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TOXICITY AND PHYTOCHEMICAL CONSTITUENTS OF AQUEOUS EXTRACT OF *Ocimum gratissimum* LEAF

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SUMMARY

In this study, we reported on the toxicity and phytochemical constituents of the aqueous extract of *Ocimum gratissimum* leaf. The extract was exhaustively extracted and the phytochemical constituents revealed several reducing sugar (free and combined), tannins, saponins, cardiac glycosides, terpenes, steroids, flavonoids and alkaloids. The acute toxicity study revealed an LD_{50} of 120 mg/kg in adult albino rats. The study also evaluated the effect of prolonged intraperitoneal injections of the extract on packed cell volume, haemoglobin concentration, white blood cell and live weight of the rats. The extract did not adversely affect the haematological parameters and weight of the treated animals. Gross and microscopic changes in the liver, lungs, kidneys and intestine showed mononuclear cellular infiltration, congestion, oedema and necrosis. These lesions may be associated with the active principles in the leaf of O. *gratissimum*. The results suggest that this plant is potentially toxic and should be used with caution.

KEY WORDS: *Ocimum gratissimum*, Phytochemical, Toxicity, Rat

INTRODUCTION

The plant, *Ocimum gratissimum* also known as the African basil or curry, belongs to the mint family, *Lamiaceae*. It is a non-woody perennial shrub that is native to Africa and other tropical and subtropical regions of the world (Tucker and Baggio, 2000). The plant has several uses, ranging from culinary, economic and nutritional to medicinal (Memphill, 2000; Tucker and Baggio, 2000; Brown, 2001).

As a medicinal plant, it has been used in the traditional treatment of various ailments such as diarrhoea, fever, pneumonia and skin diseases (Holetz *et al.*, 2003). Also, several workers have reported on the antimicrobial/antibacterial and antifungal properties of the plant (Sofowora, 1993; Nwosu and Okafor, 1995; Offiah and Chikwendu, 1999; Holm, 1999; Ngassoum *et al.*, 2003; Iwalokun *et al.*, 2003). The plant also has anthelmintic activity against *Haemonchus contortus* and other strongylid nematodes of man and animals (Pessoa *et al.*, 2002; Nwosu *et al.*, 2005).

However, in spite of the various traditional medicinal uses of the plant, its great enthnobotanical potentials are yet to be confirmed in controlled scientific studies. This study was therefore conducted to evaluate the toxicity and phytochemical constituents of the aqueous extract of *O. gratissimum* leaf.

MATERIALS AND METHODS

Collection of plant material

The leaves of the plant *O. gratissimum* were collected from private gardens within the University of Maiduguri campus. The identity of the plant was authenticated by a plant taxonomist in the Department of Biological Sciences, University of Maiduguri where voucher specimen was also deposited.

Source of experimental animals

Adult albino rats of both sexes, weighing 100-200g, obtained from the Laboratory animals House of the Department of Biochemistry, University of Maiduguri were used for the study. They were transferred to the Parasitology Laboratory of the

Department of Microbiology and Parasitology in the same University where the study was conducted. The animals were placed in rat cages with sawdust litter that was changed every two days. They were maintained on standard pelleted feed (Vital Animal Feed, Nigeria). Water was provided *ad libitum*. The rats were allowed two weeks to acclimatize to their new environment before the commencement of the experiment.

Preparation of plant extract

The collected leaves were air-dried in the laboratory for two weeks and ground into powder using a pestle and mortar. The powder was exhaustively extracted using soxhlet apparatus in water for six hours at 100°C (Nwosu *et al.*, 2005). The extract was then concentrated in *vacuo* using a rotary evaporator and stored at 4°C until used.

Phytochemical analyses of the leaf extract

The aqueous leaf extract was analyzed for phytochemical constituents such as tannins, saponins, ketones, anthraquinone derivatives, cardiac glycosides, terpenes, steroids, alkaloids, flavonoids, free and combined reducing sugars as described by Sofowora (1993).

Acute toxicity

Thirty adult albino rats of both sexes were randomly separated into 6 groups (A, B, C, D, E and F) of five rats each. The animals were starved overnight and thereafter those in groups A, B, C, D and E were respectively treated with single intraperitoneal injections of graded doses (12.5, 25, 50, 100 and 200) mg/kg of the aqueous extract of *O. gratissimum* leaf. Group F served as untreated controls and were given physiological saline equivalent to the highest volume of extract administered.

The rats were observed over a period of 24 hours for clinical signs of toxicity and death. The mean lethal dose (LD_{50}) of the aqueous extract of *O. gratissimum* leaf was calculated using the arithmetic method of Karber as modified by Aliu and Nwude (1982). Post-mortem examinations were performed on the dead animals and tissue samples of the intestine, lungs trachea, kidneys, and liver were obtained for histological examinations.

Effects of prolonged administration of extract

Twenty-five rats were divided into 5 groups (G, H, I, J and K) of 5 rats each. Group G served as untreated control while groups H, I, J and K respectively received daily intraperitoneal injections of graded doses (30, 40, 50 and 60mg/kg) of the extract for 21 days.

Blood samples were collected every 4 days from the tail vein of each rat for the determination of haematologcial parameters. The packed cell volume (PCV), the haemoglobin concentration (Hb), and white blood cell (WBC) counts were determined using standard methods (Schalm *et al.*, 1995).

The live weights of the experimental rats were recorded every four days using the triple beam balance 700 series, 2,610g capacity (OHAUS Marca, Florham Park, USA).

The rats that died during the experiment and those sacrificed at the end of this study were subjected to necropsy. Samples of the liver, trachea, kidney, spleen, lungs, heart and intestine were carefully dissected out, fixed in 10% formal saline, embedded in paraffin wax, cut at 5 micron thickness and stained with Haematoxylin and Eosin (H and E) as described by Drury and Wallington (1976). Tissue sections were examined under light microscopy.

Statistical analysis of data

Data collected during the study were summarized as means ± standard deviations (Mean ± S.D) and differences between and within the means were analyzed using the paired Student's t-test and repeated ANOVA at the 5% level of significance (GraphPad, 2003).

RESULTS

Phytochemical screening

The results showed that the leaf of *O. gratissimum* contains varying, concentration of the following active principles: reducing sugars (free and combined), tannins, saponins, cardiac glycosides, terpenes, steroids, flavonoids, and alkaloids (Table I). However, the extract did not contain ketones and anthroquinone derivatives.

Acute toxicity

Following the intraperitoneal administration of the aqueous extract of the leaf of *O. gratissimum*, the rats manifested various clinical signs, including anorexia, rough hair coat, depression, weakness of the hindlimbs leading to paresis, difficulty in respiration, with breathing becoming abdominal. These signs appeared a few minutes after the administration of extract but gradually subsided with time. Anorexia however continued to the end of the study. The clinical signs were dose dependent as the rats that received the higher doses of the extract manifested the more severe clinical signs. Mortalities occurred only in groups D and E given the 100 (40%) and 200 (100%) mg/kg doses of the extract (Table II). The LD_{50} of the aqueous extract of the leaf of *O. gratissimum* was calculated to be 120mg/kg.

TABLE I: Phytochemical constituents of aqueous extract of *Ocimum gratissimum* leaf

Constituent	Test method	Result*
Free reducing sugars	Fehling's	+
Combined reducing sugars	Fehling's	++
Ketones	Salvanoff's	-
Tannins	Ferric chloride	+
	Formaldehyde	
	Chlorogenic	-
Anthraquinone derivatives	Bortrager's	-
Saponins	Froth's	+++
Cardiac glycosides		+
Terpenes and steroids	Lieberman-burchard	+
Flavonoids	Salkonoski's	
	Lead acetate	++
	Sodium hydroxide	-
	Ferric chloride	+
	Paw	+
Alkaloids	Meyer's	+
	Dragendorf's	-

*Interpretation of results: - = Negative; + = Mildly positive; ++ = Moderately positive; +++ = Copiously positive

TABLE II: Mortality pattern of rats treated with a single injection of the water extract of *Ocimum gratissimum* leaf.

Group	Dose (m/kg)	No. in Group	Number (%) Dead
A	12.5	5	0
B	25	5	0
C	50	5	0
D	100	5	2 (40)
E	200	5	5 (100)
F	0	5	0

The gross pathological lesions observed in the treated rats included haemorrhages of the intestinal mucosa and congestion of the lungs and trachea. The degree of congestion of the lungs and the haemorrhages seen in organs were dose dependent as the lesions were more severe in the rats that received the higher doses of the extract.

The kidney showed necrosis of the renal tubular epithelial cells and mild mononuclear cellular infiltration of the interstices (Plate 1). The intestine showed extensive epithelial necrosis, villus atrophy and infiltration of eosinophils in the submucosa suggestive of eosinophilic enteritis (Plate 2). The lungs showed acute non-suppurative bronchiolitis with bronchiolar epithelial necrosis, mononuclear cellular infiltration into the submucosa and the separation of the epithelium by inflammatory oedema from the underlying mucosa (Plate 3). The bronchiolar lumen contained necrotic debris.

PLATE 1: Photomicrograph of the kidney showing acute toxic renal tubular necrosis (arrows), glomerulus (a) and infiltration of mononuclear cells into the interstitial areas (b) in a rat following treatment with the leaf of *Ocimum gratissimum* at 200 mg/kg (x 200)

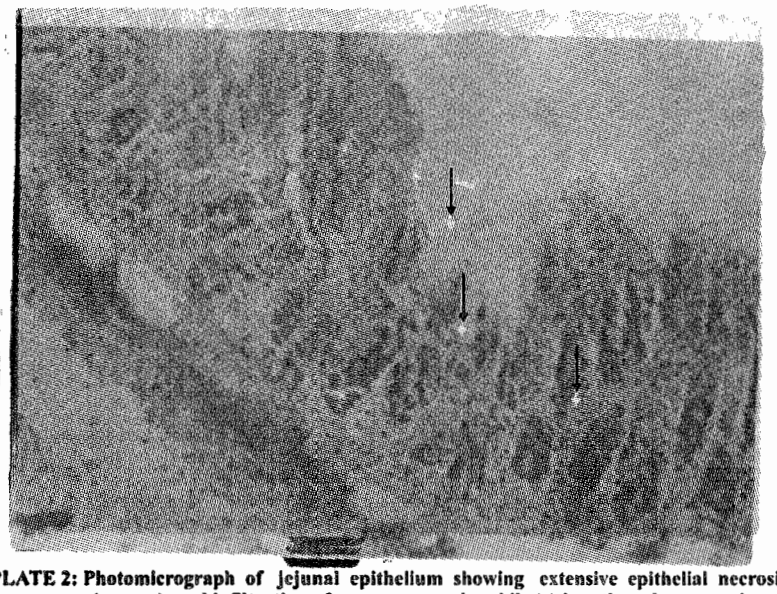

PLATE 2: Photomicrograph of jejunal epithelium showing extensive epithelial necrosis (arrows) and infiltration of numerous eosinophils (a) into the submucosa in a rat treated with the leaf of *Ocimum gratissimum* at 100 mg/kg (x 200).

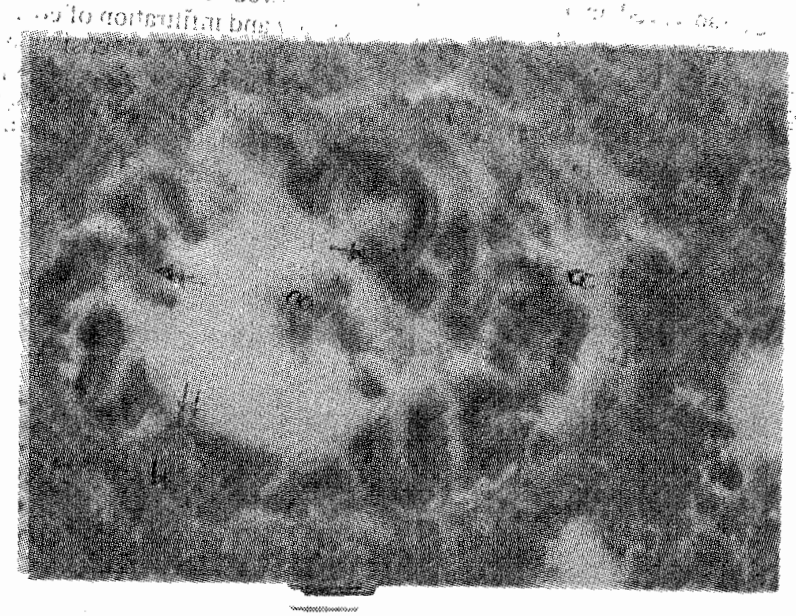

PLATE 3: Photomicrograph of the lung of a rat showing acute non-suppurative bronchiolitis with bronchiolar epithelial necrosis (arrows), mononuclear cell infiltration into the submucosa (b) and separation of the epithelium by inflammatory oedema (c) from the underlying sub-mucosa. The bronchiolar lumen contains necrotic debris (a) following treatment with the leaf of *Ocimum gratissimum* at 100 mg/kg (x 400).

Effects of prolonged administration of extract

The rats treated daily with graded doses of the aqueous extract of *O. gratissimum* leaf for 21 days manifested varying degrees of clinical signs including inappetence, rough hair coat, inactivity, laboured breathing and death. However, the clinical signs subsided and then disappeared following the termination of the extract administration. Mortalities were observed only in the groups treated with 40mg/kg (40%), 50mg/kg (60%) and 60mg/kg (80%) of the extract (TableII).

The PCV and Hb values of both the treated and control groups were not significantly ($P<0.05$) different from the pre-treatment values (Tables III and IV). The WBC counts of the untreated control group increased slightly during the first 12 days of the study before returning to the pre-treatment levels for the rest of the study period. All the treated groups showed similar increases in WBC counts and these became significantly ($P<0.05$) higher than those of the other experimental groups on days 12 (30mg/kg) and 20 (all the treated groups) post-treatment. In addition, the increase in WBC counts of the group treated with 30mg/kg of the extract was significantly ($P<0.05$) higher than those of all the other treatment groups 12 days post treatment (Table V).

TABLE III: Effect of water extract of *Ocimum gratissimum* leaf on packed cell volume (%) of rats (Mean ±S.E.M.)

Extract dose (mg/kg)	Days of treatment							
	0	4	8	12	16	20	24	
30	40.13±2.008(5)*	37.06±3.88(5)	42.43±1.05(5)	37.10±0.87(5)	45.62±1.57(5)	45.10±1.35(5)	45.23±1.30(5)	
40	43.97±1.80(5)	43.97±7.81(5)	38.70±3.45(5)	35.34±0.82(4)	48.38±3.02(4)	38.78±1.04(4)	39.38±0.77(3)	
50	39.94±2.68(5)	40.32±6.03(5)	42.80±6.86(5)	37.69±0.83(4)	47.90±1.08(4)	47.37±1.09(3)	46.18±0.25(2)	
60	43.45±1.96(5)	38.78±3.88(5)	36.16±4.27(5)	42.46±0.48(4)	47.65±0.78(4)	41.76±2.04(2)	38.95(1)	
Control	42.62±1.46(5)	42.82±0.53(5)	49.22±3.80(5)	45.89±3.35(5)	36.67±1.97(5)	45.93±3.40(5)	45.90±2.90(5)	

*Figures in parenthesis indicate number of animals ($P>0.05$).

TABLE IV: Effect of water extract of *Ocimum gratissimum* leaf on haemoglobin concentration (g/dl) of rats (Mean ± S.E.M.)

Extract dose (mg/kg)	Days of treatment						
	0	4	8	12	16	20	24
30	13.38±0.70(5)*	12.35±1.29(5)	14.14±0.35(5)	12.36±0.29(5)	15.21±0.52(5)	15.02±0.45(5)	15.08±0.43(5)
40	13.52±0.70(5)	13.44±2.70(5)	12.58±1.46(5)	11.78±0.27(4)	16.41±1.30(4)	12.93±0.35(4)	13.13±0.25(3)
50	13.20±0.89(5)	13.63±1.37(5)	13.11±0.65(5)	12.58±0.26(4)	15.97±0.36(4)	15.79±0.63(3)	15.30±0.14(2)
60	13.08±1.69(5)	12.93±1.29(5)	13.40±1.41(5)	14.30±0.15(4)	15.88±0.27(4)	13.92±0.68(2)	13.0(1)
Control	14.21±0.49(5)	14.27±0.18(5)	16.41±1.27(5)	15.30±1.12(5)	12.22±0.66(5)	15.71±0.93(5)	15.30±0.97(5)

*parenthesis indicate number of animals (P>0.05)

TABLE V: Effect of water extract of *Ocimum gratissimum* leaf on white blood cell count ($X10^3$/mm^3) of rats (Mean ± S.E.M.)

Extract dose (mg/kg)	Days of treatment						
	0	4	8	12	16	20	24
30	8.26±1.55(5)*	8.49±0.40(5)	11.02±0.82(5)	17.48±0.36(5)	11.60±0.12(5)	16.20±0.16(5)	14.08±1.12(5)
40	7.90±1.58(5)	8.50±0.22(5)	9.38±0.22(5)	10.79±0.13(4)	11.20±0.20(4)	14.40±0.33(4)	13.30±1.04(3)
50	7.90±0.46(5)	9.37±0.13(5)	9.28±0.25(5)	9.85±0.13(4)	10.79±0.11(4)	15.13±0.03(3)	14.40±0.78(2)
60	7.55±0.48(5)	8.78±0.65(5)	9.18±0.35(5)	9.80±0.08(4)	11.20±0.21(4)	15.18±0.04(2)	14.95(1)
Control	8.74±1.68(5)	7.66±0.27(5)	10.89±0.56(5)	11.51±1.13(5)	10.48±1.65(5)	8.38±1.50(5)	9.36±0.97(5)

*figure in parenthesis indicate number of animals (P>0.05)

There were no significant changes in the live weights in both treated and untreated control rats throughout the study period (Table VI).

Necropsy did not reveal any gross pathological changes in the tissues of the untreated control rats. However, in all the treated groups, the lungs were slightly congested and had pin-point whittish spots, while the intestines and the kidneys were respectively congested and had areas of haemorrhages. The severity of the lesions increased with increasing dose of the extract.

Histologically, the kidneys showed acute toxic renal tubular necrosis in the cortex and mononuclear cellular infiltration into the areas (Plate 4). The lungs had interstitial pneumonia characterized by presence of inflammatory oedema and macrophages in the alveolar lumen coupled with thickening of the alveolar septae by congestion and exudation (Pate 5). There was necrosis and desquamation of the bronchiole epithelium and the bronchiole contained necrotic debris. The peri-bronchiolar area was densely infiltrated with macrophages and lymphocytes that were focally arranged. The liver showed haemorrhages, focal areas of necrosis, mononuclear cellular infiltration and hepatocytes undergoing vacuolar changes (Plate 6).

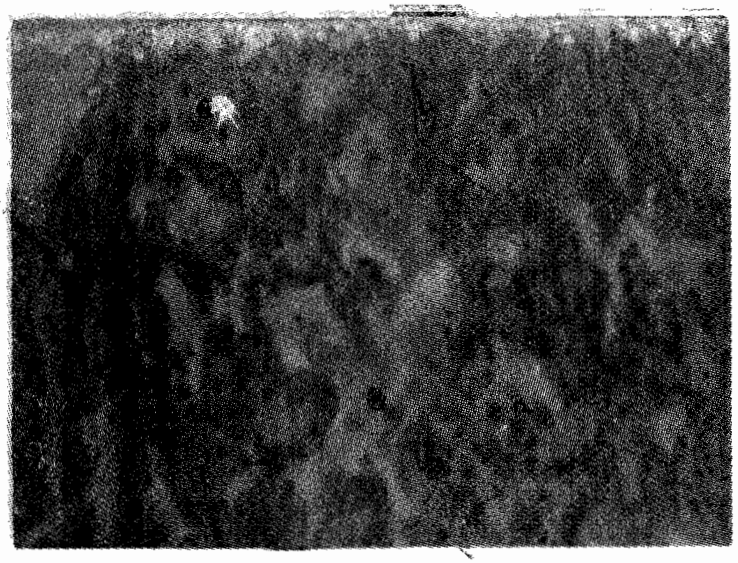

PLATE 4: Photomicrograph of the renal cortex showing desquamated necrotic renal epithelial cells (a) and infiltration of the interstices by mononuclear cells (b) in a rat following prolonged treatment with the leaf of *Ocimum gratissimum* at 60 mg/kg (x 200).

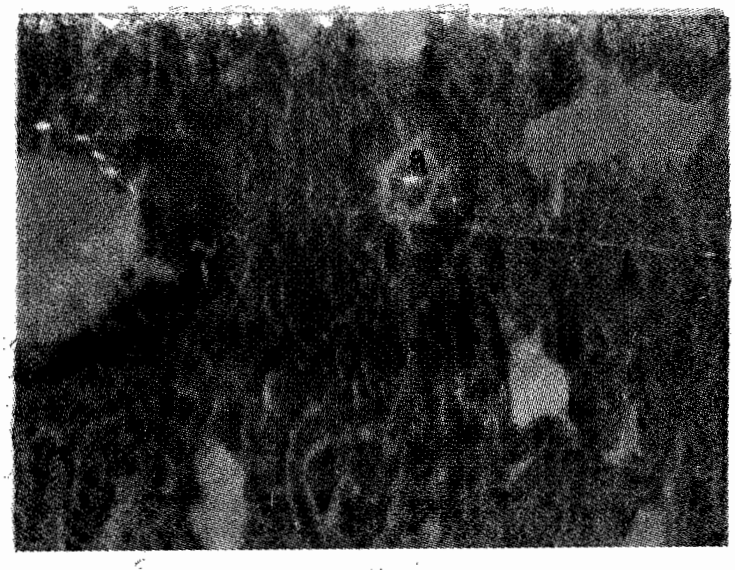

PLATE 5: Photomicrograph of the lung of a rat showing the bronchiole filled with necrotic debris (a) with peribronchiolar infiltration of mononuclear cells (arrows). The interalveolar septae are thickened by exudates (b) following prolonged treatment with the leaf of *Ocimum gratissimum* at 50 mg/kg (x 200).

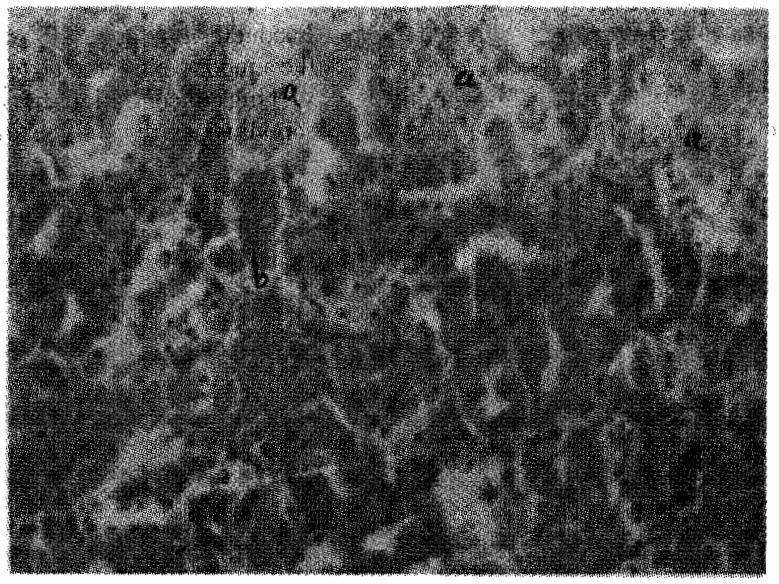

PLATE 6: Photomicrograph of the liver of a rat showing focal areas of necrosis (a) with few mononuclear cellular infiltrates and an area of haemorrhage is indicated (b) following prolonged treatment with the leaf of *Ocimum gratissimum* at 60 mg/kg (x 200).

TABLE VI: Effect of water extract of Ociumum gratissimum leaf on the live weight (g) of rats (Mean + S.E.M.)

Extract dose (mg/kg)	Days of treatment						
	0	4	8	12	16	20	24
30	97.92±10.78(5)*	96.30±5.42(5)	105.58±5.98(5)	100.62±5.62(5)	91.84±3.97(5)	90.87±40.95(5)	91.225±40.91(5)
40	96.21±12.87(5)	93.66±10.85(5)	103.84±13.01(5)	100.76±12.07(4)	93.78±10.80(4)	86.40±39.97(4)	90.70±49.83(3)
50	184.00±38.30(5)	136.30±39.03(5)	143.50±39.675)	139.43±44.39(4)	132.8±43.16(4)	130.73±30.64(3)	135.55±52.11(2)
60	120.76±13.46(5)	127.42±9.21(5)	134.84±10.80(5)	130.93±12.55(4)	119.50±11.05(4)	116.95±13.5(2)	114.70±13.15(1)
Control	126.44±12.5(5)	128.52±11.62(5)	128.52±11.62(5)	127.54±12.34(5)	126.98±12.20(5)	127.52±11.62(5)	127.68±11.58(5)

*Figure in parenthesis indicate number of animals (P>0.05)

DISCUSSION

The results of this investigation revealed that the leaf of *O. gratissimum* contain many preliminary active substances some of which have been reported previously. Offiah and Chikwendu (1999) reported the presence of tannins, steroids, triterpenes and carbohydrates as the main constituents in the leaf of the plant.

The LD_{50} of 120mg/kg obtained in this study is an indication that the plant is very toxic since any plant with LD_{50} of 500 mg/kg and below is regarded as extremely toxic (Clark and Clark, 1977). Clinical manifestations suggestive of toxicity were also noted in rats following a single or prolonged intraperitoneal administration of the extract. Pathological lesions were observed mainly in the liver, lungs and intestines of the treated rats. The lesions observed in the liver were mainly those related to degeneration due to the possible metabolism of the active principles of the plant in the organ or their interference with fat metabolism. However, these lesions are not specific to the extract of *O. gratissimum* leaf since toxins are generally known to produce degenerative changes in the liver (Smith *et al.*, 1972), which is the major organ for biotransformation in the body (Baggot, 1984).

The lesions observed in the kidneys suggest that some of the active principles in the plant are excreted through the kidneys and thus produce toxic effects in the renal tubules. The oedema, interstitial pneumonia and congestion seen in the lungs showed that the active principles in the plant extract are widely distributed in the body. The respiratory difficulties encountered by the rats given a single or prolonged administration of the extract were probably associated with the lesions produced by the extract within the respiratory system.

The prolonged administration of the extract did not adversely affect the haematological parameters of the treated rats.

CONCLUSION

In conclusion, the results of this study revealed that the aqueous extract of *O. gratissimum* leaf contains varying concentrations of reducing sugars, tannins, saponins, cardiac glycosides, terpenes, steroids, flavonoids and alkaloids which may be responsible for the clinical manifestations and the pathological changes observed in the organs and tissues of the treated rats.

REFERENCES

ALIU, Y.O. and NWUDE, N. (1982): Veterinary Pharmacology and Toxicology Experiments. Ahmadu Bello University Press, Zaria: 104-110.

BAGGOT, J.D. (1984): Disposition and fate of drugs in the body. In: John's Veterinary Pharmacology and Therapeutics (Edited by BOOTH, W. H. and MC DONALD, L. E), Kalyani Publishers Ludhiana, New Delhi: 36-70.

BROWN, D. (2001): The Herb Society of America New Encyclopedia of Herbs and their Uses. New York. D. K.: 31.

CLARK, E.G.C. and CLARK, M.L. (1977): Carleton's Toxicology. First edition. Bailliere Tindall: 10.

DRURY, R.A.B. and WALLINGTON, E.A. (1976): Carleton's Histological Techniques. 4th Ed. Oxford University Press London: 21-70.

GRAPHPAD (2003): Graphpad Instat version 3.05 for windows 95,Graphpad software San Diego California United States of America.

MEMPHILL, I. (2000): The Spice and Herb Bible. Robert Rose, Toronto: 120.

HOLETZ, F. K., VEDA-NAKAMURA, T., FILHO, B.P.D., CORTEZ, D.A.G., MORGADO-DIAZ, J. A. and NAKAMURA, C.V. (2003): Effects of essential oil of *Ocimum gratissimum* on the

trypanosomatid *Herpetomonas samuelpessoai*. Acta protozoologica, **42**:269-276.

HOLM, Y. (1999): Bioactivity of basil.In : Basil, The Genus *Ocimum* Edited by RATMO HILTUMEN and YVONNE HOLM. Harwood Academic Publishers Australia: 20-24.

IWALOKUN, B. A., GBENLE, G. O., ADEWOLE, T.A., SMITH, S. L., AKINSINDE, K. A. and OMONIGBEHIN, E.O. (2003): Effects of *Ocimum gratissimum I.* essential oil at subinhibitory concentrations on virulent and multidrug resistant *Shigella* strains from Lagos Nigeria. APMIS **III** (4): 477-82.

NGOSSOUM, M.B., NGANG, J.J., TATSADJIEU, L.N., JIROVETZ, L., BUCHBAUER, G., ADJOUDJI, O. (2003): Antimicrobial study of essential oils of *Ocimum gratissimum* leaves and *Zanthoxylum Xanthoxylides* fruits from Cameroun. Fitoterapia, **74**(3):284-287.

NWOSU, C.O., EKE, N. C. and ADAMU, M. (2005): Anthelminthic efficacy of aqueous extract of *Ocimum gratissimum* Linnaeus (Labiata) against nematode parasite of small ruminants. Nig. J. Exptl. Appl. Biol., **6**(2): 145-148.

NWOSU, M.O. and OKAFOR, J.I. (1995): Preliminary studies of the antifungal activities of some medicinal plants against basidiobulus and some other pathogenic fungi. Mycoses **38**: 191-195.

OFFIAH, V. N and CHIKWENDU, U. A. (1999): Anti-diarrhoael effects of *Ocimum gratissimum* leaf extract in experimental animals. J. Ethnopharmacol., **68**(1-3): 327-300.

PESSOA, L.M., MORAIS, S.K ., BEVILAQUA, C.M.L. and LUCIANO, J.H.S. (2002): Anthelmintic activity of essential oil of *Ocimum gratissimum* Linn and eugenol against *Haemonchus contortus*. Vet. Parasitol., **109**:59-63.

SCHALM, O.W., JAIN, N. C. and CARROL, E. J. (1995): Veterinary Haematology 3rd Edition. Lea and Febiger, Philadelphia: 498-512.

SMITH, H. A., JONES, T.C. and HUNT, R.C. (1972): Veterinary Pathology, 4th edition. Lea and Febiger, Philadelphia: 154-189.

SOFOWORA, A. (1993): Medicinal Plants and Traditional Medicine in Africa. John Wiley and Sons Limited Ibadan: 142-146.

TIETZ, N.W. (1994): Fundamentals of Clinical Chemistry with Chemical Correlation. 1st Ed. Bailliere Tindall Ltd. London: 234.

TUCKER, A.O. and D.E. BAGGIO, T. (2000): The Big Book of Herbs, a Comprehensive Illustrated Reference to Herbs of Flavour and Fragrance. Loveland COY, Inter. Weave Press: 30-34.