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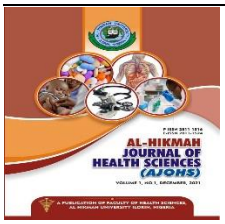

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Original Article

Effect of Aqueous Extract of *Ocimum gratisimum (Scent Leaf)* On Hepatic Profile of Male Wistar Rats

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ABSTRACT

Ocimum gratissimum (*O. gratissimum*) is an herbaceous plant commonly found in tropical Asia and in the coastal areas of Nigeria, where it is used for the treatment of ailments such as diarrhea, urinary infections, fever, and dysentery. This study thus evaluated the effect of oral administration of *O. gratissimum* on the hepatic profile of male Wistar rats. Ten (10) healthy rats weighing between 140-160 g were randomly distributed into control and test groups comprising 5 animals each. After acclimatization for two weeks, the test group was administered with 400mg/Kg crude extract of *O. gratissimum* for two weeks. The animals were euthanized through cervical dislocation at the end of the experiment and blood sample was collected via heart puncture into plain bottles for serum biochemical hepatic profile assay while the liver was excised for histological examination. The serum liver enzymes activities, bilirubin, albumin, and total protein were assayed colorimetrically while the histological examination followed the hematoxylin and eosin staining (H&E) of the liver tissue. The results of the liver enzyme activities, total protein, total and direct bilirubin levels showed a significant at $p < 0.05$ increase in the test group when compared with the controls while the observed increase in serum albumin level of the test group was not statistically significant at $p > 0.05$ compared to the controls. The histological analysis also revealed a mild lymphocytic infiltration of the liver tissue in the test group compared to controls. The observed alteration in the hepatic profile is suggestive that the toxicological effect of plant is dose dependent

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Introduction

Herbal medicine was practiced by the ancient people of Asia, Europe, and the Americas (Wargovich *et al.*, 2001). Also, over 50% of all modern clinical drugs are of natural product origin and play important roles in drug development programmers. Among the enormous number of these medicinal plants are members of the genus *O. Lamiaceae*. The genus is represented by six species in West Africa. However, only three species, *O. gratissimum, O. basilicum*, and *O. canum Sims* have been reported to have medicinal properties. *O. gratissimum* (commonly known as scent leaf) is known as Ahuji in Igbo, Effirin in Yoruba, Daidoya in Hausa, and Aramogbo in Edo (Ephrain *et al.*, 2000) which is used mainly to flavor food and meat. The plant is commonly used in folk medicine to treat different diseases such as upper respiratory tract infections, diarrhea, headache, and diseases of the eye (Adebolu *et al.*, 2005). It has been shown to possess antibacterial activity (Ofokansi *et al.*, 2003),

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antioxidant properties (Oboh *et al*., 2008), antimicrobial and antihelminthic activities (Sofowora, 2008) and hepatoprotective properties (Gbolade *et al*., 2009). *O. gratissimum* is grown for the essential oils in its leaves and stems and various research works have been designed to evaluate the various potentials of extract from the leaves of *O. gratissimum* and to explore its basis for traditional use. Overconsumption without any precautionary measures in its dosage has been reported to have adverse effects on the body system and thus, became imperative to study the effects of its oral administration on the hepatic profile of albino Wistar rats.

Materials and Method

Experimental Animals

Healthy male Wistar rats of at least 8weeks old weighing between140g-160g were used for this study, female Wistar rats was excluded to avoid interference of reproductive hormone while this experiment lasted. Ten (10) healthy male rats 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 12hours of light/day cycle, temperature of 21-31ºC and relative humidity of 45-55%. All animals were made to receive humane care following the principle of laboratory animals 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* was obtained from Ilorin township market at 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.

Experimental Design

The 10 healthy male rats were divided into 2 groups of test and control groups 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/Kg of the plant extract to the test group was done using oral gavage following OECD's guidelines (Erhirhie *et al.,* 2014). At the end of the treatment, the animals were sacrificed, blood sample was collected and liver was harvested 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 via heart puncture into plain bottle. After clotting occurred, it was centrifuged at 3000rpm for 5minutes and the serum was collected into a plain tube for quantitative estimation of serum electrolytes, urea and creatinine.

Protocol for Organs Harvesting

The animals were dissected to excise the liver and fixed immediately in 10% formol 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

Total protein estimation using the Biuret method (Gornall *et al.,* 1949)

Principle: Peptide bonds of proteins react with Cu^{2+} in alkaline solution to form a colored complex whose absorbance, proportional to the concentration of total protein in the specimen, is measured at 540 nm.

Quantitative Estimation of Albumin by Bromocresol Green Method (Bartholomew & Delaney, 1966).

Principle: The measurement of serum albumin is based on its quantitative binding to the indicator 3, 3', 5, 5'-tetrabromo-m cresol sulphonephthalein (bromocresol green, BCG). The albumin-BCG-complex absorbs maximally at 630 nm, the absorbance being directly proportional to the concentration of albumin in the sample.

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Alanine Aminotransferase enzyme activity assay by colorimetric method (IFCC, 1990)

Principle

Alanine aminotransferase catalyzes the transfer of amino group from Alanine to oxoglutarate with the formation of glutamate and pyruvate. The latter is reduced to lactate dehydrogenase in the presence of reduced nicotinamide adenine dinucleotide (NADH).

ALT

2-oxoglutarate + L-alanine ----------→L-glutamate + Pyruvate

MDH

Pyruvate + NADH + H^+——> L-lactate + NAD^+

Aspartate Aminotransferase enzyme activity assay by colorimetric method (IFCC, 1990)

Principle:

Aspartate aminotransferase catalyzes the transfer of the amino group from asparate to α-ketoglutarate with the formation of glutamate and oxaloacetate. The latter is reduced to malate by malate dehydrogenase (MDH) in the presence of reduced nicotinamide adenine dinucleotide (NADH).

AST

α-ketoglutarate + L-aspartate ——> L-glutamate + Oxaloacetate

MDH

Oxaloacetate + NADH + H^+——> L-malate + NAD^+

Alkaline Phosphatase enzyme activity assay (IFCC, 1990)

Principle:

Alkaline phosphatase hydrolyses *p*-nitrophenyl phosphate to *p*-nitrophenol and phosphate. The phosphate is transferred to AMP (2-Amino-2-methyl-1-propanol.) The increase in absorbance at 405 nm at 37^{o}C is measured and this is proportional to the amount of alkaline phosphatase that is present in the sample.

ALP

p-nitrophenyl phosphate + H_2O--------→ phosphate + p-nitrophenol

Quantitative Estimation of Bilirubin by Colorimetric Method (Jendrassik –Grof, 1938)

Principle: Direct (conjugated) bilirubin reacts with diazotized sulphanilic acid in an alkaline medium to form a blue-colored complex. Total bilirubin is determined in the presence of caffeine, which releases albumin-bound bilirubin, by the reaction with diazotized sulphanilic acid.

i. Total bilirubin:

Sulfanilic acid + NO_2^- -----→Diazo Salt + Bilirubin----------→Azobilirubin (600nm)

ii. Direct bilirubin:

Sulfanilic acid + NO_2^- ---------→Diazo Salt + Bilirubin----------→Azobilirubin (550nm)

Histological examination of tissue stained by Haematoxylin and eosin staining Technique

Thin slices (about 5mm) of the fixed liver organs were cut, put in tissue cassettes and labelled appropriately; they were processed using paraffin wax tissues processing method with the aid of LEICA PT 1020 Automatic Tissue Processor. Subsequently, the sections were stained with Mayer's Haematoxylin (Nuclear stain), differentiated with 1% acid alcohol (differentiator) and counter stained with Eosin Y (Cytoplasmic stain). The stained tissue sections were examined using x100 objective lens of the microscope for focusing and later viewed with x400 objective lens of the microscope for a higher magnification.

Statistical Analysis

Statistical package for social science (version 20) was used for the statistical analysis. The variables were expressed in Mean ± standard error of Mean (SEM). The student t-test was adopted to compare the mean of the biochemical hepatic profile between the test and control groups. Levels of significance was considered at $P<0.05$.

Results

Phytochemical Analysis Results

The results of the phytochemical analysis test performed show that the leaf of *O. gratissimum* contains flavanoids, phenolics, tannins, phlobatannins, alkaloids, terpenoids, phytic acid and steroids in varying concentrations. Table 2 below shows the qualitative and quantitative results obtained from the phytochemical components screening respectively.

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Table 2: Qualitative and Quantitative Phytochemical Results

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=5, SEM); P<0.05)

Effect *O. gratissimum* on biochemical hepatic profile

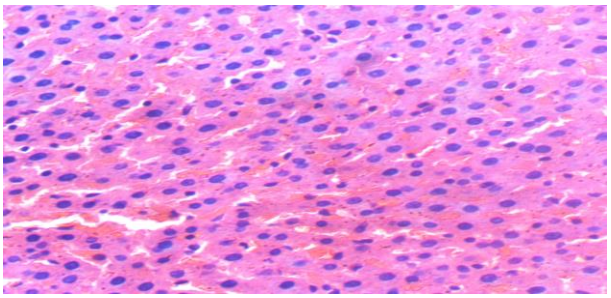
A total of 10 male Wistar rats were categorized into two different groups based on the treatment i.e., the control group (n=5) and test group (n=5) given leaf extract of *O. gratissimum*. Table 3 shows the comparison of the biochemical hepatic profile of test groups of male Wistar rats administered with aqueous extract of *O. gratissimum* and the negative control group at p>0.05 level of significance. The student t-test was used to compare the mean of the parameters and significant at p < 0.05 differences were observed in the ALT, AST, ALP, total and direct bilirubin, and total protein levels between the test and control groups while no significant difference at p >0.05 was observed in the albumin levels

Table 3: Effect of *O. gratissimum* on biochemical hepatic profile

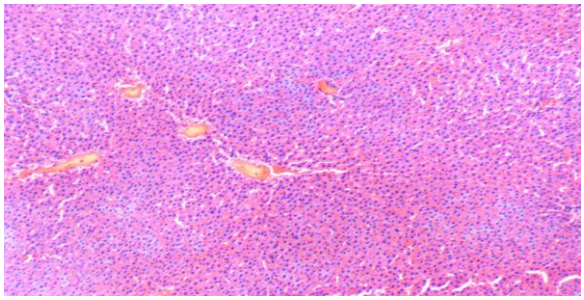
Parameters	Test Group (mean ±SD)	Control group (mean ±SD)	p-value
Total Bilirubin	37.85 ±2.03	22.80 ± 1.48	0.000*
Direct Bilirubin	21.60 ± 2.23	6.70 ± 1.25	0.000*
Albumin	41.80 ± 3.76	34.20 ± 3.55	0.310
Total Protein	78.00 ± 5.89	64.00 ±3.39	0.000*
ALT	86.75 ± 16.69	36.60 ± 4.79	0.000*
AST	24.10 ± 2.71	20.90 ± 2.92	0.000*
ALP	77.00 ± 6.27	64.40 ± 2.79	0.001*

All values are expressed as Mean ± SEM (n = 5 in each group). p<0.05 was considered significant*

Histological Examination of the Liver Tissue



A: Light photomicrographs of liver tissue sections of rats in the control group (H&E x 400).



B Light photomicrographs of sections from the liver of rats in the control group (H&E x100).

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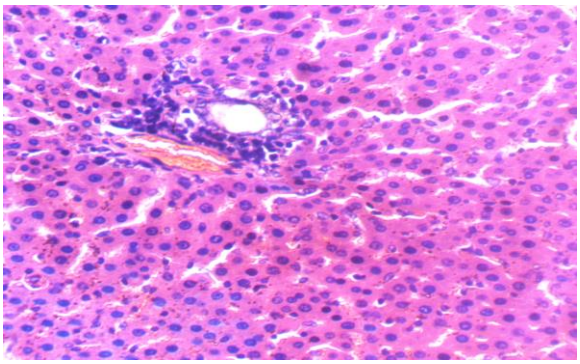

Plate C: Light photomicrograph of sections from the liver of rats administered with 400mg/kg body weight of aqueous extract of *O. gratissimum* (H&E x400).

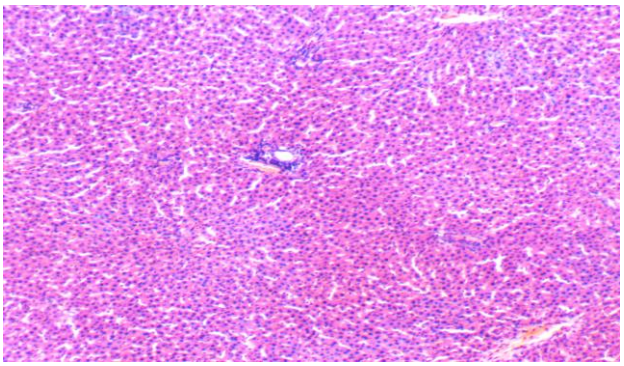

Plate D: Light photomicrograph of sections from the liver of rats administered with 400mg/kg body weight of aqueous extract of *O. gratissimum* (H&E x100).

Figure 1. Comparison of Liver Tissue sections stained with H&E in control and test groups. Plate A and B show a light photomicrograph of liver tissue sections of male Wistar rats in the control group at MG X400 and X100 respectively. The tissue sections show preserved architecture with normal hepatocytes and portal tracts. Plate C and D shows a light photomicrograph of liver tissue sections of male albino rats in the test group at MG X400 and X100 respectively. The tissue sections show preserved architecture with normal hepatocytes and portal tracts with mild lymphocytic infiltration.

Discussion

Herbal remedies are widely used for the treatment and prevention of various diseases and often contain highly active pharmacological compounds. Many medicinal herbs and pharmaceutical drugs are therapeutic at one dose and toxic at another. Toxicity related to traditional medicines is becoming more widely recognized as these remedies become popular in the Mediterranean region as well as Africa (Bashar *et al.*, 2006). The leaf of *O. gratissimum* possesses an antipyretic and anti-diarrhea activity (Sofowora, 1993) which has been used worldwide in the treatment of various ailments, including diabetes mellitus and is reported to have effect on both the hepatic and renal profile based on dose-dependence.

Phytochemical analysis revealed that *O. gratissimum* leaf contains flavanoids, phenolics, tannins, phlobatamims, alkaloids, terpenoids, phytic acid and steroids in varying concentrations which is consistent with components of the plant reported by some studies (Offiah and Chikwendu, 1999; Ojo *et al.*, 2013).

A physical reduction in the body weight was observed in the test group compared to the weight of the control group following the course of plant extract administration for two weeks which is in consonance with findings of Valey *et al.* (1995) that significant body weight reduction follows the increased intake of medicinal herbs.

This study observed marked significant at $p < 0.05$ increase in the serum liver enzymes activities including alanine aminotransaminase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) of the test group compared to controls. Elevated levels of AST, ALT and ALP are indications of hepatocellular injury due to derailment of hepatocytes which is in agreement with the study of Ajibade *et al.* (2011), who reported an elevated liver enzymes activity as indication of liver damage leading to reduced liver function. However, in contrast to this study, Yung *et al.* (2014) reported that *O. gratissimum* caused a significant reduction in the ALT and AST levels in male wistar rats.

We also observed a significant at $p < 0.05$ increase in the total and conjugated bilirubin levels of rats administered with aqueous extract of *O. gratissimum* when compared with the controls which is in consonance with the study of Egesie *et al.* (2006), who reported a rise in the concentration of serum bilirubin is suggestive of liver damage since the liver serves as an excretory unit rather than a distributing unit for bilirubin. It is removed from the blood by the liver; hence it is a good indicator liver function. Bilirubin concentration is elevated in the blood either by increased production of bilirubin or decreased liver uptake as result of liver disease.

More so, our study revealed a non-significant at $p > 0.05$ increase in albumin level of the test group compared to the controls. This result contradicts the work of Ojo *et al.* (2013) who reported low levels of serum albumin in rats administered with *O. gratissimum* as a result of the quantity of anti-nutrient factors that may affect the digestibility of the protein

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content of the extract. Albumin is the major protein present within the blood and represents a reliable test to assess the degree of liver damage in animals. Albumin which is manufactured by the liver is a major protein that circulates in the blood stream (Yakubu *et al*., 2003). However, the observed serum total protein levels of the test group were significantly at $p < 0.05$ increased when compared with the controls which might results from dehydration following plant extract administration.

The microscopical examination of liver tissue sections revealed the presence of lymphocytic infiltrations in test group compared to the controls and this alteration has no significant effect on the anatomical architecture of the liver. This observed lymphocytic infiltrations is in concordance with the study of Adamu *et al.* (2008), who reported observed pathological lesions in the liver might be as a result of degeneration accompanied by metabolism of active parts of the plant in the organ or their interference with fat metabolism. Thus, the observable liver pathological changes coupled with a significant increase in enzyme levels in the treated rats may have resulted from the toxicity potentials of the extract administered.

Conclusion

Results obtained in this study revealed that the aqueous extract of *O. gratissimum* administered at the investigated dose showed an adverse effect on the liver with markedly significant elevated levels of the hepatic profile of the treated animals. Thus, this study observed that the toxicological effect of the *O. gratissimum* extract on the liver enzyme activities and structure is dose-dependent.

Recommendations

It is recommended that further studies should be carried out in order to corroborate the findings revealed in this study. It is also recommended that the effect of *O. gratissimum* should be further investigated on other organs like the heart and brain.

Conflict of Interest: Nil

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