmol/L)	5.78 ± 0.36	2.47 ± 0.13	1.000
Creatinine (mmol/L)	232.50 ± 65.93	278.70 ± 49.99	0.000*

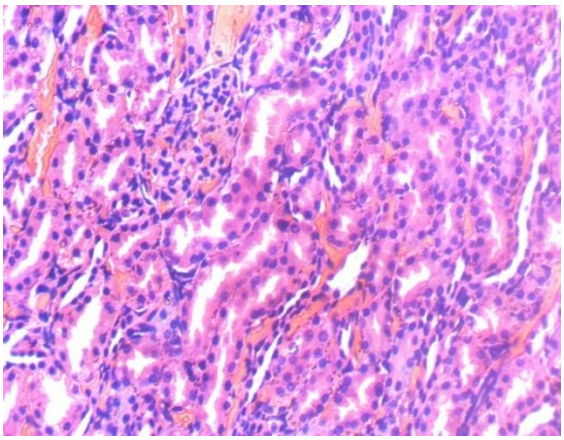
The values are expressed as mean ± SEM and the level of significance was considered at p<0.05.

Table 3 above showed the student test comparison of biochemical renal profile between the test group administered with aqueous extract of *O. gratissimum* and negative control group at P < 0.05 level of significance. The serum electrolytes and Urea were observed to be non-statistically increased at p > 0.05 in test group when compared to the control group while serum creatinine level was significantly at p < 0.05 higher in the control group compared to the test group.

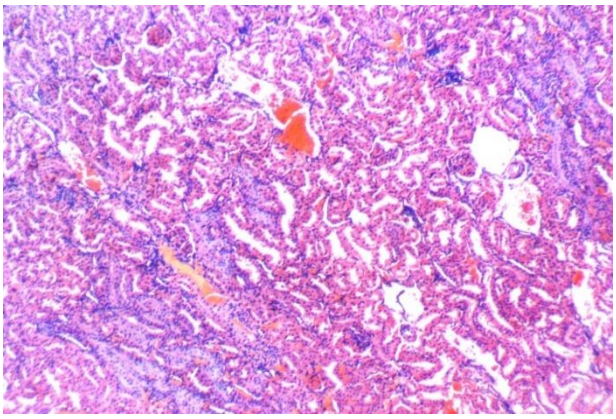
P-ISSN: 2811-1516
E-ISSN: 2811-1524



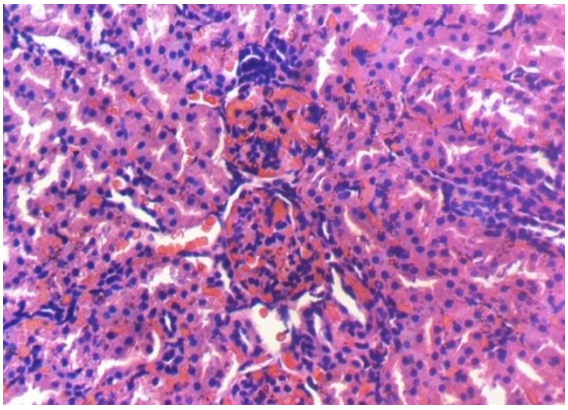
A: Light photomicrographs of kidney tissue of rats section group (H &E X100)



B: Light photomicrographs of kidney in the tissue of rats in the control group (H &E X400)



C: Light photomicrographs of kidney tissue section of rats administered with 400mg/kg/ body weight of aqueous extract of *O. gratissimum* (H &E X100)



D: Light photomicrographs of kidney tissue section of rats administered with 400mg/kg/ body weight of aqueous extract of *O. gratissimum* (H &E X400)

Figure 1: Kidney tissue sections of the test and control groups stained with H&E. Plates A and B showed light photomicrographs of kidney tissue section of rats in the control group at MG X100 and X400 respectively. The tissue sections show renal tissue with preserved architecture comprising normal glomeruli, tubules and vessels while plates C and D show light photomicrographs of kidney tissue section of rats in the test group at MG X100 and X400 respectively. The tissue sections show renal tissue with preserved architecture comprising normal glomeruli, tubules and vessels with mild lymphocytic infiltration.

Discussion

O. gratissimum is an herbaceous plant belonging to the *Labiatae* family from the genius *Ocimum* (Nadkarni, 1999). In Nigeria, the plant is used in the treatment of epilepsy, high fever and diarrhea, mental illness, fungal infection, fever, cold, catarrh and management of the baby's cord, (Ijzeh *et al*., 2005; Akinmoladun *et al*., 2007; Effaraim *et al*., 2013).

In this study, phytochemical analysis of *O. gratissimum* leaf revealed that it contains tannins, flavonoids, phenolics, pholobatannnis, alkaloids, terpenoids glycoside and phytic acid and steroid at varying concentration which is consistent with the components of the plant reported by Offiah and Chikwendu (1999). Following the course of administration of *O. gratissimum* extract for two weeks, a physical reduction was observed in the test group compared to the control that is in consonance with the finding of Valey *et al.* (1995) who reported significance reduction of body weight following increase intake of medicinal herbs.

This study observed a non-significant at $p > 0.05$ increased in serum level electrolytes in the test group compared to control group which is in agreement with the study of Abdullahi (2012) who reported a non-significant change in the serum electrolytes (Na^+, K^+, Cl^- and HCO_3^-) which indicates that *O. gratissimum* maintained electrolyte balance. Also, non-significant increase in mean serum urea level was observed in the

P-ISSN: 2811-1516
E-ISSN: 2811-1524

test group compared to the control group at p<0.05 level of significance. This finding is consistent with the studies of Pedraza-Chaverrı *et al.* (2000) and Maldonado *et al.* (2003) who established inhibition of protein synthesis in renal cells with consequent abundance of amino acid in the kidney resulting in increased urea levels. Increase urea level is a common biomarker associated with prediction of nephritis, renal ischemia, urinary tract obstruction, and extra renal diseases. However, this study is in contrast with the work of Ajith *et al.* (2007) who reported the presence of polyphenols flavonoids in *O. gratissimum* extract might be responsible for the antioxidant nephroprotective activities and the reduction of serum urea levels.

Our study observed a significant at P < 0.05 reduction in mean serum creatinine level in the test group compared to the control group that is in contrast to the research of Treasure (2003) who revealed an increased creatinine level as a common index of glomerular function in herbal administration. Kidney damage can be caused by creatinine accumulation in the blood. Hence, a high level of blood creatinine will indicate kidney damage. Creatinine is produced endogenously and released into body fluid at a constant rate and its plasma concentration is maintained within narrow limits predominately by the glomerular filtration (Ochei and Kolhaktar 2007; Tietz, 2012).

Histological analysis of the kidney tissue section revealed the presence of mild lymphocytic infiltration in test group compared to the control group. However, this observed alteration has no significance in anatomical architecture of the kidney which agrees with the study of Adamu *et al.* (2008), who reported that the pathological lesion observed in the kidney is as a result of degeneration which might be due to active properties of the plant.

Conclusion

This study observed an insignificant increase in serum electrolytes and urea with a significant reduction in serum creatinine level following the administration of aqueous extract of *O. gratissimum* which is suggestive that *O. gratissimum* is ethnomedicinal at lower doses but could be nephrotoxic at higher doses.

Conflict of Interest: None

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