



SUB-ACUTE TOXICITY STUDY OF ETHANOL LEAF EXTRACT OF *Ocimum canum* ON THE KIDNEY OF WISTAR RATS

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ABSTRACT

The use of plant in treatment of human diseases is as old as man. Medicinal plants are often consumed locally without a graded dose or expected duration of use. This can precipitate unexpected side effects on the tissue, organ or body system. *Ocimum canum* is a plant regularly consumed in many part of Sub-Sahara Africa in management of various conditions such as infection, pain and diarrhea. The aim of this study is to study sub-acute toxicity effect of the ethanol plant extract on the liver. Animals 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 histological nephrotoxic potential of the plant was studied using haematotoxilin 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. *Ocimum canum*, significantly ($p < 0.05$) increased Na level at 200 mg/kg and Creatinine level at 100 mg/kg dose levels respectively when compared to the control. Other parameters (K, CL and Urea levels) were not significantly affected. Histological study reveals slight tubular distortion. The result of the study showed that the plant could have slight effect on the kidney which suggests that the plant should be used with caution when taken for a sustained period.

KEYWORDS: OCIMUM CANUM; KIDNEY; RATS; TOXICITY

INTRODUCTION

Medicinal plants are plant used to maintain health, to be administered for a specific condition, or both, whether in modern medicine or in traditional medicine (1). Medicinal plants have been discovered and used in traditional medicine practices since prehistoric times. Plants synthesize hundreds of chemical compounds for functions including defence against insects, fungi, diseases, and herbivorous mammals (2). Numerous phytochemicals with potential or established biological activity have been identified. However, since a single plant contains widely diverse phytochemicals, the effects of using a whole plant as medicine are uncertain. Furthermore, the

phytochemical content and pharmacological actions, of many plants having medicinal properties have not been rigorously assessed to define their safety. Medicinal plants are widely used in non-industrialized societies, mainly because they are readily available and cheaper than modern medicines (2). However, the rationale for the utilization of medicinal plants has rested largely on long-term clinical experience with little or no scientific data on their efficacy and safety (3). Medicinal herbs have their use as medicament based simply on a traditional folk use that has been perpetuated along several generations. With the upsurge in the use of herbal medicines a thorough scientific investigation of these plants is imperative, based on the need to validate their folkloric usage

(4). Herbs are supposed to be safe but many unsafe and fatal side effects have been reported (5). These could be direct toxic effects, allergic reactions, effects from contaminants and/or interactions with drugs and other herbs (6). Phytotherapeutic products are many times, mistakenly regarded as less toxic because they are 'natural'. Nevertheless, those products contain bioactive principles with potential to cause adverse effects (7).

Ocimum Canum belongs to the Lamiaceae family. This annual plant is native to the African continent and grows to a height of 2 feet. It is also known as the African basil with a distinct mint flavor, with hairy leaves and scented flowers (8). *Ocimum Canum* is grown for its medicinal and culinary value and it is highly useful in treating various types of diseases and in lowering blood glucose, especially in type 2 diabetes levels (9). The herb can be used to treat colds, fevers, parasitic infestations on the body and inflammation of joints and headaches (10). The traditional medicine, its value is recognize in the treatment of fevers, dysentery and tooth problems. It was used as an insect repellent to counter the insect damages postharvest (8). In this work sub-acute toxicity study of *Ocimum Canum* was carried out on the kidney.

MATERIALS AND METHOD

Animals

Male and female Wistar 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 (127-293 g) were selected and randomized into four groups of six rats per group. Group 1 served as the control and received normal saline (10 ml/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 D₀ while the day of sacrifice was designated as D₂₉.

Haematological analysis

The rats were sacrificed on the 29th day of experiment. Blood samples were collected via cardiac puncture. One portion of 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).

Kidney Function Test

The following biochemical parameters were assayed as markers of kidney function using diagnostic kits; Level of electrolytes (Na⁺, K⁺, Cl⁻, and HCO³⁻), creatinine and blood urea. The above parameters were determined at the Chemical Pathology Department of University of Jos Teaching Hospital.

Kidney harvested were preserved in 10% formal saline solution, processed, sectioned and stained with Heamatoxylin and eosin (H&E) according to standard procedures at Department of Chemical Pathology, University of Jos Teaching Hospital, Jos.

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 ≤ 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, neutrophils, eosinophils and lymphocytes were however not significantly ($p < 0.05$) affected by mean corpuscular hemoglobin concentration

Effect of 28 days oral administration of *Ocimum canum* on renal indices and electrolytes in Wistar rats

Ocimum canum significantly ($p < 0.05$) increased Na level at 200 mg/kg and Creatinine level at 100 mg/kg dose levels respectively when compared to the control. Other parameters (K, CL and Urea levels) were not significantly affected.

Histopathological Investigations of the effect of 28 days oral administration of *Ocimum canum* on renal indices and electrolytes in Wistar rats

The kidney showed slight tubular distortion and glomerular necrosis at 200 mg/kg and 400 mg/kg. There was also, Slight tubular necrosis with lymphocyte hyperplasia at 100 mg/kg. Normal renal histological features were observed in the control group.

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

| Hematological parameters | Treatment (mg/kg) | | | |
|----------------------------|--------------------|--------------------|---------------------|--------------------|
| | DW(1ml/kg) | 100 mg/kg | 200 mg/kg | 400 mg/kg |
| WBC ($\times 10^9/L$) | 8.167 \pm 0.772 | 6.740 \pm 1.419 | 3.700 \pm 0.657* | 7.220 \pm 1.085 |
| RBC ($\times 10^{12}/L$) | 8.30 \pm 0.34 | 8.65 \pm 0.66 | 6.11 \pm 0.55* | 7.71 \pm 0.21 |
| HGB (g/dL) | 15.95 \pm 0.56 | 15.24 \pm 0.66 | 11.33 \pm 0.86* | 14.58 \pm 0.36 |
| HCT (g/dL) | 55.18 \pm 2.03 | 56.60 \pm 3.74 | 34.67 \pm 3.18* | 53.40 \pm 1.81 |
| MCV (fL) | 66.62 \pm 0.93 | 65.40 \pm 1.44 | 57.17 \pm 0.31* | 69.60 \pm 1.72 |
| MCH (pg) | 19.17 \pm 0.17 | 17.80 \pm 1.02 | 18.83 \pm 0.37 | 18.80 \pm 0.20 |
| MCHC (g/dL) | 29.17 \pm 0.17 | 27.40 \pm 1.12 | 32.50 \pm 0.62* | 27.60 \pm 0.68 |
| PLT ($\times 10^9/L$) | 620.83 \pm 52.81 | 567.00 \pm 96.41 | 252.00 \pm 50.38* | 670.40 \pm 55.72 |
| LYM (%) | 86.83 \pm 4.06 | 85.00 \pm 4.18 | 82.83 \pm 5.89 | 86.40 \pm 3.14 |
| NEUT ($\times 10^9/L$) | 10.83 \pm 3.67 | 10.83 \pm 3.68 | 15.40 \pm 5.60 | 11.20 \pm 3.02 |
| EOSI ($\times 10^9/L$) | 1.50 \pm 0.34 | 2.40 \pm 0.75 | 1.80 \pm 0.47 | 1.20 \pm 0.20 |
| BASO ($\times 10^9/L$) | 1.00 \pm 0.28 | 2.00 \pm 0.55 | 2.50 \pm 1.50 | 3.30 \pm 2.20 |

Data presented as Mean \pm SEM: n = 6, One way ANOVA, followed by Dunnett's post hoc for multiple comparison *significantly different from the distilled water (DW) control at $p < 0.05$. SHBP = Safi® herbal blood purifier, 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 ethanol leaf extract *Ocimum canum* on renal indices and electrolytes in wistar rats

| Renal indices and electrolytes | Treatment (mg/kg) | | | |
|--------------------------------|-------------------|--------------|--------------|-------------|
| | DW (10ml/kg) | 100 mg/kg | 200 mg/kg | 400 mg/kg |
| Potassium (mmol/L) | 6.00±0.57 | 7.66±0.69 | 5.32±0.38 | 5.68±0.15 |
| Sodium (mmol/L) | 137.00±2.90 | 142.20±2.82 | 147.00±1.95* | 144.25±1.88 |
| Chloride (mmol/L) | 100.00±5.83 | 99.80±6.61 | 107.20±2.28 | 100.50±2.65 |
| Urea (mmol/L) | 8.46±0.29 | 8.46±0.69 | 7.86±0.20 | 7.68±0.33 |
| Creatinine (µmol/L) | 63.40±9.63 | 83.80±12.44* | 54.80±16.93 | 71.75±6.13 |

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. SHBP = Safi® herbal blood purifier, DW = distilled water.

DISCUSSION

The phytochemicals in medicinal plants have been reported to be the active compounds responsible for the pharmacological potentials of medicinal plants (11). *Ocimum canum* Linn belongs to the Family- Lamiaceae (Labiatae). Many species of Lamiaceae have long history of uses in culinary spices and folk medicine (12). Medicinal properties of the plant would be due to the presence of alkaloids, flavonoids, phenolic, tannins, and other phytoconstituents. Several reports have demonstrated that secondary plant metabolites exert diverse medicinal biological effects¹³. In the study, Wistar rats were used to screen the safety at various dose level of the plant extract with hematological and biochemical estimation from blood and histopathology of kidney for 28 days. Ethanol extract of leaves of *O. canum* resulted in significant (*p<0.05) decrease in the red blood cell, hemoglobin and platelet when compared to the control group of rats. This indicated that the plant may suppress the production of red blood cells, decrease the lifespan of red blood cells or causes problems with how the body uses iron. The result of this study contradict what was previously done by Rai, Ghosh and Basheer(13) that *Ocimum canum* resulted in increased hemoglobin level indicating enhanced formation of hemoglobin. Also, the level of basophiles, neutrophils, 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. The study also showed that *O. canum* caused slight increase in

serum Na and creatinine level. This may be due to disruptive effect of the plant. An increase creatinine level can be observe in some kidney diseases, due to loss of normal excretory function of the creatinine (14), when there is a muscular cells damage or following an incompatible medication interfering with the normal functioning of the kidney (15). Creatinine, is mostly derived from endogenous sources by tissue creatinine breakdown (16). Thus serum urea concentration is often considered a more reliable renal function predictor than serum creatinine (17). In this study, serum urea was unaffected suggesting that the plant may cause slight damage to the kidney.

The histopathological analysis, showed that in all groups after 28 days administration of ethanol extract of *Ocimum canum* the kidney there was slight changes at the cellular level in comparison to control. This resonates with other parameters that the leaves of the plant slightly have nephrotoxic effect.

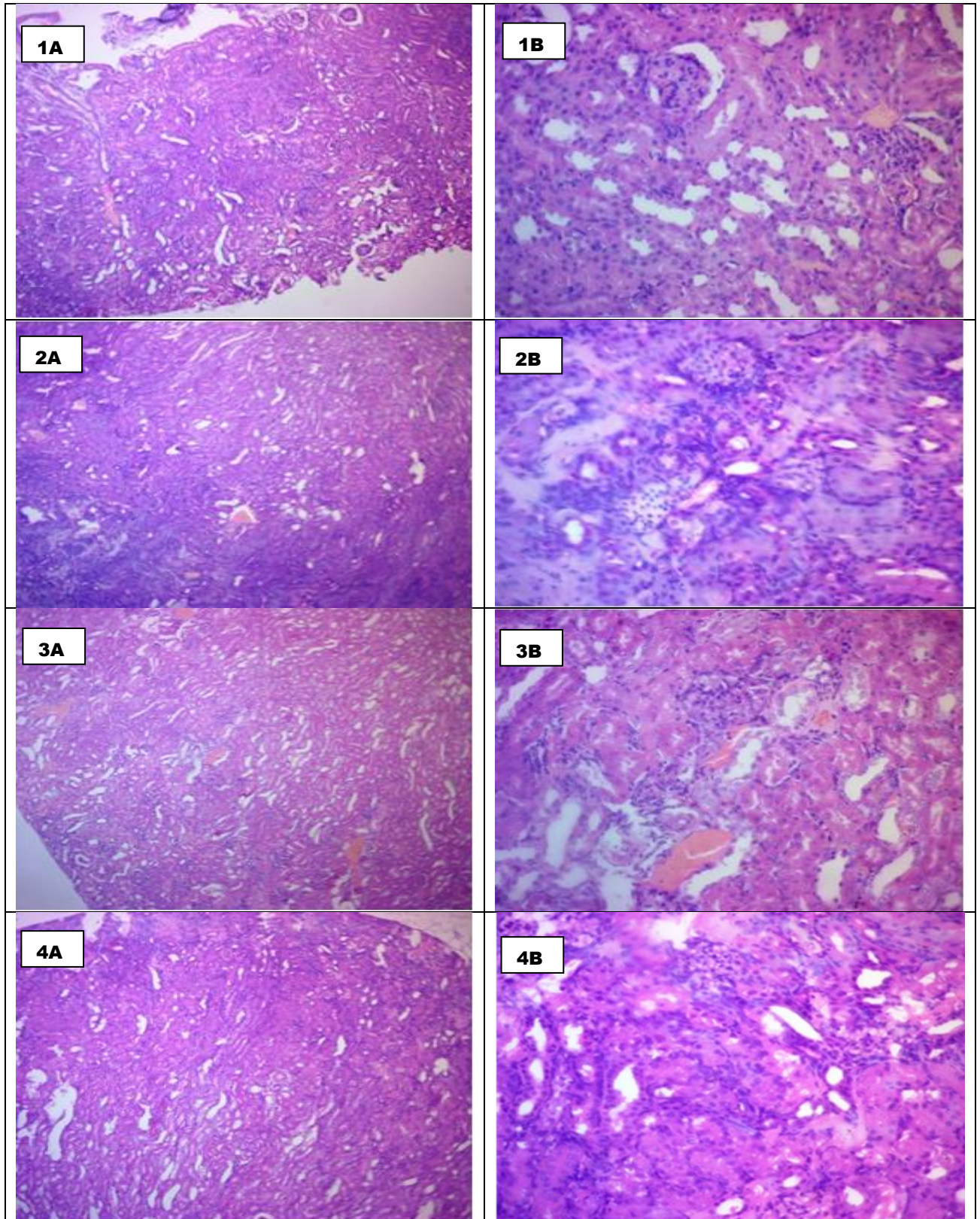


Figure 1-4: Histological sections of Kidneys of rats treated with Normal saline 10 ml/kg (1), *Ocimum canum* 200 mg/kg (2), *Ocimum canum* 200 mg/kg bw (3) and *Ocimum canum* 400 mg/kg at magnification A (x100) and B(x400) stained with H&E Technique.

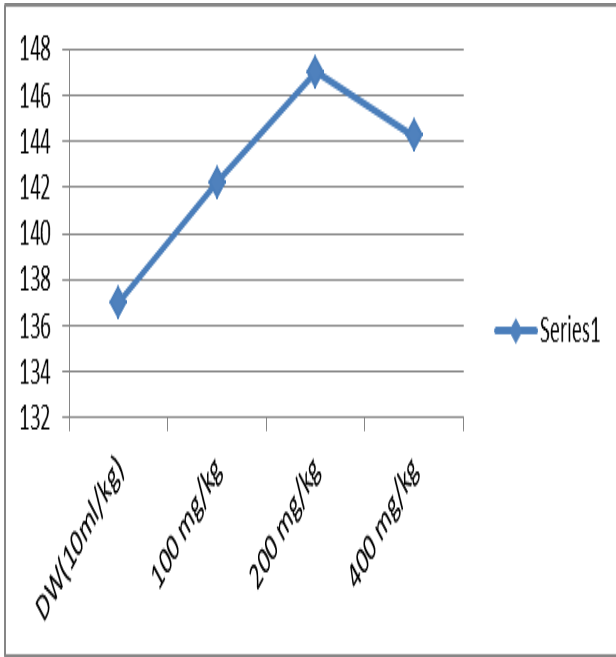


Fig 5: The effect of the ethanol leaf extract of *Ocimum canum* serum sodium level in rats

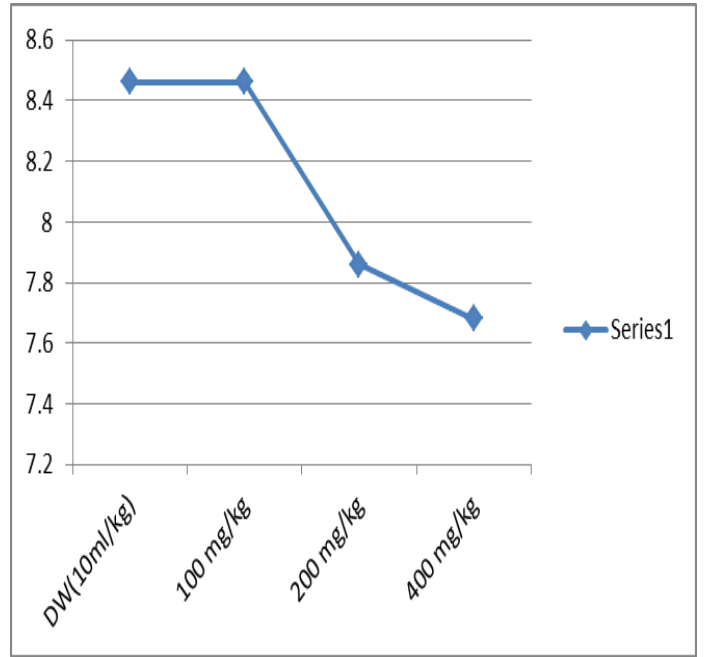


Fig 6: The effect of the ethanol leaf extract of *Ocimum canum* serum urea level in rats

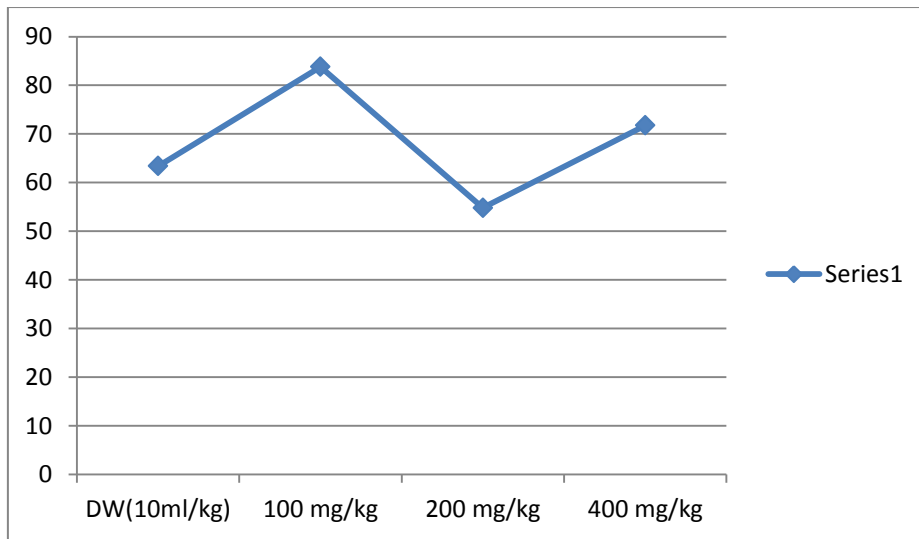


Fig 7: graph showing effect of the ethanol leaf extract of *Ocimum canum* serum urea level in

CONCLUSION

Result from biochemical parameters and histological study shows that the plant possesses chemical constituent that has slight nephrotoxic potential precipitating suggestion that caution should be taken while consumed for sustained period.

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