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SUB-ACUTE TOXICITY STUDY OF ETHANOL LEAF EXTRACT OF *Ocimum canum* ON BRAIN, LUNGS, STOMACH AND SPLEEN OF WISTER RATS

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ABSTRACT

All substances have the potential to be toxic if given to humans and other living organisms in certain conditions and at certain doses or levels. The toxic and adverse effect of traditional medicines may persist long and may not be immediately detected, even, when the desire effect is forgotten. There is therefore the need to evaluate the safety profile of this plant on different body organs. In this study the effect of sub-acute administration of *Ocimum canum* on the brain, spleen, lungs and stomach were evaluated. Rats of either sex were selected. Group 1 received distilled water (10 ml/kg), while group 2, 3 and 4 received *Ocimum canum* 100, 200 and 400 mg/kg respectively. Animals were kept in standard cages and given access to the extract, water and food orally for 28 days, after which they were weighed and sacrificed. Blood was collected by cardiac puncture and taken immediately for hematological and chemo pathological analysis. The brain, spleen, lungs and stomach were also harvested for histological study of the effect of the plant using haematotoxylin and eosin (H&E) staining technique. There was Significant ($P<0.05$) decrease in RBC, HGB, MCV, while there was no change in the level of neutrophiles, basophiles, eosinophiles and platelets. The brain, lungs, spleen and stomach were observed to be slightly significantly ($p<0.05$) increased in rats with dose level of 100 mg/kg. Histopathological examination of the brain, stomach and lungs showed normal features at all doses. The spleen showed Slight lymphocyte hyperplasia at all doses and normal features at the control (10 ml/kg). Though result of the study suggests that the plant may be safe, care should still be ensured when consumed for a sustained period of time.

KEYWORDS: **Brain; Lungs; Ocimum canum; Rat**

INTRODUCTION

The lungs are the primary organs of the respiratory system in humans and many other animals (1). Humans have two lungs, a right lung and a left lung. They are situated within the thoracic cavity of the chest. The right lung is bigger than the left, which shares space in the chest with the heart. The lungs together weigh approximately 1.3 kilograms (2.9 lb), and the right is heavier. The lungs are part of the lower respiratory tract that begins at the trachea and branches into the bronchi and bronchioles, and which receive air breathed in via the conducting zone (1).

The brain is an organ that serves as the center of the nervous system in all vertebrate and most invertebrate animals. The brain is located in the head, usually close to the sensory organs for senses such as vision (2). Physiologically, the function of the brain is to exert centralized control over the other organs of the body. The brain acts on the rest of the body both by generating patterns of muscle activity and by driving the secretion of chemicals called hormones. This centralized control allows rapid and coordinated responses to changes in the environment. Some basic types of responsiveness such as reflexes can be mediated by the spinal cord or peripheral ganglia, but

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sophisticated purposeful control of behavior based on complex sensory input requires the information integrating capabilities of a centralized brain(2).*Ocimum canum* Sims. (Hairy Basil) is a traditional medicinal plant distributes throughout sub-Saharan Africa and very well known in northern Nigeria[3]. The plant branches out from its base, with angle stems and open foliage. It is not often used as a culinary herb, unlike the related basil species O. basilicum, but more often as a medicinal plant[4]. The essential oils found in this species have strong fungicidal activity against certain plant pathogens[5]. In Africa, leaves of *O. canum* have been used as an insecticide for the protection against post-harvest insect damage especially that by bruchid beetles [6]. Medicinal properties may be associated with the external flavonoids, as some specimens produce very high levels of these compounds, especially nevadensin, which has antioxidant activity [7].

The leaves of the plant is used specially for treating various types of diseases and lowering blood glucose and also treats cold, fever, parasitic infestations on the body and inflammation of joints and headaches[8]. Essential oil from the leaves of O. canum possesses antibacterial and insecticidal properties[9]. In this study effect of Ocimum canum on lung, stomach, spleen and brain was studied after 28 days of oral administration of the ethanol leaf extract of the plant.

MATERIALS AND METHOD

Animals

Male and female wister rats were obtained from Bingham University, Animal House. They were maintained on standard animal pellets and given water *ad libitum*. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee of Bingham University.

Plant collection

Leaves of *Ocimum canum* were collected from its natural habitat from nearby Karu village, Nasarawa State, Nigeria. The plant was authenticated from Department of Botany, Bingham University, Nasarawa State, Nigeria.

Plant extraction

The leaves were shadow dried for two weeks. The dried plant material was further reduced into small pieces and pulverized. The powdered material was macerated in 70% ethanol. The liquid filtrates were concentrated and evaporated to dryness at 40°C *in vacuum* using rotary evaporator. The ethanol extract was stored at -4°C until used.

Animal study

Twenty four (24) rats of either sex (127-293g) were selected and randomized into four groups of six rats per group. Group 1 served as the control and received normal saline (10ml/kg) while the rats in groups 2, 3 and 4 were giving 100, 200, and 400 mg/kg of extract respectively. The weights of the rats were recorded at the beginning of the experiment and at weekly intervals. The first day of dosing was taken as D_0 while the day of sacrifice was designated as D_{29}.

Haematological analysis

The rats were sacrificed on the 29th day of experiment. Blood samples were collected via cardiac puncture. The blood was collected into sample bottles containing EDTA for hematological analysis such as Hemoglobin concentration, white blood cell counts (WBC), differentials (neutrophils, eosinophils, basophils, lymphocyte and monocyte), red blood cell count (RBC), platelets and hemoglobin (Hb) concentration using automated Haematology machine (Cell-Dyn, Abbott, USA).

Food and water consumption

The amounts of feed and water consumed were measured daily as the difference between the quantity of feed and water supplied each day and the amount remaining after 24hours.The rats were sacrificed on the 29th day of experiment organs were harvested for further gross histo-pathological analysis.

Statistical analysis

Data were expressed as the Mean ±Standard Error of the Mean (SEM). Data were analyzed statistically using one-way Analysis of Variance (ANOVA) followed by Dunnett's post hoc test for multiple comparisons between the control and treated groups. Values of $P \leq 0.05$ were considered significant.

RESULT

Effect of 28 days oral administration of *Ocimum canum* on hematological parameters in rats

Ocimum canum caused significant ($p<0.05$) decrease in the level of red blood cell, hemoglobin, platelet etc. and significantly ($p<0.05$) caused an increase in mean corpuscular hemoglobin concentration in the rats at the dose level of 200 mg/kg compared to the control. The level of basophiles, neutrophiles, eosinophils and lymphocytes were however not significantly ($p<0.05$) affected by mean corpuscular hemoglobin concentration (Table 1).

Effect of 28 days oral administration of *Ocimum canum* on body weight (g) in rats

In the 1st, 3rd and 4th week significant ($p<0.05$) increase was observed at 100 mg/kg dose level by ethanol leaf extract of *Ocimum canum*, while in the 2nd week there was increase in body weight by the extract but not significant when compared with the control (Table: 2).

Effect of 28 days oral administration of *Ocimum canum* on relative organ to body weight ratio in rats.

The brain, lungs, spleen and stomach were observed to be significantly ($p<0.05$) increased in rats with dose level of 100 mg/kg of the ethanol plant extract. At higher doses of 200 and 400 mg/kg there was no significant ($p<0.05$) change (Table 3).

Effect of oral administration of ethanol leaf extract of *Ocimum canum* on histology of Brain, Lungs, Spleen and Stomach of rats

Histopathological examination of the brain showed normal features at all doses and slight vacuolation at 400 mg/kg dose of the extract (Plate VI). The lungs showed normal features at all doses respectively (Plate x). The spleen showed Slight lymphocyte hyperplasia at all doses and normal features at the control (10 ml/kg). The stomach showed normal features at all doses of the extract administered.

Table 1: Effect of 28 days oral administration of ethanol leaf extract of *Ocimum canum* on hematological parameters in wistar rats

	Treatment (mg/kg)			
Hematological parameters	DW(10ml/kg)	100 mg/kg	200 mg/kg	400 mg/kg
WBC ($\times10^9$/L)	8.167±0.772	6.740±1.419	3.700±0.657*	7.220±1.085
RBC ($\times10^{12}$/L)	8.30±0.34	8.65±0.66	6.11±0.55*	7.71±0.21
HGB (g/dL)	15.95±0.56	15.24±0.66	11.33±0.86*	14.58±0.36
HCT (g/dL)	55.18±2.03	56.60±3.74	34.67±3.18*	53.40±1.81
MCV (fL)	66.62±0.93	65.40±1.44	57.17±0.31*	69.60±1.72
MCH (pg)	19.17±0.17	17.80±1.02	18.83±0.37	18.80±0.20
MCHC (g/dL)	29.17±0.17	27.40±1.12	32.50±0.62*	27.60±0.68
PLT ($\times10^9$/L)	620.83±52.81	567.00±96.41	252.00±50.38*	670.40±55.72
LYM (%)	86.83±4.06	85.00±4.18	82.83±5.89	86.40±3.14
NEUT ($\times10^9$/L)	10.83±3.67	10.83±3.68	15.40±5.60	11.20±3.02
EOSI ($\times10^9$/L)	1.50±0.34	2.40±0.75	1.80±0.47	1.20±0.20
BASO ($\times10^9$/L)	1.00±0.28	2.00±0.55	2.50±1.50	3.30±2.20

Data presented as Mean ± SEM: n = 6, One way ANOVA, followed by Dunnett's post hoc for multiple comparison *significantly different from the distilled water (DW) control at $p<0.05$. DW = distilled water (WBC = white blood cells, RBC = red blood cells, HGB = hemoglobin, HCT = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, PLT = platelet, LYM = lymphocyte, NEUT = neutrophils, EOSI = eosinophils, BASO = basophils).

Table 2: Effect of 28 days oral administration of *Ocimum canum* on body weight (g) in rats

Treatment (mg/kg)	Week 1	Week 2	Week 3	Week 4
DW (10ml/kg)	162.95±6.71	176.10±6.35	184.75±7.30	174.72±9.73
100 mg/kg	168.33±10.17	186.48±11.58	171.98±6.52	158.60±9.61
200 mg/kg	226.17±19.51*	239.67±19.56	240.83±20.44*	240.83±20.44*
400 mg/kg	168.68±11.39	148.48±8.94	168.47±9.57	159.54±8.30

*Significantly different from the distilled water (DW) control at p<0.05. DW = distilled water

Table 3: Effect of 28 days oral administration of *Ocimum canum* on relative organ to body weight ratio in rats

	Relative organ to Body weight Ratio%			
Treatment(mg/kg))	**BRAIN**	**LUNGS**	**STOMACH**	**SPLEEN**
DW(10 ml/kg)	0.808±0.02	0.905±0.179	0.908±0.063	0.429±0.018
100 mg/kg	0.943±0.098	0.753±0.063	0.839±0.027	0.429±0.060
200 mg/kg	1.291±0.191*	1.681±0.345*	1.318±0.120*	0.934±0.099*
400 mg/kg	0.961±0.068	0.743±0.079	0.874±0.041	0.548±0.029

*Significantly different from the distilled water (DW) control at p<0.05. DW = distilled water

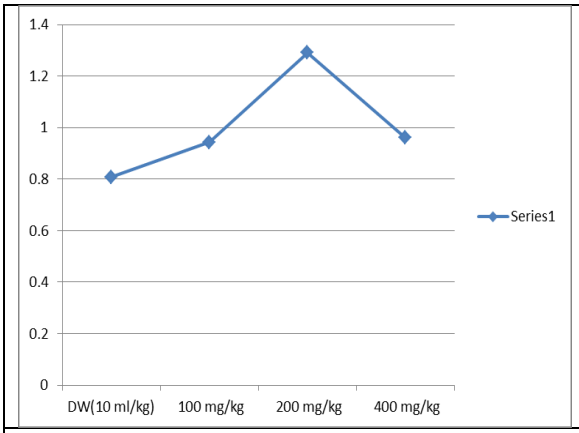

Fig. 1: Effect of Ocimum canum on relative organ and body weight ratio of the brain

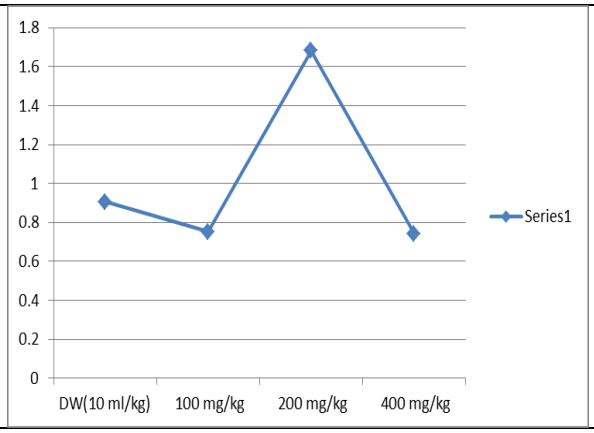

Fig. 2: Effect of *Ocimum canum* on relative organ and body weight ratio of the lung

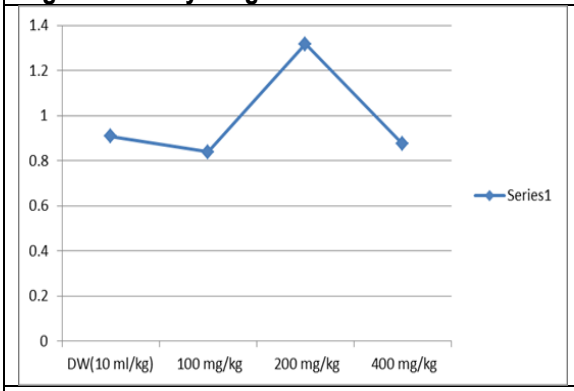

Fig. 3: Effect of *Ocimum canum* on relative organ and body weight ratio of the stomach

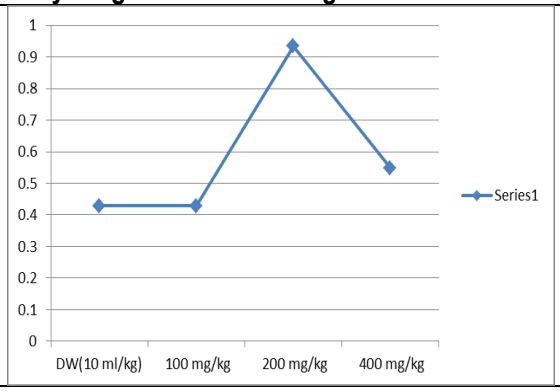

Fig. 4: Effect of *Ocimum canum* on relative organ and body weight ratio of the spleen

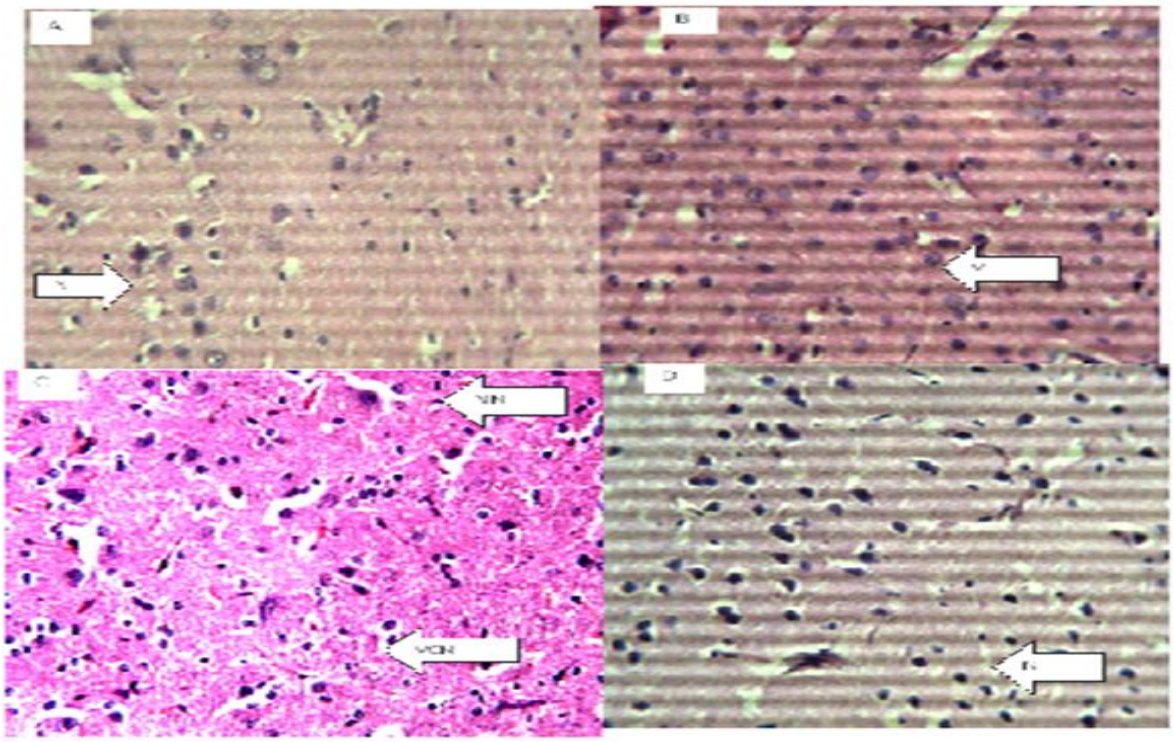

Figure 5. **Photomicrograph of the brain (Hematoxylin and eosin.** H and E ×100). (a) Control group, Shows normal neurons (N). (b) 100 mg/kg. (c) 200 mg/kg (d) 400 mg/kg of ethanol stem extract of *Ocimum canum*

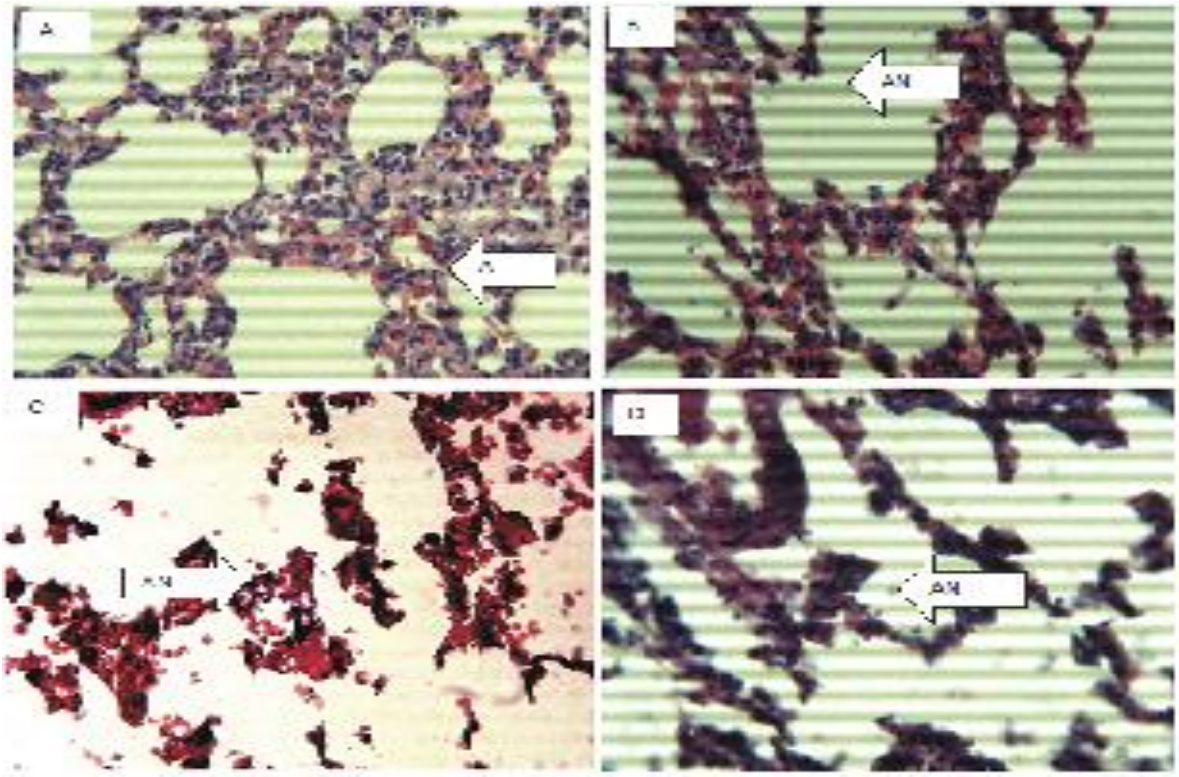

Figure 6. Photomicrograph of the lungs (Hematoxylin and eosin. H and E ×100). (a) Control group, shows normal alveoli (A). (b) 100 mg/kg (c) 200 mg/kg (d) 400 mg/kg ethanol leaf extract of *Ocimum canum*.

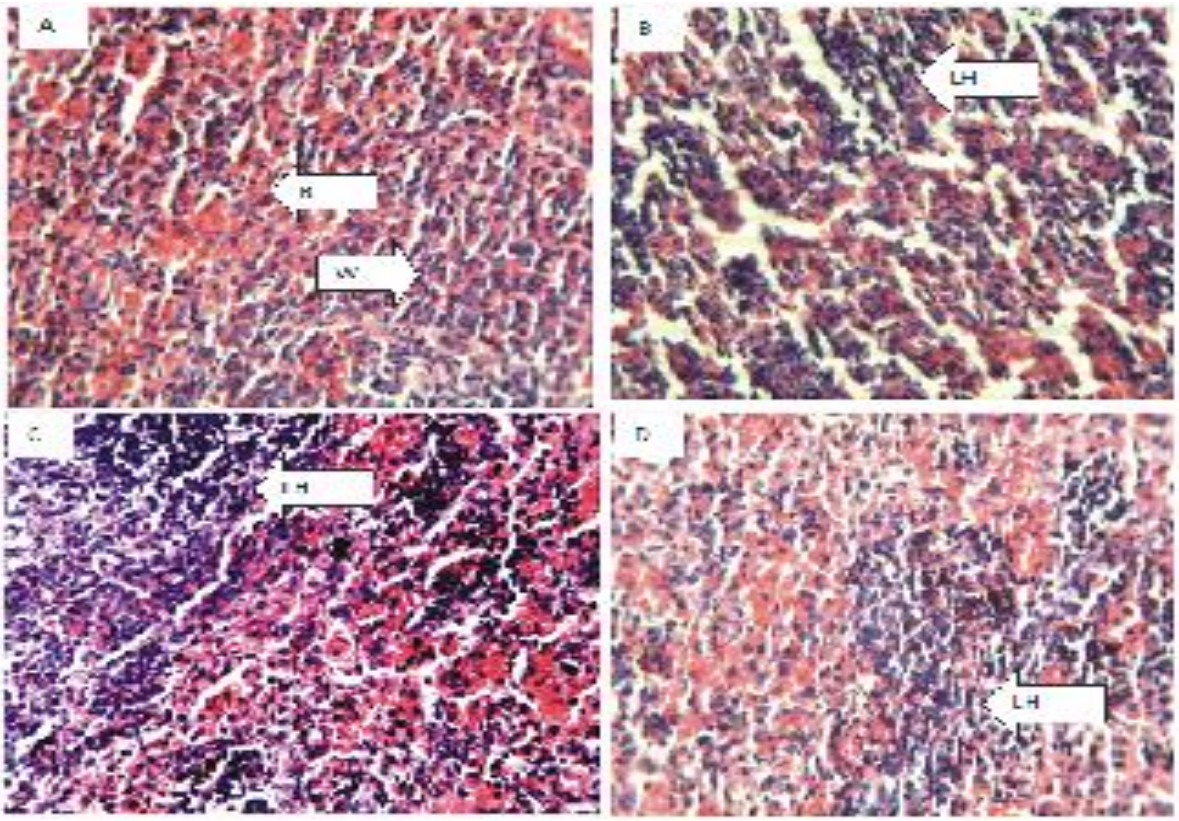

Figure 7. Photomicrograph of the Stomach (Hematoxylin and eosin. H and E ×100). (a) control group, shows normal red (R) and white (W) pulp. (b) 100 mg/kg (c) 200 mg/kg, (d) 400 mg/kg of ethanol leaf extract of *Ocimum canum*.

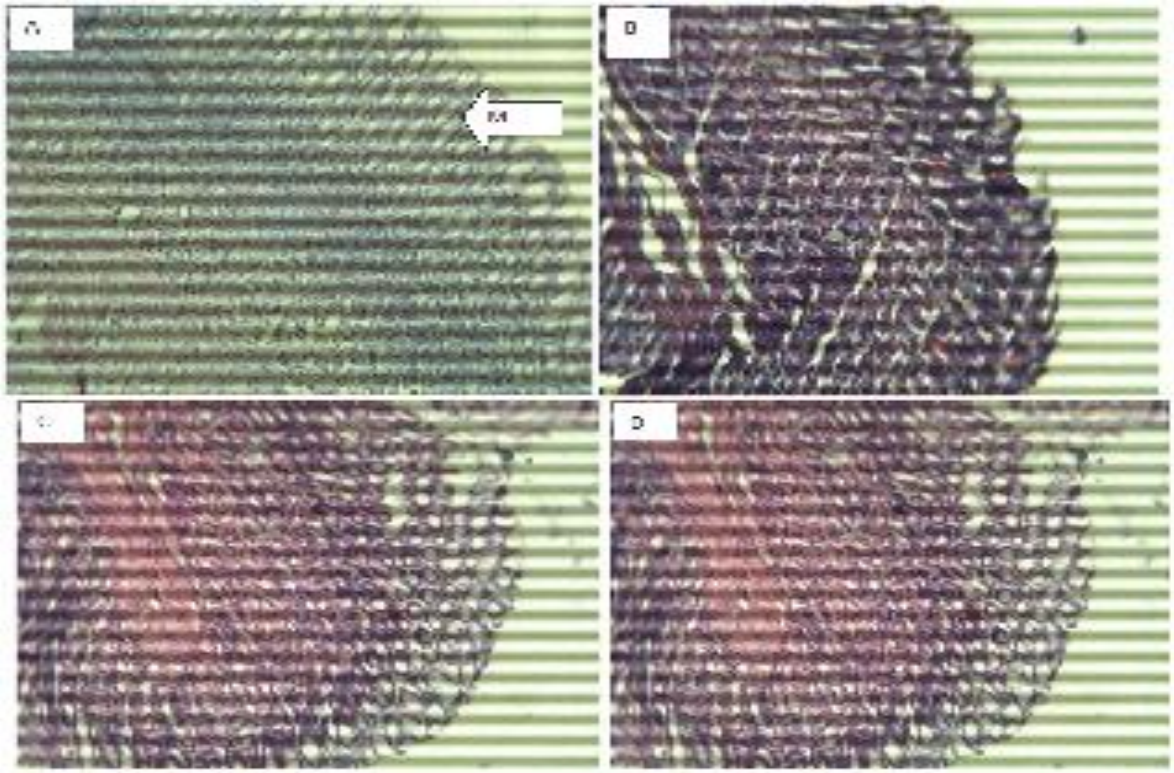

Figure 8. **Photomicrograph of the Spleen** (Hematoxylin and eosin. H and E ×100). (a) Control group, shows normal stomach mucosa (M). (b) 100 mg/kg, shows normal features. (c) 200 mg/kg, shows normal features. (d) 400 mg/kg, shows normal features.

DISCUSSION

Over the past few years, toxicology has played a vital part in the screening of newly developed drugs before it can be used on humans. Through incorporation of physiological, hematological, biochemical, pathological, and histopathological investigations in simplified test procedures with small numbers of animals, it is possible to markedly increase the informational content of the results with regard to the toxicological spectrum and the target organs of toxicity. There are many commonly grown plants that are quite poisonous. Medicinal plants, either as an extract, pure compound or as a derivative, offer unlimited opportunities for the discovery of new drugs [(10)]. Most of the natural products used in folk remedy have solid scientific evidence with regard to their biological activities. However, there is little information or evidence available concerning the possible toxicity that medicinal plants may cause to the consumers [(11)].
Subchronic studies assess the undesirable effects of continuous or repeated exposure of plant extracts or compounds over a portion of the average life span of experimental animals, such as rodents. Specifically, they provide information on target organ toxicity and are designed to identify noobservable adverse effect level[(12)]. The body weight changes serve as a sensitive indication of the general health status of animals[(13)]. Weight gains were observed in all animals administered with *O. canum* extract. It can be stated that the *O. canum* extract did not interfere with the normal metabolism of animals as corroborated by the non-significant effect from animals in the vehicle control group. The significant increment in food and water intake is considered as being responsible for the increment in body weight gain. As mentioned earlier the loss of appetite is often synonymous with weight loss due to disturbances in the metabolism of carbohydrate, protein, or fat [(14)]. Therefore, the normal food and water intake ($P > 0.05$) without loss of appetite are suggested as being responsible for the observed increment in body weight in this study. Fatness is characterized by increased adipose tissue mass that results from both increased fat cell number and increased fat cell size. Adipose tissue is a dynamic organ that plays an important role in energy balance and changes in mass according to the metabolic requirements of the organism[(15)]. Moreover, it was reported that excess energy intake and reduced energy expenditure result in abnormal excessive growth of white adipose tissue (WAT), which can lead to the development of obesity[(16)] . The current research findings did not suggest that *Ocimum canum* extract at the various doses administered may prevent the accumulation of WAT in rats. Also, the level of basophiles, neutrophiles, eosinophils and lymphocytes were not affected by the extract. This indicates that the plant may not affect the body immune. It could also suggest that the plant may have immunomodulatory property. Agents that activate host defense mechanisms in the presence of an impaired immune responsiveness can provide supportive therapy to conventional chemotherapy [(17)]. The study shows that the extract does not stimulate or activate the immune system. Histological analysis does not reveal significant damage to any of the organ. This suggests that the ethanol extract of the plant at the maximum dose administered, which is equivalent to the extrapolated dose consumed by human, it may be safe. Though, biochemical parameters which are essential for normal physiological functions of each organ were not determine, hematological, anatomical and histopathological analysis suggest that the plant may be relatively safe at the doses administered. Further research needs to be undertaken to determine subsequent effect with higher doses.

CONCLUSION

Result from the study indicates that the plant may be orally safe for consumption.

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