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PHYTOCHEMICAL ANALYSIS OF AQUEOUS LEAF EXTRACT OF *Olea hochstetteri* BAK. (*OLEACEAE*) AND ITS EFFECTS ON HAEMATOLOGICAL PARAMETERS IN RATS

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SUMMARY

The aqueous leaf extract of *Olea hochstetteri* was studied for its acute toxicity, its effects on haematological parameters due to prolonged oral administration in rats and its phytochemical constituents. The results of this investigation showed that the LD_{50} of the aqueous leaf extract following intraperitoneal administration was estimated to be 1280 mg/kg showing low toxicity. The extract showed a dose - dependent decrease in packed cell volume; haemoglobin concentration and erythrocyte count ($P<0.05$) on days 14 and 21 of extract treatment. There was also a dose - dependent decrease ($P<0.05$) in total leucocyte and lymphocyte counts on days 7, 14 and 21 of extract treatment. However, 7 to 14 days after withdrawal of the extract treatment, the values rose considerably. Monocytes, neutrophils, eosinophil and basophil counts did not show any difference ($P>0.05$). The extract showed the presence of gallic tannins, saponins, reducing sugars, alkaloids, flavone aglycones, sterols and triterpenes, emodols and cardiac glycosides. The elemental analysis of the leaf revealed high concentration of sodium, followed by iron, magnesium, zinc, cadmium, manganese, and potassium. Calcium, cupper, lead and arsenic were absent. In conclusion, the extract contains important chemical constituents possessing pharmacological activities. It has adverse effects on haematological parameters following prolonged oral administration beyond 7 days, and it is recommended that the extract should not be administered beyond 3 to 4 days during treatment.

KEY WORDS: Haematological parameters, *Olea hochstetteri*, Phytochemical constituents

INTRODUCTION

Olea hochstetteri Bak. (English: Olive; Arabic: Zaitun), a member of the family Oleaceae, has been identified by Hutchinson and Dalziel (1963) as a small tree in Montane forest. In Nigeria, it is locally known as *Zaitun* in Hausa, Kanuri, Shuwa and Fulfulde, it is called *ewe olifi* in Yoruba. The indigenes use the aqueous leaf extract of the plant for the treatment of febrile illnesses, severe headache, psychiatric illness, abdominal pain and relief from witchcraft and other evil spirits (Personal observation).

It has been reported that, a handful of the leaves is boiled in a quart of water and used for treatment of febrile illnesses, hypertension, arteriosclerosis, diabetes and HIV AIDS (Privitera, 1996). Olive leaf poultices are among the oldest therapies for infections of the skin. *Olea hochstetteri* is widely employed in traditional medicine in Africa. It is one of the browse plants present in the savannah region of Nigeria.

Scientists have isolated a bitter substance from the Olive leaf and named it Oleuropein (Veer,

1957). It was found to be one ingredient in a compound produced by the Olive tree that makes it particularly robust and resistant against insect and bacterial damage (Privitera, 1996). Oleuropein is an iridoid, a structural class of chemical compounds found in plants (Petkov and Manolov, 1972), and has been found to lower systemic blood pressure in animals (Samuelsson, 1951). It increases blood flow in the coronary arteries, relieve arrhythmias and prevent intestinal muscle spasms (Privitera, 1996).

The cultivation of Olive tree has produced over 900 varieties of Olives among which *Olea europaea* is the most widely studied. Although Olea *hochstetter is* widely used among the indigenes of Northern Nigeria for a variety of animal and human diseases, information on its acute toxicity, effects on blood and phytochemical properties is limited. The objectives of this study, therefore, was to determine the acute toxicity of the aqueous leaf extract, its phytochemical and elemental constituents as well as its effects due to prolonged oral administration on haematological parameters in rats.

MATERIALS AND METHODS

Plant collection, identification and extract preparation

Fresh leaves of *Olea hochstetteri* Bak. *(Oleaceae)* were collected from Mafa Local Government Area of Borno State, Nigeria. The plant was identified and authenticated by Dr. S.S. Sanusi of the Department of Biological Sciences, University of Maiduguri, Nigeria and a set of voucher herbarium (Species Vet. 206 A) was deposited in the Department of Veterinary Physiology and Pharmacology, University of Maiduguri, Maiduguri. The air - dried leaves were crushed into fine powder. The powder was dissolved and then filtered using Whatman No.1 filter paper. The filtrate was concentrated in a rotary evaporator and stored at 4^0C in a concentration of 0.5g/ml until used.

Experimental animals

Forty-five albino rats of both sexes weighing between 105 g and 337 g were used. The rats were kept in plastic cages and were allowed to adjust to the laboratory environment over a period of three weeks before commencement of the experiments. They were given access to feeds and water *ad libitum*. The animals were handled according to the International Guiding Principles for Biomedical Research Involving Animals (C.I.O.M.S., 1985).

Acute toxicity test

Twenty five albino rats of both sexes weighing between 217 g and 337 g were separated at random into five groups (A, B, C, D, and E) of five rats per group. The animals in groups A, B, C and D were treated intraperitoneally with single dose of 200, 400, 800 and 1600 mg/kg body weight of aqueous leaf extract of *Olea hochstettteri*, respectively, while the rats in group E were given only distilled water by the same route. They were monitored over a period of 24 hours for clinical signs and death. The LD_{50} of the aqueous leaf extract of *Olea hochstetteri* was calculated using arithmetic method of Aliu and Nwude (1982).

Effects of the extract following prolonged oral administration

Twenty albino rats of both sexes weighing between 105 g and 171 g were separated at random into four groups (A, B, C, and D) of five rats per group. Graded doses of 200, 500 and 1000 mg/kg body weight of the extract was administered orally and daily for 21 days to group A, B and C, respectively. Rats in group D served as control and they were administered with distilled water. Every week, 1 ml of blood was obtained from the tail of each rat into commercially prepared bottles with ethylene diamine tetra-acetic acid as anticoagulant (1mg/ml) and used for the determination of haematological parameters such as packed cell volume (PCV); haemoglobin (Hb) concentration, red blood cells count (RBC); total and differential white blood Cells count (WBC). Animals in each group were weighed weekly and their mean body

weight calculated and recorded. The PCV was determined by microhaematocrit method (Coles, 1974); The Hb concentration was measured calorimetrically by cyanmethaemoglobin method (Coles, 1974); The RBC and total WBC counts were done by haemocytometry (Brown, 1976). Blood films were stained with Leishman's stain and used for the differential WBC counts as described by Coles (1974). The mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were calculated using standard formulae (Schalm *et al., 1975)*

Phytochemical analysis

The aqueous extract is freshly prepared and divided into different test tubes and various chemical constituents were analysed according to methods described by Allen (1974) and Harbone (1976). The different chemical constituents tested for included polyuronides, reducing sugars, gallic tannins and catechol tannins, saponins, alkaloids, sterols and triterpenes, flavone aglycones, emodols and cardiac glycosides.

Elemental analysis

The elemental content of the leaf was determined using the standard calibration curve method according to the methods of Sunderman (1973) and Kalthoff and Elving (1976). Flame emission spectrometer (FES) (Gallenkamp FGA 330) was used to determine sodium and potassium. Magnesium, calcium, iron, lead, zinc, manganese, cadmium, copper and arsenic were determined by Atomic Absorption Spectrophotometer (SPG Unicam Model No.1) at the appropriate wave length, temperature and lamp current for each element (WHO, 1976).

Statistical analysis

All values obtained were expressed as mean ± standard deviation. Analysis of Variance (ANOVA) was used to compare the observations among the various groups. P values less than or equal to 0.05 were considered significant. Graphpad Instat 3.0 for windows USA® computer software was used to analyse the data.

RESULTS

Extraction

The aqueous extract was light green and tasteless. The yield was 6.1% (w/w).

Acute toxicity Studies

One hour after the administration of the aqueous leaf extract of *Olea hochsteteri,* the rats were sedated. The effect of the extract was dose - dependent and at a dose of 1600 mg/kg, the rats were deeply sedated with locomotion greatly decreased. Mortality in groups A, B and C was 0%, with 80% in group D. Death occurred within twenty two hours after administration of the extract. Signs observed before death include anorexia, starry hair coat and paralysis of the hind limbs which progressed to the forelimbs, dyspnea and coma. The LD_{50} was calculated to be 1280 mg/kg.

Effects of the aqueous leaf extract of *Olea hochstetteri* on haematological parameters

The changes in mean values of PCV, RBC, Hb, MCV, MCHC and total WBC counts in rats exposed to aqueous leaf extract of *Olea hochstetteri* are presented in Table I. These haematological parameters were observed to be significantly ($P < 0.05$) lower in the exposed animals on the 14th and 21st day of treatments compared to those of the control animals. However, normal values of the PCV, RBC, WBC and Hb concentration were promptly re-established and maintained following the withdrawal of extract treatments.

TABLE I: Effect of aqueous leaf extract of *Olea hochstetteri* on some haematological parameters in rats

Parameters	Dose (mg/kg body weight)	Days of treatment (Mean ± SD)			Days of post-treatment (Mean ±SD)	
		7	14	21	7	14
Packed cell volume (%)	Distilled water (control)	44.0 ±5.63	44.8±1.79	44.8±1.79	44.0 ± 5.63	43.2±2.86
	200	43.3±2.89	41.0±2.35[a]	40.0±1.58[a]	41.8±1.10	42.6±0.55
	500	42.0±3.08	41.0±0.89[a]	39.8±1.92[a]	-	-
	1000	41.6±7.30	40.0±1.48[a]	39.6±2.88	-	-
Haemoglobin concentration(gm/dl)	Distilled water (control)	10.3±1.14	11.1 ± 1.32	10.7± 0.35	11.2 ±0.43	10.2 ±1.56
	200	10.2 ±2.14	8.8 ±1.07[a]	7.3 ±0.14[a]	11.0 ±0.36	10.5±0.75
	500	10.1±1.78	6.5 ±2.47[a]	6.2±1.56[a]	-	-
	1000	9.7± 2.49	6.2 ±1.80[a]	5.3±1.49[a]	-	-
Red blood cells count (10^{12}/L)	Distilled water (control)	6.9 ±1.17	6.5 ±0.17	6.5 ±0.78	6.3±0.36	6.8±0.78
	200	6.6 ± 0.66	6.4±0.74	5.1 ± 0.26[a]	6.6 ±0.78	7.3 ±0.60
	500	6.3 ± 0.94	5.2 ±1.23[a]	4.9 ±0.30[a]	-	-
	1000	5.6 ±1.17	5.2 ± 0,73[a]	4.8 ±0.58[a]	-	-
Mean corpuscular volume (fl)	Distilled water (control)	63.7±5.63	68.9±1.79	68.9±1.79	69.8±5.63	63.5±2.86
	200	65.6±2.89	64.0±2.35	78.4±1.58[a]	62.1±1.10	58.4±0.55
	500	66.7±3.08	78.8±0.89[a]	79.5±1.92[a]	-	-
	1000	74.3±7.30[a]	76.9±1.48[a]	82.0±2.88[a]	-	-
Mean corpuscular haemoglobin concentration(gldl)	Distilled water (control)	23.4±1.14	24.7±1.32	23.8±0.35	25.5±0.43	23.6±1.56
	200	23.5±2.14	21.5±1.07	18.3±1.79[a]	26.3±0.36	24.6±0.75
	500	24.0±1.78	15.9±2.47[a]	15.6±1.56[a]	-	-
	1000	23.3±2.49	15.5±1.80[a]	13.4±1.49[a]	-	-
White blood cells count(10^9/L)	Distilled water (control)	15.4 ±3.75	11.5 ±2.48	11.3 ±2.48	12.6 ±2.08	10.3 ±1.48
	200	10.6 ± 1.12[a]	7.50 ± 2.90[a]	8.4 ± 1.04[a]	10.4 ± 1.98	9.70 ± 1.13
	500	9.80 ±1.56[a]	6.30 ± 0,87[a]	6.60 ± 1.63[a]	-	-
	1000	9.60 ± 1.18[a]	5.1 ±2.04[a]	5.78 ±1.71[a]	-	-

[a] **Values significantly different (P<0.05) from those of control; SD, Standard Deviation; -, All the animals in this group died by this time**

Effect of the aqueous leaf extract of *Olea hochstetteri* on differential leucocyte count

The changes in the lymphocyte counts are presented in Table II. The lymphocytes showed significant ($P < 0.05$) decrease on days 7, 14, and 21 of treatment. Following withdrawal of treatments the lymphocytes production was re-established and maintained neutrophils, eosinophils and basophils counts did not show any difference ($P > 0.05$).

Chemical composition of the aqueous leaf extract of *Olea hochstetteri*

The results of the phytochemical analysis of the leaf extract of *Olea hochstetteri* are shown in Table III. The results showed that the colour of the aqueous leaf extract was light green and the pH was 5.4. It also revealed high presence of reducing sugars, garlic tannins, saponins and alkaloids, while sterols and triterpenes were in moderate concentration and flavones aglycones, emodols and cardiac glycosides were in low concentrations. The results of the elemental analysis of the leaf extract of *Olea hochstetteri* are shown in TableIV. The elemental analysis of the leaf showed high concentration of sodium followed by iron, magnesium, zinc, cadmium, manganese and potassium. Calcium, copper, lead and arsenic were found to be absent.

TABLE II: Effect of aqueous leaf extract of *Olea hochstetteri* on differential leucocyte count in rats

Parameters (10^9/L)	Dose (mg/kg body weight)	Days of treatment (Mean ± SD)			Days of post treatment (Mean ± SD)	
		7	14	21	7	14
Neutrophils	Distilled water (control)	4.26 ± 0.85	3.97 ± 0.59	3.10 ± 0.79	3.39 ± 0.55	3.84 ±0.44
	200	4.67 ± 0.93	3. 33 ± 0.31	3.06 ± 0.93	3.79 ± 0.72	3.69 ± 0.56
	500	3.91 ± 0.54	3.33 ±0.72	3.29 ± 0.31	-	-
	1000	3.54 ± 0.57	3.17 ± 0.29	3.30 ± 0.64	-	-
Eosinophils	Distilled water (control)	0.28 ± 0.08	0. 25 ± 0.13	0.23 ± 0.05	0.23 ±0.08	0.28 ± 0.06
	200	0.31 ±0.04	0.27 ± 0.04	0.25 ±0. 15	0. 34 ± 0.12	0.28 ±0.11
	500	0.29 ± 0.04	0.26 ± 0.08	0.21 ± 0.04	-	-
	1000	0.23 ± 0.46	0.17 ±0.06	0.16 ± 0.06	-	-
Basophils	Distilled water (control)	0.16± 0.04	0.12 ± 0.03	0.11 ±0.02	0.09 ±0. 06	0.07 ±0.07
	200	0.16 ± 0.12	0.11 ± 0.08	0.08 ± 0.08	0.08 ±0:05	0.02 ± 0.04
	500	0.13 ± 0.19	0.08 ± 0.05	0.07 ± 0.02	-	-
	1000	0.08 ± 0.05	0.08 ± 0.03	0.06 ±0.0	-	-
Monocytes	Distilled water (control)	0.10 ± 0.04	0.09 ± 0.10	0.09 ±0.06	0.15 ± 0.11	0.10 ± 0.01
	200	0.12 ± 0.03	0.11 ±0.13	0.08 ±0.05	0.15 ±0.04	0.11 ±0.02
	500	0.10 ±0.05	0.08 ± 0.09	0.07 ± 0.02	-	-
	1000	0.08 ± 0.03	0.08 ± 0.03	0.07 ±0.02	-	-
Lymphocytes	Distilled water (control)	10.2 ± 2.70	10.1 ± 0.79	8.10 ± 1.82	8.70 ±1.50	8.50 ± 4.10
	200	10.1 ± 0.79	6.30 ±0.87[a]	5.27 ± 0.92[a]	8.10 ± 1.56	7.60 ± 0.09
	500	5.90 ± 1.09[a]	5.10 ± 2.98	4.35 ± 1.12[a]	-	-
	1000	5.10 ± 0.86[a]	5.0 ± 2.04[a]	3.74 ± 1.17[a]	-	-

[a] Values significantly different (P<0.05) from those of control; SD, Standard Deviation; - All the animals in this group died by this time

TABLE III: Phytochemical screening of the leaf extract of *Olea hochstetteri*

Chemical Components	Extract
P^H	5.4
Colour	light green
Polyuronides (mucilage)	-
Reducing sugars	+++
Gallic tannins	+++
Catechol tannins	-
Saponins	+++
Alkaloids	+++
Sterols and triterpenes	++
Flavone aglycones	+
Emodols	+
Cardiac glycosides	+

+, Positive test; -, negative test and +++, quantitative presence

TABLE IV: Elemental analysis of the leaf extract of *Olea hochstetteri*

Elements	Concentration (mg/L x 10^{-2})	Standard concentration (mg/Lx 10^{-2})
Calcium	00.000	36000 80000
Copper	00.00	100 300
Lead	00.00	100 200
Arsenic	00.00	2 700
Potassium	2.00	10 100
Manganese	2.00	1000 20000
Cadmium	10.13	1000- 3500
Zinc	15.45	1500- 2000
Magnesium	19.53	-
Iron	58.80	50 5000
Sodium	2990	400 500

WHO Standard Concentration (WHO, 1996)

DISCUSSION

From the acute toxicity study, the intraperitoneal LD_{50} of aqueous leaf extract of *Olea hochstetteri* in rats was calculated to be 1280 mg/kg. This is an indication that the extract has low toxicity. According to the classification of Clarke and Clarke (1977), substances that have an intraperitoneal LD_{50} between 50 and 500 mg/kg are considered toxic and Onyeyili *et al.* (2000) categorized an intraperitoneal LD_{50} of 1440 mg/kg under low toxicity. In this study, toxicity signs were dose - dependent. The fact that high LD_{50} was obtained is an indication that the extract could be administered with some degree of safety, especially through the oral route for one week where the absorption might not be complete due to inherent factors limiting absorption in the gastro-intestinal tract (Dennis, 1984).

The toxicity observed could result from any of the various organic chemicals found in this study. High concentration of saponins, tannins and alkaloids are indicated by the result of phytochemical tests. This is contrary to the finding that olive leaf extract is extremely safe and non-toxic, even at high doses (Privitera, 1996).This may be due to variations in the bioactive compound of the same plant found in different environment. These organic chemicals may cause haemolysis, cardiomyopathy, toxic myopathy, myodegeneration and death (Muyiba *et al., 2000).* Hence care should be taken when using this plant for medical purposes to forestall any major lethality.

It has been shown from scientific investigation that the potential use of plant extract treatment is due to the active principles or chemical compounds in its extracts. Tannins have astringent properties, which are important in wound healing (Tyler et al., 1988). They act by precipitation of proteins, thereby protecting the underlying tissues. They also inhibit microbial proliferation by denaturation of enzymes involved in microbial metabolism (Awosika, 1991). Saponins are known to have expectorant properties which are of value in the treatment of upper respiratory tract inflammation (Trease and Evans, 1989). In addition, they are known to have antibacterial activities (Birk and Petri, 1980), hence may be used in the treatment of microbial infections. Glycosides have been utilized in the treatment of congestive heart failure, constipation, oedema and microbial infections (Robinson, 1967; Frantisek, 1991). Terpenoids have also been known to induce apoptosis and to be anti-tumoral (Reves, et al., 2005). They are also found useful in the stimulation of apetite

(Frantick, 1991). Some reducing sugars can soothe the gastrointestinal tract and can help in preventing diarrhea and gastroenteritis (Dharmanada, 1991). Alkaloids are supposed to clinically relax muscles and promote sleep (Lin and Wu, 1998). Although, flavone aglycones are present in the extract,they showed little or no action on haematological parameters. According to Dharmananda (1991), flavone aglycones increase vascular integrity and act as anti-haemorrhagic. This variation may be due to the fact that the component of the extract such as flavone aglycones would be very low to exert any appreciable effects after administration on RBC values.

The presence of essential and non-essential elements in the extract may be an indication of the type of minerals present in the soil. Mineral elements are essential in many vital processes in both plants and animals. Some of these inorganic chemical elements are essential in nutrition (Hakeem, 1987). Toxic inorganic elements form a larger group, which have established toxicity as in industrial medicine and environmental studies (Kaplan and Pesche, 1989; Thomas, 1992; Blaurock Busch, 1997). The toxic inorganic metals such as lead and arsenic were found to be absent in the leaves of *Olea hochistetteri*, and cadmium was found in very low concentration. This may be an indication of low degree of pollution in the area where the plant was obtained or poor absorption of these elements by the plant root. Borno state is not an industrialized area, hence, it is expected that the production and disposal of these toxic metals will be minimal to contribute to environmental pollution. This must have contributed to the low accumulation of the toxic metals in the plant. The low extract concentration of the toxic cadmium may also be due to the low deposits of the element in the soil, since the concentration of elements in the plant is a reflection of the concentration in the soil (Clarke and Clarke, 1977).The implication of the low concentration of the heavy metals in the extract is that heavy metal toxicity following the administration of the plant extract to man and animal may be minimal.

The concentration of some trace elements in the aqueous leaf extract of *Olea hochistetteri* from elemental analysis in the study appeared to be within safety limits reported by WHO (1996). Trace elements play very important roles in health and disease. The presence of the trace elements (magnesium, zinc, manganese and iron) and essential elements like sodium and potassium in the leaves of *Olea hochstetteri* is an indication that the plant may serve as a mineral supplement in both man and animal. The sodium level in the extract was above the WHO recommended level. The presence of high level of this element in the extract could influence the level present in the body following administration. Prolonged oral administration of the aqueous leaf extract of *Olea hocheestettri* to rats at three doses (200 mg/kg, 500mg/kg and 1000mg/kg) had slightly affected the haematological parameters.

After one week of treatment, the extract decreased RBC, PCV, Hb, total WBC and lymphocytes. This could be due to the high levels of saponins, tannins and alkaloids found in the leaves in this study. The decrease in the haematological parameters was dose dependent. The highest dose produced the highest decrease in the haematological parameters when compared to those treated with lower dose. It is therefore suggested that the extract may be less toxic at low dose than at high dose. Saponins have been reported to induce RBC lysis or inhibition of RBC synthesis (Irvine, 1961; Effraim *et al.*, 1999). Possible reason may be the interaction of saponins with micronutrients which makes the nutrient unavailable and therefore affect haemopoesis (Xing *et al.*, 1965).

Furthermore, although iron is important in haemopoesis, extremely high level could be inhibitory to RBC production (Clarke and Clarke, 1977), which is indicated in the extract in the present study. After one week of treatment, there was a significant variation in the MCV and MCHC indicating

macrocytic-hypochromic erythrocyte morphology. This may be due to interaction of saponins with micronutrients essential to erythrogenesis.

There was progressive and significant decrease in total WBC count of all rats treated with aqueous leaf extract of *Olea hochstetteri*. This decrease may suggest a possible suppression of some immune pathways. The decrease in total WBC count observed in the present study was due to lymphocytopenia. Cadmium has been reported to have immunosuppressive action (Tanasa *et al.*, 2003). Although the cadmium level in the extract was within the WHO recommended level, it could be that the higher dosages of the extract administered for a long period may affect lymphocyte production.

The fact that the physiological processes of the affected rats re-established promptly and maintained within the normal range following the withdrawal of treatments is an indication that the effect of prolonged oral administration of the extract on haematological parameters can be reversed.

CONCLUSION

From this study, it is obvious that *Olea* hochstetteri in dosages above 200 mg/kg administered beyond one week appeared to be toxic in rats. The plant contains important chemical constituents possessing pharmacological actions, and it is recommended that the extract should not be administered beyond 3 to 4 days during treatment.

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