

Evaluation of Subchronic toxicity and phytochemical analysis of aqueous extract of *Ocimum suave* (Lamiales)

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ABSTRACT

Evaluation of phytochemical content, acute and sub-chronic toxicity of the aqueous leaf extract of *Ocimum suave* in Wistar rats was carried out. Experimental rats were administered extracts daily for 31 days at dose levels of 200, 400 and 800 mg/kg. Acute toxicity and phytochemical screening of aqueous leaf extract were done according to method of Lorke's Trease and Evans. Biochemical analysis was equally done at the end of the administration of extracts using standard methods. At the end of the study, animals were sacrificed and blood samples taken for biochemical assay. The LD₅₀ was found to be greater than 10,000 mg/kg body weight. Results showed the presence of most phytochemicals tested for. The aqueous extract caused a decrease in ALT activity but AST and CK activities were higher in the treated groups in a dose dependent manner relative to the control. The aqueous leaf extract of *Ocimum suave* affected mean cell volume, mean cell hemoglobin and mean cell hemoglobin concentration. The aqueous leaf extract caused mean body weight gain, but decrease in relative organ weight. The study suggests that the aqueous extract administered at normal therapeutic doses is not likely to produce severe toxic effects on some hematological and biochemical indices in rats and the organs tested. Chronic toxicity of aqueous leaf extract of *Ocimum suave* should be done for histological, haematological and biochemical evaluation.

Key Words: *Ocimum suave*, Toxicity, Wistar Rats, Biochemical assay, Phytochemical, Extract

INTRODUCTION

Majority of the world's population in developing countries rely on plant medicine for their healthcare needs either due to unavailability of or lack of trust in orthodox medicine among the local people [1,2]. Natural plant herbs have played and continues to play very important role in medicine and healthcare. Plants and natural products have been shown to possess numerous health beneficial and medicinal properties [3] They have been shown to be able to ameliorate several health conditions such as malaria, ulcer, diabetes, diarrhea, fever, arthritis, epilepsy, worm infestation among others [4] They are also known to be effective in managing immuno neurodegenerative conditions, cancer and pathologic inflammatory conditions. These properties have been attributed to abundance of phytochemicals possessed by these plants. Health efficacy is equally attributed to vitamins and minerals content.

Ocimum suave is used as a traditional medicine for the treatment of stomach ache, cough and influenza. It is also used as a perfume, an insect

repellent (particularly against mosquitoes) and a grain protectant [5]. Another species *Ocimum kilimandscharicum* Baker ex Gurke Traditionally is used for the treatment of serious colds and coughs, abdominal pains, measles and mild diarrhoea in children. It is also used as a grain protectant in East Africa. *Ocimum canum* Sims leaves are used as a traditional medicine in West Africa for the treatment of fevers, dysentery and to relieve toothache the *Ocimum sanctum* alcoholic leaf extract shows significant hepatoprotective activity and synergism with silymarin [6].

Despite huge medicinal benefits, some plant decoctions are known to have serious toxic side effects. This is due to the fact that they exist in crude impure form usually in combination with several unwanted components. [7]. There is ongoing need to scientifically evaluate these plants and screen them for any side toxicity [8] This plant is particularly being used widely by the local populace without knowledge of its proper

dose and toxicity. This lack of knowledge may be causing huge problems among the people without any prior note. Therefore, there is need to evaluate possible toxic profile of this plant [9,10]. The purpose of the study was to ascertain the acute toxicity, possible biochemical and hematological effects of aqueous extract of the leaves of *Occimum suave* and further identify the compounds responsible for the effects. Unfortunately, the

required information on the local species of this plant is greatly unavailable; also administration and dosage of these drugs have not been established. Therefore, in order to fill this gap in knowledge, preliminary studies have to be done to evaluate and ascertain possible risks such as undesirable effects, overdose or poisoning so that may be associated with administration and usage of the plant extract using rats as experimental model.

METHODS

Plant material identification

The *ocimum suave* plant was taxonomically identified by a botanist from Kampala International

University-Western Campus and a voucher specimen prepared and deposited in the herbarium of the School of Pharmacy.

Extract preparation

The leaves of *Ocimum suave* plant were collected and dried under shade, then ground into

powder. Extraction was by boiling in water (decoction) and this is how it was used traditionally. The extract was filtered then the filtrate evaporated and dried in water bath.

Phytochemical Screening

Secondary metabolites screening were carried out according to standard procedures as described by Sofowara [11], Trease and Evans [12]

Laboratory animal acquisition and maintenance

Male Wistar rats (weighing not less than 100g) were used for this study. The animals were bred and housed in the Animal Facility Centre of the School of Pharmacy, Kampala International University-Western Campus. The animals were then kept in a cage lined with sawdust, at room temperature with adequate ventilation, under a naturally illuminated environment with 12 hours of light/dark cycle. They were fed with standard diet (Nuvita (R) Animal Feed Ltd, Jinja Uganda) and had access to clean drinking water ad libitum. The animal experiment was conducted according to the National Institute of Health Guide for the care and use of laboratory animals and ethical guidelines for investigation of experimental pain in conscious animals [13]

Toxicity Evaluation (Acute toxicity)

This involved observation for signs of acute toxicity and other effects of the extract following administration. Acute toxicity referred to the effects on the whole body of a single dose of a chemical (or

several doses within a 24- hour period), which was manifested over a period of 14 days. Determination of acute oral toxicity was an initial screening step in the assessment and evaluation of the toxic characteristics of all compounds. Acute toxicity data were used to provide a rough guide for dose selection among many other factors. The LD₅₀ test was used to determine the therapeutic index, i.e. ratio between the lethal dose and the pharmacologically effective dose in the same strain and species (LD₅₀/ED₅₀). The greater the index, the safer the compound was. LD₅₀ with confidence limits was to be established on one common laboratory species such as mice using the Lorke's method.

Biochemical assay

Serum and liver alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed by the method described by Reitman and Frankel [14]. Gamma glutamyl transpeptidase (GGT) [15] LDH and CK were analyzed using standard methods [16]

Hematological studies

Blood samples were collected into heparinized tubes and used for the estimation of Complete Blood Count using an automated cyflow machine. All data generated was presented as Mean ± Standard Error of the Mean (SEM) and statistical comparisons were performed using descriptive statistics and ANOVA-repeated measures $p < 0.05$ was considered as statistically significant.

Data analysis

The data collected from the study was analyzed by using the Statistical Package for Social Scientists (SPSS) version 8.0 for windows to obtain descriptive statistical correlation and the results were presented in form of tables and figures. Statistician was consulted during data analysis.

RESULTS

Acute Toxicity

Ocimum suave leaf extract administered orally up to 10,000 mg/kg to rats was found to cause no death in

the two phases of the test. Thus, the LD₅₀ of *Ocimum suave* extract in rats was estimated to be greater than 10,000 mg/kg.

Phytochemical Analysis

Phytochemical analysis of *Ocimum suave* aqueous leaf extract gave positive reactions to

phlobatannins, steroids, terpenoids, cardiac glycosides, reducing sugars and tannins.

Table 1: showing the bioactive compounds of *Ocimum suave* aqueous leaf extract

PHYTOCHEMICAL CONSTITUENTS	RELATIVE PRESENCE
Tannins	Positive
Phlobatannins	Positive
Saponins	Negative
Flavonoids	Negative
Steroids	Positive
Terpenoids	Positive
Cardiac glycosides	Positive
Reducing sugars	Positive

Biochemical parameters.

Results expressed as Mean±SEM. Statistical significance tested with student's t-test, p<0.05.

ALP levels were found to be higher in the 800mg/kg OSE treated group as compared to the control group with a decrease in 200 and 400mg/kg OSE treated groups. However, the increase was not statistically significant at p<0.05. CK levels were found to be higher in the OSE treated groups as compared to the control group with a decrease in 400mg/kg OSE treated group. The increase of CK

levels appeared to be dose dependent. The increase was not statistically significant at p<0.05. ASAT levels were higher in the 800mg/kg OSE treated group as compared to the control group with a decrease in 200 and 400mg/kg OSE treated groups. GGT levels were found to be high in all OSE treated groups as compared to the control group. The increase of GGT levels appeared to be dose dependent. The increase was not statistically significant at p<0.05. ALAT levels were found to have decreased in all OSE treated groups as compared to the control group.

Table 2 showing the Biochemical parameters.

BIOCHEMICAL PARAMETERS.	TREATMENT			
	10 ml/kg (control)	200 mg/kg	400 mg/kg	800 mg/kg
ALPDGKC U/L	252.2±72.652	158.6±70.84	208.8±112.495	365±105.754
CK ₂ RU/L	2287.2±600.45	3864.6±2570.29	1060±528.708	3388±1248.286
GOT-ASATU/L	128.60±114.583	94.4±37.098	92.20±43.929	141.8±55.674
GGTU/L	26.2±8.224	46.80±18.148	69.4±43.929	55.6±22.065
GPT- ALATU/L	189.8±22.758	92.2±37.090	101.2±29.006	136.00±27.395

Hematological values.

Results expressed as Mean±SEM. Statistical significance tested with student's t-test, $p < 0.05$.

WBC increased in the 800mg/kg OSE treated group as compared to the control group with a decrease in 200 and 400mg/kg OSE treated groups. RBC decreased in all OSE treated groups as compared to the control group. Hemoglobin levels were found to be higher in the 800mg/kg OSE treated group as compared to the control group with a decrease in 200 and 400mg/kg OSE treated groups. Hematocrit percentage was found to be higher in the 800mg/kg OSE treated group as compared to the control group with a decrease in 200 and 400mg/kg OSE treated groups. Mean cell volume increased in the 800mg/kg OSE treated group as compared to the control group with a decrease in 200 and 400mg/kg OSE treated groups. Mean cell hemoglobin decreased in the 200 and 400mg/kg OSE treated groups as compared to the control group with no slight increase in 800mg/kg OSE treated group as

compared to the control group. Mean cell hemoglobin concentration decreased in all OSE treated groups as compared to the control group. Platelet count was found to be higher in the 800mg/kg OSE treated group as compared to the control group with a decrease in 200 and 400mg/kg OSE treated groups. Neutrophil percentage was found to be higher in 400 and 800mg/kg OSE treated groups as compared to the control group with a decrease in 200mg/kg group. Lymphocyte percentage was higher in the 800mg/kg as compared to the control group with a decrease in 200 and 400mg/kg OSE treated group. Monocyte percentage was found to be lower in 200 and 800mg/kg groups as compared to the control group. Eosinophil percentage decreased in all OSE treated groups as compared to the control group. Basophil percentage increased in all OSE treated groups as compared to the control group.

Table 3 showing the Hematological values and treatment

Hematological values	TREATMENT			
	10ML/KG (conti-ol)	200MG/KG	400MG/KG	800MG/KG
WBC $10^3/\mu\text{L}$	9.74±1.697	9.70±3.112	7.42±2.352	12.62±1.705
RBC $10^6/\mu\text{L}$	8.69±0.328	6.79±1.733	6.89±1.759	8.65±0.327
HGB g/dl	14.8±0.586	11.72±2.974	11.72±2.961	15.06±0.453
HCT o/o	46.18±1.893	36.98±9.370	36.90±9.365	47.62±1.271
MCVfI	53.2±1.393	43.8±10.901	42.4±1.656	54.40±2.074
MCHpg	17.42±0.493	13.82±3.465	13.62±3.414	17.46±0.256
MCHCg/dl	32.22 ±0.073	25.38±6.358	25.46±6.377	32.02±0.136
RDW o/o	11.56±0.282	8.98±2.292	9.38±2.414	11.48±0.538
PLT $10^3/\mu\text{L}$	583.8±128.2575	535.6±182.704	527.2±144.527	616.6±115.289
MPV fl	7.61±0.938	5.50±1.401	7.02±1.771	8.48±0.381
NE%	11.72±1.976	10.42±3.025	14.88±5.068	14.34±3.057
LY%	65.34±5.464	53.22±13.682	44.92±11.647	68.78±5.556
MO%	17.9±3.851	13.72±4.078	17.90±4.506	14.72±2.426
Eo%	4.74±0.946	1.82±0.681	1.66±0.797	2.32±1.063
BA%	0.68±0.37	0.82±0.256	0.84±0.254	1.04±0.268

Results expressed as Mean±SEM. Statistical significance tested with student's t-Test, $p < 0.05$.

There was weight gain between day1 and day7 in 200mg/kg and 400mg/kg OSE-treated groups

compared to the control group with a decrease in day 1 800mg/kg OSE- treated group. Between day7 and day 14 weight gain increased in the OSE-treated groups compared to the control group. Between day14 and day 21 weight gain also increased in the OSE-treated groups compared to the control group. There was weight gain between

day 21 and day28 in 200mg/kg and 400mg/kg OSE-treated groups compared to the control group with a decrease in day28 800mg/kg OSE-treated group. Between day28 and day 31 weight gain increased in the 200mg/kg and 400mg/kg OSE-treated groups compared to the control group, with a decrease in day28.

Table 4: shows mean body weight of rats
MEAN BODY WEIGHT OF RATS

DURATION	Treatment			
	10ml/kg	200mg/kg	400mg/kg	800mg/kg
Day1	191.53±1.545	225.1±16.883	219.77±1.048	202.7±1.572
Day 7	210.9±6.31	268.87±7.85	257.87±2.392	234.93±4.129
Day 14	220.2±4.187	288.4±7.275	264.53±5.716	235.7±3.74
Day 21	225.8±0.275	291.57±15.306	280.37±4.715	252.23±7.464
Day 28	243.3±5.285	307.53±16.36	289.37±3.077	239±5.903
Day 31	248.7±3.044	319.2±24.652	289.37±1.184	258.97±6.939

Results expressed as Mean±SEM. Statistical significance tested with student's t- Test, $p < 0.05$.

There was decrease in relative organ weight of liver in 200mg/kg and 400mg/kg OSE treated groups compared to the control group, with slight increase in 800mg/kg OSE-treated group. Relative organ weight of lungs decreased in 200mg/kg and 400mg/kg OSE-treated groups

compared to the control group. There was decrease in relative organ weight of kidney in 200mg/kg and 400mg/kg OSE- treated groups compared to the control group, with an increase in 800mg/kg OSE- treated group. Relative organ weight of testes and stomach decreased in the OSE-treated groups compared to the control group.

Table 5: Shows the relative organ weight
RELATIVE ORGANWEIGHT

Mean organ weight \pm S.E.M (Values are in grams)				
Relative body organ	10mVkg	200mg/kg	400mg/kg	800mg/kg
Liver	2.37 \pm 0.67	2.01 \pm 0.070	2.13 \pm 0.033	2.4 \pm 0.058
Lungs	0.47 \pm 0.033	0.44 \pm 0.031	0.37 \pm 0.033	0.47 \pm 0.033
Kidney	0.47 \pm 0.033	0.41 \pm 0.007	0.43 \pm 0.033	0.57 \pm 0.038
Testes	1.83 \pm 0.033	1.26 \pm 0.057	1.43 \pm 0.033	1.58 \pm 0.033
Stomach	1.6 \pm 0.0	0.67 \pm 0.033	0.67 \pm 0.033	0.6 \pm 0.0

Values presented as mean \pm standard error of mean (S.E.M)

DISCUSSION

The safety of drugs and plant products for human use can be determined using toxicological evaluation which is usually carried out in various experimental animals to predict toxicity and to provide guidelines for selecting a safe dose in humans [3,17, 22-30]. The acute toxicity studies showed that the aqueous leaf extract of *Ocimum suave* has a high safety profile when given orally with an LD50 value greater than 10000 mg/kg body weight. Therefore, the medicinal plant in its local formulation can be categorized as relatively non-toxic based on the method proposed by Lorke (Lorke 1983). Acute toxicity study is a quick and reliable method of assessing the toxic potentials of natural products [5,18,19]. Analysis of blood parameters is relevant in risk evaluation as changes in the hematological system have higher predictive value for toxicity studies [30-35]. WBC increased in the 800mg/kg OSE treated group as compared to the control group. The increase in WBC might be due to the increase of the dose. From the observed values of WBC, it is clear

that an increase in the WBC count is a normal reaction of rats to foreign substances, which alter their normal physiological process [35-40]. RBC decreased in all OSE treated groups as compared to the control group. The decreased levels of RBC may be due to the low rate of erythropoiesis occurring in the bone marrow of the rats [40-43]. Hemoglobin levels were found to be higher in the 800mg/kg OSE treated group as compared to the control group and the increase might be due to the increase of the dose. Increase in hemoglobin mainly occurs as a result of high altitudes and sickle cell disease. Hematocrit percentage was found to be higher in the 800mg/kg OSE treated group as compared to the control group and the increase as in previous cases might be due to the increase in the dose. Mean cell volume increased in 800mg/kg OSE treated group which was as a result of dose increment. This could have indicated the possibility of liver damage or disease. Platelet count also was found to be higher in the 800mg/kg OSE treated group as compared to the

control group and this indicated platelet aggregates or macroplatelet. Neutrophil percentage increased in the treated groups [400 and 800mg/kg] and this indicated neutrophilia. Neutrophilia occurs as a result of bacterial infections where the neutrophils are involved to fight the infection. Lymphocyte percentage was higher in the 800mg/kg OSE treated group as compared to the control group and this was as a result of dose increment. This increment of lymphocytes indicated lymphocytosis and lymphocytosis occurs mainly when viral infections are present. Monocyte percentage was found to be lower in the treated groups [200 and 800mg/kg] and this indicated monocytopenia. Eosinophil percentage decreased in all OSE treated groups as compared to the control group and this indicated eosinopenia which showed that allergic reactions or parasitic infections were not manifested in the rats. Basophil percentage increased in all OSE treated groups as compared to the control group. This increment of basophil percentage indicated basophilia. Basophilia occurs mainly when allergic reactions are manifested.

ALP levels were found to be higher in the 800mg/kg OSE treated group as compared to the control group. Increase in ALP levels by administration of aqueous leaf extract of *Ocimum suave* showed possible cholestasis which occurred at

the dose levels tested since a rise in plasma ALP level is usually a characteristic finding in cholestatic liver disease, an indicator of hepatic toxicity [2,22]. CK levels were found to have increased in the treated groups of rats and this indicated kidney damage. Therefore, the excretory system of animals [rats] exposed to the aqueous leaf extract of *Ocimum suave* may have been slightly affected. AST levels were higher in the treated group [800mg/kg] as compared to the control group. AST is a marker of skeletal muscles damage [23]. Decreased ALT levels in the treated groups indicated that there was no hepatocellular injury or damage. ALT is a cytoplasmic enzyme found in very high concentration in the liver [24], and indicates hepatocellular damage, while AST is less specific than ALT as an indicator of liver function.

Increased or decreased organ weight has been observed as a sensitive indicator of organ toxicity. The aqueous leaf extract of *Ocimum suave* did not produce any demonstrable toxic effects to the relative organ weights of liver, lungs, kidney, testes and stomach. The 200 mg/kg dose decreased the relative organ weights of liver, lungs, kidney, testes and stomach while 800 mg/kg dose increased the relative organ weights of liver and kidney.

CONCLUSION

In conclusion, these results provide evidence for the safety profile of the aqueous leaf extract of *Ocimum suave* in the treatment of the various ailments because the aqueous extract did not produce any significant toxic effects in the organs tested and some haematological and biochemical indices in rats but caution needs to be taken on the

appropriate dosage as higher concentration could induce renal toxicity. It can also be concluded that aqueous leaf extract of *Ocimum suave* contains the following secondary metabolites: tannins, phlobatannins, terpenoids, steroids, reducing sugars and cardiac glycosides.

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