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Toxicity Studies of the Crude Aqueous Leaves Extracts of *Ocimum gratissimum* in Albino Rats

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Abstract
Objective: *Aqueous leaves extracts of Ocimum gratissimum has been reported to possess hypoglycaemic effects. Hence, the present study was aimed to investigated the LD_{50} of the crude aqueous leaves extracts of Ocimum gratissimum and the effect of sub-chronic doses of the extract on liver and kidney function parameters of albino rats.*
Materials and Methods: *Phytochemical and acute evaluation of the possible toxicity risk of Ocimum gratissimum aqueous leaves extracts were investigated in this study. Sixteen albino rats were randomly assigned to four experimental groups of four marked as control group, groups A, B, and C respectively. Groups A, B and C were treated with oral administration of aqueous extract of Ocimum gratissimum at 100mg, 200mg and 400mg/kg body weight daily respectively for two weeks. Control group received no treatment for subchronic toxicity.*
Results: *Phytochemical constituents were detected in the leaves extracts. The median lethal dose (LD_{50}) of the aqueous leaves extracts was calculated to be 4.24 µg/kg body weight. It result showed that there was significant ($p<0.05$) decreased in the total bilirubin and conjugated bilirubin levels of rats administered with O. gratissimum when compared with those of the control. Significant reduction in the serum albumin was also observed while the serum total protein was insignificant. Results also showed that treatment of rats with the respective doses of the extract did not significantly alter the serum and liver levels of Alanine Transaminase and Aspartate Transaminase in all test groups. There was a significant increase in the activities ($P<0.05$) of AST and ALT in the kidney and serum which might be caused by activation of enzymes synthesis in renal cells.*
Conclusion: *These investigations thus indicate the toxic effects of the aqueous leaves extracts of Ocimum gratissimum at 4.5µg/kg and these could be attributed to the combined toxicity of the phytochemical constituents such as tannin, saponins, glycosides and alkaloids*
Keywords: *Aqueous Ocimum gratissimum extract, Phytochemical, LD_{50}, sub-chronic toxicity, albino rats, toxicological effect.*

I. Introduction

Herbal plants produce and contain a variety of chemical substances with varied physiological effects. They are huge reservoir of various chemical substances with potential therapeutic properties [1]. Herbal plants are being increasingly utilized to treat a wide variety of clinical diseases [2]. Herbs have been used by all cultures throughout history and thus, herbal medicine is the oldest form of health care known to mankind. It was an integral part of the development of modern civilization. Many drugs commonly used today are of herbal origin. Higher plants as source of medicinal compound continue to play a dominant role in maintenance of human health since antiquities [3]. The primary benefit of using plant derived-medicine is that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and affordable treatment [4]. However, it must be noted that not all medicinal plants are safe for consumption in the crude form. Some level of toxicity may arise as a result of potential toxic compounds they contain and pesticide application during cultivation [5, 6]. The therapeutic properties of medicinal plants used by traditional medical practitioners may be due to one or more of the many compounds of the plant material. These phytochemicals include complex carbohydrates, alkaloids, glycopeptides, phenols, terpenes, cardiac glycosides, terpenoids, tannins, cyanogens, peptides and amines, steroids, flavonoids, lipids, coumarins, sulphur compounds and inorganic ions among numerous others. Some of these compounds may be toxic, and thus the plants containing them, when consumed could confer varied levels of toxicity to the individual [7]. The growing interest in herbal medicine therefore demands toxicity risk assessment of the various indigenous preparations used in the treatment of diseases [8]. Phytochemical screening of the leaf extract of *Ocimum gratissimum* (OG) had shown the plant to contain alkaloids, saponins, tannins, alkaloids, anthraquinone, flavonoids, steroids, terpenoids and cardiac glycosides [11,10,12]. Furthermore, OG had been shown to possess diverse pharmacological properties which may be attributed to its usefulness in folk medicine. These properties include antioxidant [12,13,14] chemotherapeutic

[15]; antimutagenic [16]; antidiarrhoeal [9, 17, 18] antinociceptive [19] insecticidal [20] hypotensive [21] and antihelmintic [22,23]. The present study investigated the acute and sub-chronic toxicity effects of the crude aqueous leaves extract of Ocimum gratissimum in rats.

II. Materials And Methods

Collection and identification of plant materials

Fresh leaves of *O. gratissimum* were bought in the market at Ado-Ekiti, Nigeria. The plant was identified and authenticated by a plant scientist in the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria and a voucher specimen number U.H.A.E 15 was deposited accordingly at the herbarium of the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria.

Preparation of aqueous leaf extract of Ocimum Gratissimum

The leaves were air-dried in the laboratory at ambient temperature (30 ± 2°C) for 10 days, pulverized using a laboratory mechanical grinder (Christy and Norris limited, machine type 8) and the fine powders obtained stored until further use. 50g of the powdered sample was extracted with distilled water of 500mls (via maceration) for 48hrs. The mixture was decanted and filtered using sterile whatman paper No 1. The filterate measured up to 425mls and evaporated to dryness using a freeze dryer to obtain 8 % yield aqueous residue. The stock solution of the extract was prepared by dissolving 1g of extract in 10ml of water to give a concentration of 10mg/ml. The stock solution was labelled appropriately and refrigerated at 4℃ until required for use.

Phytochemical analysis of Ocimum Gratissimum

Chemicals tests were carried out on the extract using standard procedure to identify the constituents as described by [24, 25, 26].

Experimental Animals

After Institutional Animal Ethics approval, a total number of sixteen [16] inbred albino male rats weighing between 100-190 g were used in this study. The animals were obtained from the animal house of the Department of Chemical Sciences, Afe Babalola University, Ado-Ekiti, Nigeria. The animals were randomly distributed into cages and allowed to acclimatize for 7 days in a well ventilated room at a room temperature of 20.0±2.0°C under natural lighting condition. The animals were allowed free access to standard rat chow (Topfeeds Ltd., Ado-Ekiti, Nigeria) and distilled water *ad libitum*. Experimental procedure involving animals and their care were employed in conformity with guidelines for care and use of laboratory animals and the procedure approved by the Ethical Committee.

Acute toxicity study (Determination of LD_{50})

The median lethal dose (LD_{50}) of the plant extract was determined by method of Lorke (1983) [27] using twelve [12] rats weighing between 100 – 190g. In the first phase, four rats were divided into two groups of two rats each and were treated with the aqueous leaf extract of the plant at dosages of 1600 and 2000mg/kg body weight intra-peritoneally. They were observed for 24 hours for signs of toxicity. In the second phase, eight rats were again divided into four groups of two rats each and were also treated with aqueous extract of *Ocimum gratissimum* at dosages of 3000, 3200, 4000 and 4500 mg/kg body weight intra-peritoneally. The median lethal dose (LD_{50}) was calculated using the second phase.

Experimental protocol

Animals were divided into four groups- A, B, C and control group respectively. Group A was given single daily doses of 100 mg kg^{-1} of OG for 14 days. Group B received single daily doses of 200 mg kg^{-1} of OG for 14 days. Group C was given single daily doses of 400 mg kg^{-1} of OG for14 days. The control group (group D), containing four animals, was given only distilled water daily for 14 days. OG was administered orally using a calibrated 1 mL syringe with attached polythene cannula. At the end of the treatment period, the animals were sacrificed using cervical dislocation. Blood samples were collected into EDTA sterile bottles. These were used for biochemical assay of alanine aminotransaminase (ALT) and aspartase aminotransaminase (AST) following the methods of [28] and [29].

Determination of Liver function Indices

Serum total protein was determined by Biuret method as reported by [30]. Albumin was determined according to method of [31]. Bilirubin (Colorimetric method) was carried out according to method of [32] and [33].

Statistical analysis

Data were expressed as Mean±SEM of mean. Comparisons between control values and values of treated groups of albino rats were performed with one-way Analysis of Variance (ANOVA) with the help of software SPSS 16.0 for windows. Statistical significance was set at $p<0.05$.

III. Results

Phytochemical screening: Alkaloid, saponin, phenol, tannin, cardiac glycoside, steroid, flavonoids, anthraquinones, terpenes and phlobatannins were detected in the aqueous leaves extracts while cardenolides and chalcones were absent. The result is shown in table 1.

Table 1: Phytochemical Screening of Leaf Extracts of *Ocimum gratissimum*.

Phytochemical	Extract Content
Alkaloids	+++
Tannin	++
Phlobatannin	++
Saponin	+++
Flavonoids	+
Anthraquinones	++
Steroids	+
Terpenes	+
Cardenolides	-
Phenol	+++
Chalcones	-
Cardiac glycosides	++

+ = Trace amount Present ++ = Moderate amount present, +++ = Appreciable amount present - = Completely Absent

Acute toxicity study (LD_{50})

While animals that received very high dosage died, other signs of toxicity were noticed 2 - 4 hours after extract administration. There were decrease locomotion, writhing, constipation and decreased in sensitivity to touch. Also, there was decreased feed intake and prostration 15 hours after extract administration. The median lethal dose was calculated as follows:

$LD_{50} = \sqrt{D_0} \text{ X } D_{100}$

Where D_0 = Dosage of 0 per cent mortality

D_{100} = Dosage of 100 per cent mortality

Thus,

$LD_{50} = \sqrt{4000} \text{ x } 4500$

$= \sqrt{18,000,000}$

LD_{50} = 4242.64 mg/kg.

Liver function indices

Liver function indices of rats administered with aqueous extract of *O. gratissimum* is shown in Table 2. There was no significant difference ($p>0.05$) in the total protein level of rats administered with *O. gratissimum* when compared with those of the control group. However significant ($p<0.05$) decreased was observed in the total bilirubin and conjugated bilirubin levels of rats administered with *O. gratissimum* when compared with those of the control. There was a significant ($p<0.05$) difference in the albumin levels when compared group 1 (100mg/kg) with those of control group. But no significant ($p>0.05$) difference when compared group 2 (200mg/kg), group 3 (400mg/kg) with those of group 1 (100mg/kg) and control group.

Table 3 shows the ALT Activity (U/L) of liver, kidney and serum of rats administered with *O. gratissimum* for 2 weeks. Significant ($p<0.05$) increase and decreased was observed in the ALT activities in liver of rats administered with *O. gratissimum* when compared with those of the control group. However, significant ($p<0.05$) increase and decreased was observed in the ALT activities in the kidney of rats administered with *O. gratissimum* when compared with those of the control group. Though, significant ($p<0.05$) increase was observed in the ALT activities in serum of rats administered with *O. gratissimum* when compared with those of the control group.

Table 4 shows the AST activity of liver, kidney and serum of rats administered with *O. gratissimum* for 2 weeks is shown in Table 3. When compared with those of the control, significant ($p<0.05$) increased were observed in

the activities of AST in the liver of rats administered with *O. gratissimum*, but there was no significant ($p>0.05$) difference in the activities of AST in the three groups. When compared with those of the control group, significant ($p<0.05$) increased and decreased were observed in the activities of AST in the kidney of rats administered with *O. gratissimum*. When compared with those of the control group, significant ($p<0.05$) increased was observed in the AST activities in the serum of rats administered with *O. gratissimum*.

Table 2: Liver function indices of rats administered with *Ocimum gratissimum*

Parameter	Control	Group 1 (100mg/kg)	Group 2 (200mg/kg)	Group 3 (400mg/kg)
Total bilirubin (mg/dl)	6.39 ± 0.12^{b}	4.99 ± 0.35^{a}	5.65 ± 0.19^{b}	4.90 ± 0.32^{a}
Conjugated bilirubin (mg/dl)	4.90 ± 0.04^{c}	3.60 ± 0.04^{a}	3.80 ± 0.08^{a}	4.20 ± 0.24^{b}
Albumin (g/l)	38.73 ± 0.62^{b}	33.13 ± 0.39^{a}	36.58 ± 2.13^{ab}	35.60 ± 1.57^{ab}
Total protein (g/l)	58.50 ± 1.64^{a}	59.35 ± 0.10^{a}	56.07 ± 0.74^{a}	56.60 ± 1.04^{a}

Values are expressed as mean of three determinations ± SEM
Row values with different superscripts are significantly ($p<0.05$) different

Table 3: ALT Activity (U/L) of Liver, Kidney and serum of Rats administered with *Ocimum gratissimum* for 2 weeks.

Tissues	Control	Group 1 (100mg/kg)	Group 2 (200mg/kg)	Group 3 (400mg/kg)
Liver	103.25 ± 2.18^{a}	114.55 ± 2.23^{b}	113.75 ± 0.38^{b}	101.05 ± 2.00^{a}
Kidney	52.45 ± 4.18^{b}	36.30 ± 4.80^{a}	73.73 ± 4.97^{c}	69.10 ± 2.03^{c}
Serum	10.50 ± 0.77^{a}	24.75 ± 3.50^{b}	56.50 ± 5.75^{c}	54.00 ± 1.58^{c}

Values are expressed as mean of three determinations ± SEM
Row values with different superscripts are significantly ($p<0.05$) different

Table 4: AST Activity (U/L) of Liver, Kidney and serum of Rats administered with *Ocimum gratissimum* for 2 weeks.

Tissues	Control	Group 1 (100mg/kg)	Group 2 (200mg/kg)	Group 3 (400mg/kg)
Liver	59.93 ± 0.13^{a}	68.83 ± 1.22^{b}	66.00 ± 1.55^{b}	68.93 ± 0.47^{b}
Kidney	56.88 ± 1.60^{a}	63.05 ± 4.35^{a}	55.00 ± 2.03^{a}	72.15 ± 0.10^{b}
Serum	21.60 ± 1.22^{a}	25.18 ± 0.38^{a}	61.13 ± 4.36^{b}	64.78 ± 2.67^{b}

Values are expressed as mean of three determinations ± SEM
Row values with different superscripts are significantly ($p<0.05$) different

IV. Discussion

The result of the Phytochemical screening of the aqueous leaf extract of *Ocimum gratissimum* showed no major differences in the Phytochemical constituents from the results of other previous investigations. Saponins, flavonoids, alkaloids, phenol, terpenes and steroids, tannins and cardiac glycosides were found to be present [12]. Tannins have been reported to act on proteins to form protective layer on mucus membranes [37]. Flavonoids have been found to have membrane stabilizing properties and also affect some process of intermediary metabolism and inhibit lipid peroxidation in different systems [34]. Phenols have antioxidant properties which carry out their protective activity on cells either by preventing the production of free radicals or by scavenging free radicals produced in the body [35,36]. Almost all the photo constituents of *Ocimum gratissimum* confirmed in this study are known to influence biological system activities. The median lethal dose was calculated to be 4.24 µg/kg body weight. A dosage of 1600 and 2000 mg/kg body weight of the aqueous leaf extract of *Ocimum gratissimum* was administered per oral to the test groups. No animal died following the administration of the dose used for the study. [37] had earlier administered a dosage of 250 – 1500 mg/kg body weight to achieve a dose dependent blood glucose reduction in STZ-induced diabetic rats [37]. It is clear from the present findings that this plant has no clinical safety at higher doses especially at the acute administration of the extract to rats. The concentrations of albumin and bilirubin in the serum of the animals can indicate the secretory and synthetic functioning of the liver and can be used to ascertain types of liver damage [38]. Bilirubin is the major breakdown product that results from the destruction of old red blood cells. It is removed from the blood by the liver; hence it is a good indication of the function of liver. Bilirubin concentration is elevated in the blood either by increased production of bilirubin or decreased liver uptake (as a result of liver disease). [39], reported that a rise in the concentration of serum bilirubin indicate or suggests liver damage since the liver serves as an excretory unit rather than a distributing unit for bilirubin. The result showed that there was significant ($p<0.05$) decreased in the total bilirubin and conjugated bilirubin levels of rats administered with *O. gratissimum* when compared with those of the control. This observation might suggest that the extract had no adverse effect on the liver.

Other important biochemical indices that can be used to assess the health status of liver are the serum levels of albumin. 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 [40]. Low serum albumin has also been associated with low protein intake. Animals that grow at a faster rate than others sometimes have higher serum albumin, Hb, glucose and low concentration of potassium [41]. Therefore, significant decrease in serum albumin was observed in rats administered with *O. gratissimum* when compared to the control group and this may be due to the quantity of antinutrient factors which affect the digestibility of the protein content of the extract. Though, the serum total protein of rats administered with *O. gratissimum* compared to the control group was insignificant. The measurement of the activities of 'marker' or diagnostic enzymes in tissues and body fluids play a significant and well known role in diagnostic disease investigation and in the assessment of drug or plant extract for safety/ toxicity risk [38]. Most of the enzymes tests used in diagnostic or as markers for cellular damage depend on the very high concentration of such enzymes within the cell relative to that in plasma or serum. Consequently, cellular damage arising from drug or chemical toxicity and diseases often result in measurable increase in enzyme activity in the extracellular fluid as the enzyme is released from the damage cell. Their presence in the serum may give information on tissue injury or organ dysfunction [42]. Aminotransferase which include Alanine aminotransferase (ALT) otherwise referred to as glutamate pyruvate transaminase (GPT) and Aspartate aminotransferase (AST) otherwise referred to as glutamate oxaloacetate transaminase (GOT) are enzymes located in the cytosol and mitonchondria where they are involved in the transfer of amino group from α-amino to α-keto acids. They are also involved in the biochemical regulation of intracellular amino acid pool [43]. These aminotransferase belong to the plasma non-functional enzymes which are normally localized within the cells of liver, heart, kidney and muscles. Their presence in serum may give information on tissue injury or organ dysfunction [42]. Blood and tissues levels of ALT and AST can be used to assess the toxic impact of chemical compound. Significant increased observed in the ALT activity of the liver of rats administered with *Ocimum gratissimum* extract compared to the control diet might be due to *de novo* synthesis of the enzymes or sharp increase in the metabolic activities of the liver in response to the administered extract. The reduction in the liver ALT activity following administration of the extract for to group 3 (400mg/kg) may be attributed to reduced rate of synthesis of the liver enzyme. It may also be that the extract has caused leakage of the enzyme into the blood via altered membrane permeability [44]. Cellular damage arising from plant extract administration can result in leakage of the marker enzymes to the extracellular fluid. However, significant increase in the ALT activities in the serum of rats administered with *Ocimum gratissimum* extract compared to the control diet might confirm that damage has been inflicted on the plasma membrane of the liver which might lead to the compromise of its integrity [45]. A significant increase ($p<0.05$) in AST activity in the liver of rats administered with *Ocimum gratissimum* extracts compared to the control diet. This results signify that the liver is intact and increase in AST activities observed in the liver may be due to *de novo* synthesis of the enzymes [46]. Generally decrease in ALT and AST in the serum may perhaps suggests that the administered extract confer protection on the liver tissues against injury, damage or disease, which are often the direct cause of elevation of the enzymes in the blood stream [47]. The fact that the levels of ALT and AST in liver, kidney and serum of both control and treated groups were similar implies that *Ocimum gratissimum* may not pose any toxicological threat to the liver when used in traditional medicine at the doses investigated.

V. Conclusion

It can be concluded that ingestion of aqueous extract of *Ocimum gratissimum* could confer protection on the liver tissues against injury, damage or disease and the extract may not be toxic at the doses investigated. But at a higher dosage of 4500mg/kg and above it could indicate toxic effects.

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