Sub-Acute Toxicity Study of Ethanol Leaf Extract of *Ocimum Canum* on Liver of Wister Rats

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Abstract

Background/Aim: The liver plays an extremely important role in the body. It ensures the removal of toxins and has numerous other functions, including: Fat metabolism, carbohydrate metabolism and protein metabolism. The function and integrity of this organ is regularly challenged by endogenous and environmental substances. *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 determine the sub - acute effect of *Ocimum canum* on the liver of Wister rats.

Method: 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 hepatotoxic potential of the plant was studied using haematotoxylin and eosin (H&E) staining technique.

Result: 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 extract did not produce any significant change (P<0.05) in the level of ALB, AST and ALT when compared to the control. At 100 mg/kg dose level, *Ocimum canum* produced a decrease in BILD concentration in the treated rats while at 200 mg/kg dose level it caused slight increase in the levels of ALP, BILD AND BILT concentration. Histopathological observation also agrees with other parameters.

Conclusion: the result of the study suggests that the plant may be safe for consumption as it is been used by the locals to achieve it intended purpose.

I. INTRODUCTION

Toxicity testing is paramount in the screening of newly developed drugs before it can be used on humans. Toxicity testing is the determination of potential hazards a test substance may likely produced and the characterization of its action, most of the toxicity testing is carried out on experimental animals[1]. The advantages of using animal models in toxicity testing are enormous. These advantages include the possibility of clearly defined genetic constitution and their amenity to controlled exposure, controlled duration of exposure, and the possibility of detailed examination of all tissues following necropsy[2]. The information obtained can serves as the basis for hazard classification and labeling of chemicals in commerce[3].

Drug-Induced Liver Injury (DILI) occurs when the consumption of a substance, such as a drug, nutritional supplement, medicinal herb, or plant, causes direct damage to the liver. In some cases, there might not be any symptoms, which can allow damage to go unnoticed[4]. While some drugs, such as acetaminophen, elicit predictable and dose-dependent effects on the liver, others may have unforeseeable results, oftentimes unrelated to dose. DILI typically occurs within three months of beginning the drug, but it can vary from a couple hours to a year after drug initiation [4]. One example of DILI is drug-induced hepatitis, which is characterized by inflammation of the liver [5]. This condition can be caused by a number of different drugs[5].

Ocimum canum, known as American basil or "hoary basil"[6], is an annual herb with white or lavender flowers. It is used for medicinal purposes[7]. Despite the misleading name, it is native to Africa, the Indian Subcontinent, China, Southeast Asia[8]. *Ocimum canum* Sims. (Hairy Basil) is a traditional medicinal plant distributes throughout sub-Saharan Africa and very well known in northern Nigeria[9]. 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[10]. The essential oils found in this species have strong fungicidal activity against certain plant pathogens[11]. 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[12]. 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[13].

The leaves of the plant has been used specially for managing various types of diseases and lowering blood glucose and also treats cold, fever, parasitic infestations on the body and inflammation of joints and headaches[14]. Essential oil from the leaves of *O. canum* possesses antibacterial and insecticidal properties[15]. In this study effect of *Ocimum canum* on liver

was investigated after 28 days of oral administration of the ethanol leaf extract of the plant.

II. 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.

Chempathology analysis

Sera were separated from the blood samples and were stored at -20oC until used for biochemical investigations such as measuring total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total cholesterol, and total and direct bilirubin

Histology study

The livers of the animals were surgically removed and weighed and a part of each was fixed in 10% formaldehyde for histological processes.

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.

III. RESULT

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

There was no significant difference in the relative organ to body weight ratio of the liver when compared to the control

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

Effect of 28 days oral administration of ocimum canum on hepatic indices in rats.

At 100 mg/kg dose level, *Ocimum canum* produced significant ($p<0.05$) decrease in BILD concentration in the treated rats while at 200 mg/kg dose significant ($p<0.05$) increase was obtained in ALP levels, BILD and BILT concentrations when compared to the control. All other parameters studied were however, not significantly affected

Effect of 28 days oral administration of ethanol leaf extract of Ocimum canum on histology Liver of rats.

The liver showed vascular congestion, slight hepatic necrosis with sinusoidal congestion and lymphocyte hyperplasia at 100 mg/kg and 200 mg/kg, Sinusoidal congestion at 200 mg/kg and Moderate hepatic necrosis and vascular congestion at the control (10ml/kg).

Table 1: effect of 28 days oral administration of Ocimum canum on relative organ to body weight ratio in rats.

Treatment(mg/kg)	LIVER
DW(10 ml/kg)	3.623±0.273
100	3.151±0.169
200	3.591±0.234
400	0.961±0.068

Blood purifier, DW = distilled water

Table 2: Effect of 28 days oral administration of Ocimum canum on hematological parameters in wistar rats.

Hematological parameters	Treatment (mg/kg)			
	DW(1ml/kg)	100	200	400
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 $p<0.05$. = 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 3: Effect of 28 days oral administration of Ocimum canum on hepatic indices in wistar rats.

Hepatic indices	Treatment (mg/kg)			
	DW(10 ml/kg)	100	200	400
ALB (g/L)	39.60±1.72	43.20±0.97	35.20±1.59	41.75±2.18
ALP (IU/L)	113.20±6.73	152.00±8.99	370.00±43.37*	125.50±6.93
ALT (IU/L)	59.80±3.01	67.80±10.28	91.40±10.99	87.75±27.67
AST (IU/L)	292.80±79.90	297.20±57.60	171.20±30.88	213.00±10.75
BILD (µmol/L)	0.22±0.07	0.16±0.07*	0.60±0.14*	0.60±0.13
BILT (µmol/L)	2.14±0.50	2.56±0.25	3.46±0.80*	2.73±0.56
TP (g/L)	79.60±3.08	6.07±2.71	11.33±5.07	80.25±2.02

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 (ALB = albumin, ALP = alanine phosphatase, ALT = alanine transaminase, BILD = unconjugated bilirubin, BILT = conjugated bilirubin, TP = total protein).

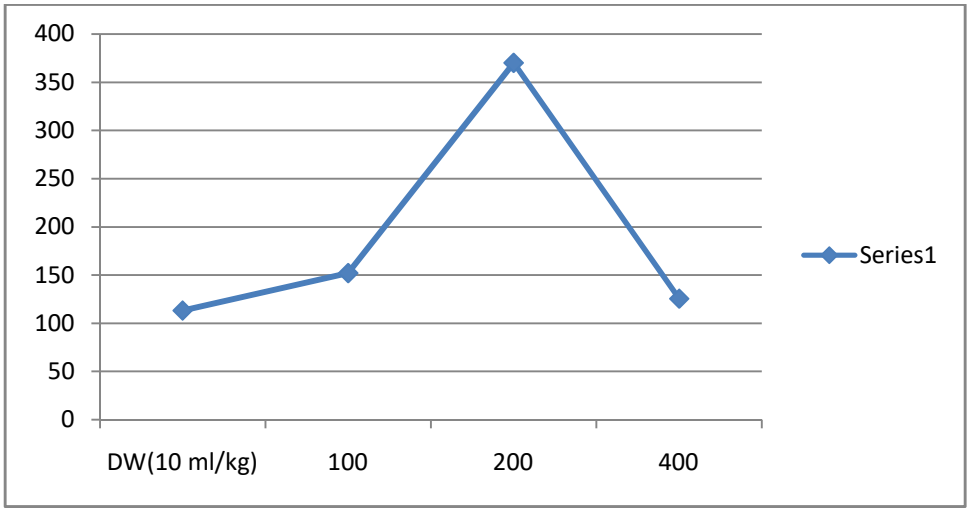

Figure 1: Effect of Ocimum canum on ALP level in rat

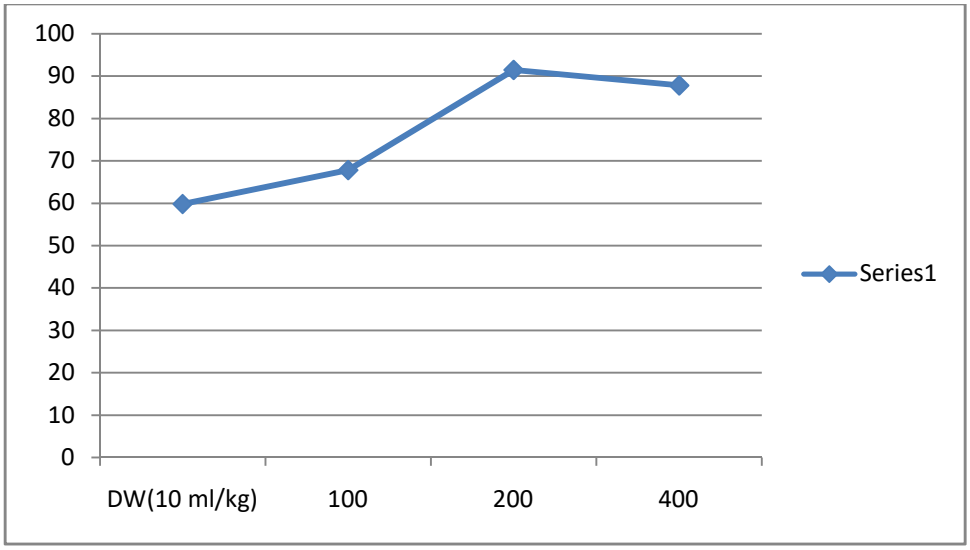

Figure 2: Effect of Ocimum canum on ALT level in rat

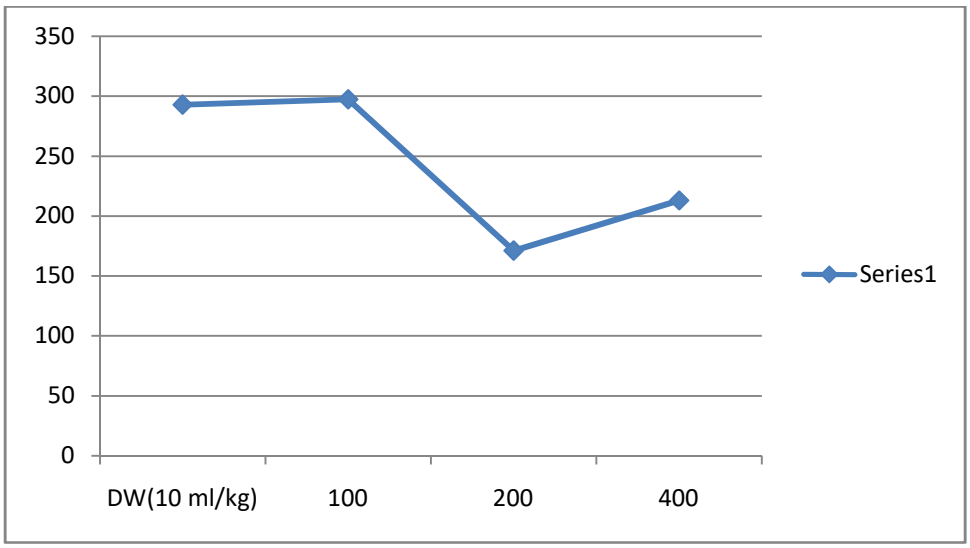

Figure 3: effect of Ocimum canum on level of AST level on rat

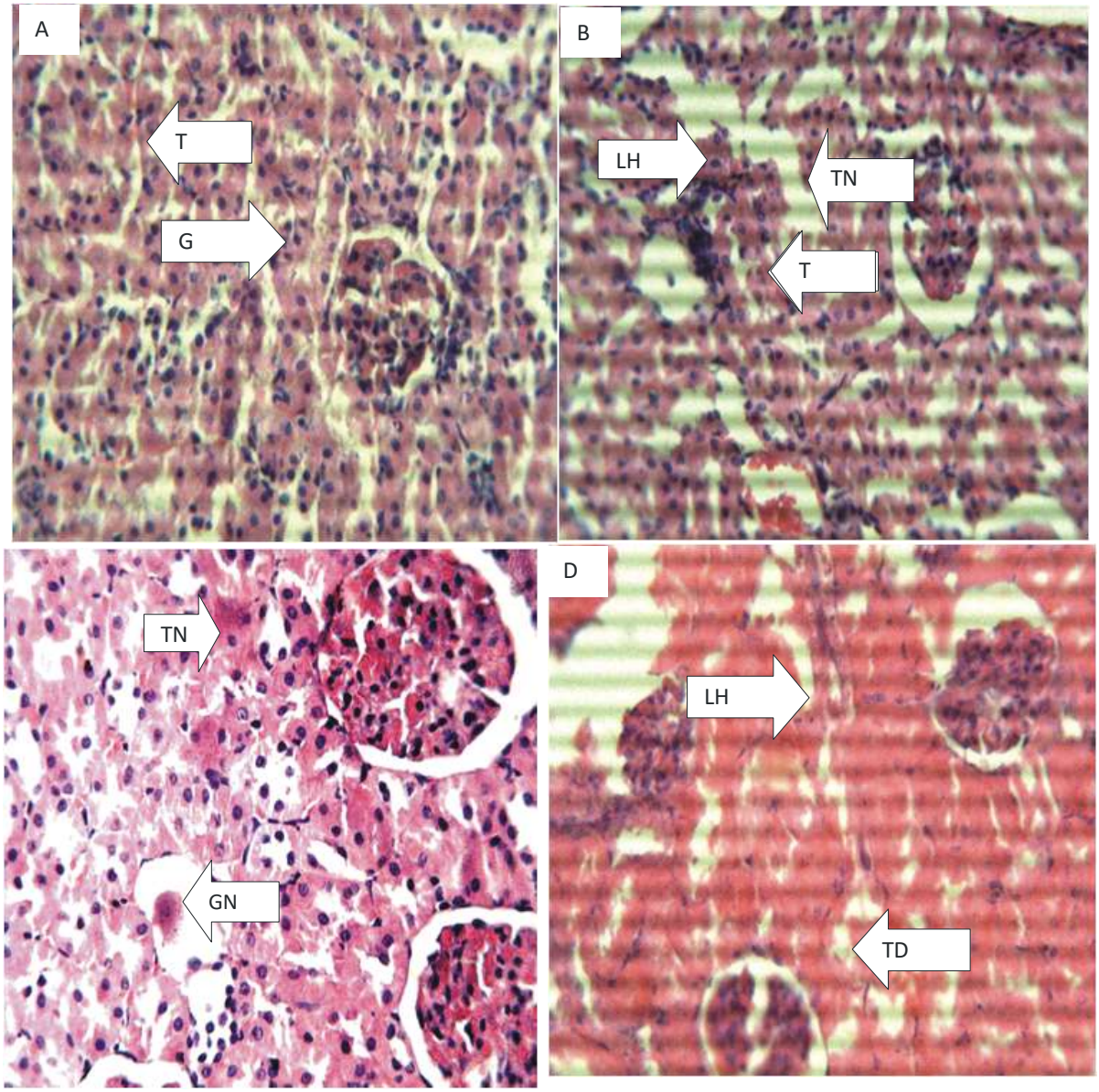

Figure 4:. figure of the liver (Hematoxylin and eosin. H and E ×100). (a) Control group, shows normal hepatocyte (H). (b) OC 100 mg/kg, vascular congestion (VC), slight hepatic necrosis (HN) with sinusoidal congestion (SC) and lymphocyte hyperplasia (LH). (c) OC 200 mg/kg, moderate vacuolation (V) with sinusoidal congestion (SC) and moderate hepatic necrosis (HN). 400 mg/kg vascular congestion (VC) with sinusoidal congestion (SC) with slight hepatic necrosis (HN).

IV. DISCUSSION

Hematological parameters are useful indices that can be employed to assess the toxic potentials of plant extracts in living systems[16]. They can also be used to explain blood relating functions of chemical compound/plant extract. From the results of this study, administration of ethanol leaf extract of *Ocimum canum* led to a decrease in platelet counts, red blood cell and haemoglobin in rats. Reduction in platelets count in experimental animals has been reported to indicate adverse effect on the oxygen carrying capacity of the blood as well as thrombopoietin[17]. Reduction in platelets counts obtained from the results of this study suggests that the administration of *Ocimum canum* may cause disruption in the oxygen carrying capacity of the blood. The study showed that Ocimum canum could disrupt hemoglobin production at high doses. Failure to produce hemoglobin occurs in many diseases, including iron deficiency anemia, thalassemia, and anemias associated with chronic infection or disease[18]. Also, the level of basophiles, neutrophiles, eosinophils and lymphocytes were not affected by the extract. This indicates that the plant may not interfere with the body immune.

Liver function was assessed by assaying the activities and levels of serum ALT, AST, ALP, bilirubin (total and direct), total cholesterol, total protein and albumin which are

originally present in the cytoplasm[19]. When there is hepatopathy, these enzymes and molecules leak into the blood stream which serves as an indicator for the liver damage[20]. The most commonly used indicators of liver (hepatocellular) damage are alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The ALT is felt to be a more specific indicator of liver inflammation as AST is also found in other organs such as the heart and skeletal muscle[21]. The normal levels of serum ALT, AST and total protein and albumin levels as observed in extract administered rats is indicative of the plant possessing little to no effect in inducing liver injury. Alkaline phosphatase estimation is the most frequently used test to detect obstruction in the biliary system. Bilirubin is the main bile pigment in humans which, when elevated causes the yellow discoloration of the skin called jaundice. Bilirubin is formed primarily from the breakdown of a substance called heme found in red blood cells. It is taken up from the blood, processed, and then secreted into the bile by the liver. There is normally a small amount of bilirubin in the blood in healthy individuals (<17µmol/L). Conditions which cause increased formation of bilirubin, such as destruction of red blood cells, or decrease in its removal from the blood stream as in liver dysfunction, may result in an slight increase in the level of bilirubin in the blood[19].findings from the study revealed that there was significant increase in the level of ALP, BILD and BILT. These indicate that the plant extract may cause mild biliary obstruction, destruction of red blood cells and/or decrease in RBC removal from the blood stream. Slight hepatic necrosis with other normal hepatic features in histopathological study shows agreement with other parameters

V. CONCLUSION

The results of the study shows that the Ocimum canum causes relative no damage to the liver even when taken for a prolonged period of time

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