



## Article Info

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## Toxicological Evaluation of Sub-Chronic Administration of Ethanol Leaf Extract of *Ocimum canum* on Lipid Parameters, Heart Histology and Feed Consumption of Wistar Rats

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*Ocimum canum* is a plant that is commonly used in many parts of Sub-Saharan Africa to treat ailments such as infection, discomfort, and diarrhea. The study was aimed to evaluate the sub-chronic toxicity of *Ocimum canum* on the heart, lipid profile and feed consumption in Wistar rats after oral administration of the leaf extract. Animals of either sexes were chosen. Group 1 received distilled water (10 ml/kg), while groups 2, 3, and 4 received 100, 200, and 400 mg/kg of *Ocimum canum*, respectively. Animals were kept in conventional cages and provided oral access to the extract, water, and food for 28 days before being weighed and slaughtered. Blood was drawn via heart puncture and immediately sent for hematological and chemopathological investigation. The plant's histological cardiotoxic potential was investigated using the haematoxylin and eosin (H&E) staining technique. There was a significant ( $P < 0.05$ ) drop in RBC, HGB, and MCV, but no change in neutrophils, basophils, eosinophils, or platelets. The extract did not cause a significant ( $P < 0.05$ ) change in cholesterol, LDL, or triglyceride levels, but it did induce a substantial increase in HDL levels. Histological examination of cardiac tissue confirms other parameters. The results indicated that the plant may not be cardiotoxic.

**Keywords:** *Ocimum canum*, Heart, Sub-chronic, toxicity.

## 1. Introduction

The human heart is located in the middle mediastinum, between thoracic vertebrae T5 and T8 [1]. A double-membraned sac that joins to the mediastinum surrounds the heart. The heart's back surface is near the spinal column, while its front surface is behind the sternum and rib cartilages [1]. Several significant blood arteries, including the vena cavae, aorta, and pulmonary trunk, attach to the upper region of the heart. The upper half of the heart is placed at the third costal cartilage level [2]. The apex of the heart is located to the left of the sternum (8 to 9 cm from the midsternal line) between the fourth and fifth ribs at their articulation with the costal cartilages. Atherosclerosis is a disorder that occurs when a material called plaque accumulates in the artery walls. This accumulation narrows the arteries, making blood flow more difficult (Moore). When a blood clot forms, it can obstruct blood flow. This can result in a heart attack or a stroke<sup>6</sup>. Appropriate heart failure management entails not only optimizing pharmacotherapy as indicated in

published national recommendations, but also following suitable nonpharmacologic measures [3]. One critical strategy for managing these patients is to avoid or limit the use of medications that can induce or worsen heart failure [4].

*Ocimum canum*, also known as "American basil" or "hoary basil," is an annual herb with white or lavender blooms. It has medicinal properties. Despite its misleading moniker, it is native to Africa, India, China, and Southeast Asia. *Ocimum canum* Sims. (Hairy Basil) is a traditional medicinal plant found throughout Sub-Saharan Africa that is especially popular in northern Nigeria [5]. With angle stems and open foliage, the plant branches out from its base. In contrast to the allied basil species *O. basilicum*, it is more commonly utilized as a medicinal plant [6]. This species' essential oils exhibit high fungicidal effect against certain plant pathogens (Moore). *O. canum* leaves have been used as an insecticide in Africa to defend against post-

harvest insect damage, particularly that caused by bruchid beetles [6]. External flavonoids may be connected with medicinal capabilities, as some specimens produce extremely high quantities of these chemicals, particularly nevadensin, which has antioxidant activity [7].

The plant's leaves have been used specifically for treating numerous ailments and decreasing blood glucose levels, as well as treating colds, fevers, parasitic infestations on the body, joint pain, and headaches [8]. The essential oil extracted from the leaves of *O. canum* has antibacterial and insecticidal properties [9,10]. The purpose of this study is to evaluate how *Ocimum canum* affects the heart, lipid profile and feed consumption of Wister rats after 28 days of oral treatment of the extract.

## 2. Materials and Methods

### 2.1 Plant collection

Fresh leaves of *Ocimum canum* were collected from its natural habitat in Karu village, Nasarawa State, Nigeria. The plant was authenticated by Kelvin Amadi at the Department of Botany, Bingham University-Nasarawa State, Nigeria. A voucher specimen with number BU1145 was prepared and then kept at the herbarium of the department for reference.

### 2.2 Plant extraction

The leaves were shade dried for two weeks. The dried plant material was then crushed to coarse powder using pestle and mortar. The powder plant (200 g) was extracted by percolation using 100 mL of 70% ethanol at room temperature for several hours. The liquid extract was filtered and then evaporated to dryness in a vacuum at 40°C using a rotary evaporator. The ethanol extract was kept at -4°C until it was required usage.

### 2.3 Qualitative phytochemical screening

The test was carried out according to the procedures outlined by Trease and Evans (1968) and Harbourne (2003). Ten percent (10%) preparation of the extract in distilled water was considered as the test samples. Distilled water was used as a negative control throughout the phytochemical tests.

### 2.4 Laboratory Animals

Male and female Wister rats were obtained from Bingham University's Animal House. They were fed with standard animal pellets purchased from

Grand Cereals Limited and given unlimited water. The Animal Ethics Committee of Bingham University College of Health Sciences issued authorization and approval for animal studies (BU/2021/1132). The rats ( $n = 6$ ) were randomized to different treatment groups. Animals studies were done according the established methods on scare and handling of the animals followed public health guidelines in Guide for Care and Use of Laboratory Animals (2011).

## 2.5 Acute toxicity study

### 2.5.1 LD<sub>50</sub> determination

Lorke [11] approach was used to perform an acute toxicity, LD<sub>50</sub> test. In two phases, a total of 13 rats weighing 100-120 g were employed.

The animals were separated into three groups of three mice each in the first stage, and the extract was supplied at three dose levels (10, 100, and 1000 mg/kg) body weight. The animals were kept under constant observation for 24 hours. Due to the lack of deaths in the first phase, extract doses of 2000, 3000, 4000, and 5000 mg/kg were used for four groups of one animal each. The animals were inspected again after 24 hours. The number of deaths (s) for each group was recorded, and the LD<sub>50</sub> was determined as follows;

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

Where:  $D_0$  = Highest dose that gave no mortality

$D_{100}$  = Lowest dose that produced mortality.

## 2.6 Sub-chronic toxicity study

The Organization for Economic Development (OECD) guideline no. 425 for analysis of Chemicals was employed for this study [12]. Twenty-four (24) rats of either sexes (weighing between 190 and 289g) were chosen at random. The extract was given to rats in groups 2, 3, and 4 at doses of 100, 200, and 400 mg/kg, respectively, while group 1 served as the control group and received normal saline (10 ml/kg). The weights of the rats were recorded at the start of the experiment and once a week thereafter. The day of sacrifice was designated as D29, whereas the initial day of dosing was designated as D0.

### 2.6.1 Haematological analysis

The rats were sacrificed using diethylether in accordance with protocol on the 29th day of the trial. Blood samples were slowly obtained through cardiac puncture. Blood was drawn into sample bottles containing EDTA for hematological analysis, including hemoglobin

concentration, white blood cell counts (WBC), differentials (neutrophils, eosinophils, basophils, lymphocytes, and monocytes), red blood cell counts (RBC), platelets, and hemoglobin (Hb) concentration. This was accomplished with the help of an automated haematology machine (Cell-Dyn, Abbott, USA).

### 2.6.2 Food and water consumption

The difference between the daily supply of feed and water and the amount still available after 24 hours was used to compute the daily feed and water consumption. The rats were sacrificed on the 29th day of the experiment, and their organs were removed for further gross histo-pathological investigation.

### 2.6.3 Chempathology analysis

The second portion of the blood was collected into a simple bottle, allowed to clot, then centrifuged for 10 minutes at 300rpm. The serum was utilized to calculate biochemical parameters such as cholesterol, triglycerides, high density lipoprotein (HDL), and low density lipoprotein (LDL).

### 2.6.4 Histology study

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

## 2.7 Statistical analysis

Data were expressed as the Mean  $\pm$  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.

## 3. Results and Discussion

### 3.1 Effects of 28 days oral administration of *Ocimum canum* on feed consumption (g) in rats.

Phytochemical test was carried out on the whole ethanol extract as well as the. The results are shown in Table 1. Phytochemical screening of all the crude extract of *Ocimum canum* extract showed the presence of various chemical constituents mostly Phenols, saponins, alkaloids, flavonoids, tanina, terpenoids, steroids, glycosides.

### 3.2 Acute toxicity

No deaths were recorded after 24 hours of administration of the various doses (10, 100 and

1000 mg/kg body weight) of the ethanol extract of *Ocimum canum*. In the second stage, four dose ranges were also used 2000, 3000, 4000 and 5000 mg/kg body weight and there was no death after 24 hours. Therefore, the LD<sub>50</sub> is estimated at LD<sub>50</sub>  $\geq$  5000 mg/kg b.wt. in mice. It is therefore considered as safe (Table 2).

### Phytochemical screening of *Ocimum canum*

When compared to the control, the ethanol leaf extract of *Ocimum canum* significantly ( $P < 0.05$ ) reduced feed intake at 100, 200, and 400 mg/kg dosing levels in the first week. When compared to the control, the extract's increase in the second, third, and fourth was not significant (Table 1).

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

At a dose of 200 mg/kg, administration of *Ocimum canum* extract resulted in a substantial ( $P < 0.05$ ) drop in WBC, RBC, HGB, HCT, PLT, and MCV and a significant ( $P < 0.05$ ) increase in MCHC in rats. However, extract had no effect on LYM, NEUT, EOSI, or BASO ( $P < 0.05$ ) (Table 2).

### 3.4 Effect of 28 days oral administration of *Ocimum canum* on lipid profile in wistar rats.

When 200 mg/kg dose level was compared to the control, significant ( $P < 0.05$ ) increases in total cholesterol and HDL levels were detected. When compared to the control, the extract had no significant effect on any of the other parameters evaluated (LDL, TRIG levels). (Table 3 and figure 1.2.3 and 4).

### 3.5 Effect of 28 days oral administration of *Ocimum canum* on histology of Heart in rats.

At all doses, histopathological analysis of the heart revealed mild necrosis of cardiac muscles, with normal characteristics at the control (10 ml/kg). The research uncovered normal elongated and rod-shaped cells, striated muscles, and blood arteries (Table 4 and figure 1.2.3 and 4).

Table 1: Results of Phytochemical Analysis of ethanol fruit extract of *Canscora decussate*

	Phytochemical	Crude extract
1	Tannins	+
2	Alkaloids	+
4	Flavonoids	+
5	Glycosides	+
7	Saponins	+
8	reducing sugar	-
9	Terpenoid	+

10	cardiac glycoside	–
11	Acidic compounds	–
12	Carbohydrate	+
13	Steroid	+
14	Resins	–

**Table 2: Effects of 28 days oral administration of *Ocimum canum* on feed consumption (g) in rats.**

Treatment (mg/kg)	Week 1	Week 2	Week 3	Week 4
DW (10 ml/kg)	398.9±1.3	303.9±40.0	204.7±8.4	119.10±12.2
100	286.4±4.5*	293.7±58.6	363.4±8.4	224.40±55.5
200	260.7±14.7*	262.5±63.5	237.5±37.5	229.50±4.5
200	281.7±4.5*	355.6±18.8	370.6±59.3	260.5±11.4

\*Significantly different from the distilled water (DW) Control at p<0.05. purifier, DW = distilled water

**Table 3: Effect of 28 days oral administration of *Ocimum canum* on hematological parameters in wistar rats.**

Hematological parameters	DW(10 ml/kg)	Treatment (mg/kg)		
		100	200	400
WBC (×10 <sup>9</sup> /L)	8.167±0.772	6.740±1.419	3.700±0.657*	7.220±1.085
RBC (×10 <sup>12</sup> /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 (×10 <sup>9</sup> /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 (×10 <sup>9</sup> /L)	10.83±3.67	10.83±3.68	15.40±5.60	11.20±3.02
EOSI (×10 <sup>9</sup> /L)	1.50±0.34	2.40±0.75	1.80±0.47	1.20±0.20
BASO (×10 <sup>9</sup> /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 4: Effect of 28 days oral administration of *Ocimum canum* on lipid profile in wistar rats.**

Lipid profiles	DW(10 ml/kg)	Treatment (mg/kg)		
		100	200	400
CHOL (mmol/L)	45.0±8.6	52.80±6.6	43.40±4.5	55.75±9.22
HDL (mmol/L)	38.2±3.8	55.00±3.1*	38.20±1.8	46.75±3.8
LDL (mmol/L)	7.4±1.81	7.40±1.8	8.400±4.5	4.50±2.2
TRIG (mmol/L)	56.4±2.7	52.40±8.4	67.60±10.8	61.00±3.9

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.

(CHOL = total cholesterol, HDL = high density lipoprotein, LDL = low density lipoprotein, TRIG = triglycerides.

The grid lines in the graphs should be removed.

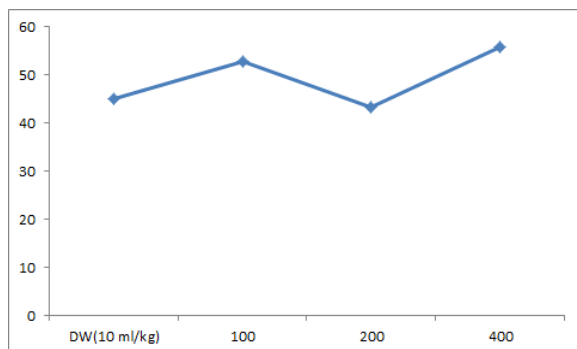


Figure 1: showing effect of *Ocimum canum* on cholesterol level in rats

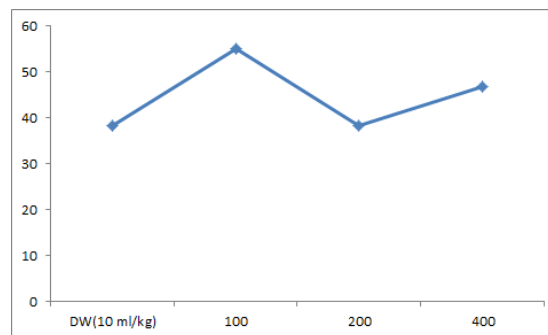


Figure 2: showing effect of *Ocimum canum* in the level of HDL in rat

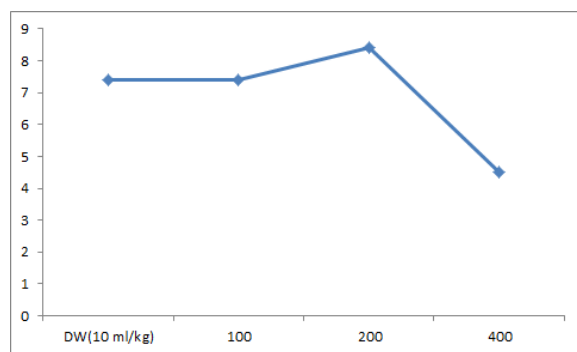


Figure 3: showing effect of *Ocimum canum* on LDL in rat

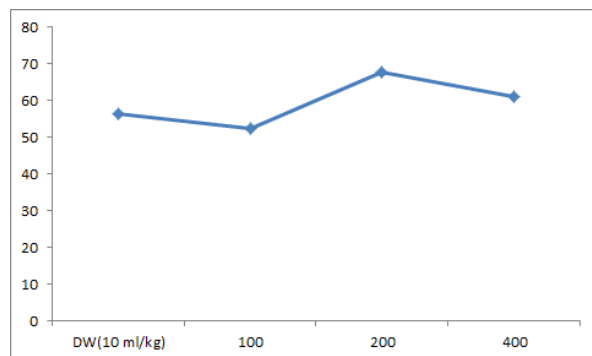
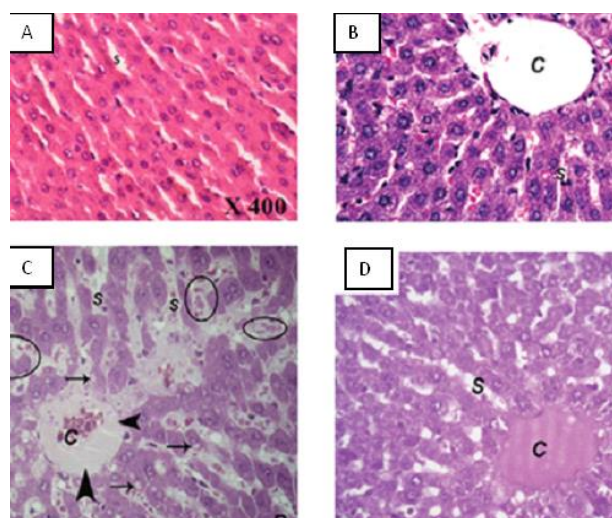


Figure 4: showing effect of *Ocimum canum* on triglyceride level in rat



**Figure 5:** Figure of the heart (Hematoxylin and eosin. H and E  $\times 100$ ). (A) Control group, Shows normal myocardium (M). (B) 100 mg/kg, slight necrosis of myocardium (MN). (C) 200 mg/kg, moderate necrosis of myocardium (M). (D) 400 mg/kg, slight necrosis of myocardium (MN).

The effect of ethanol leaf extract of *Ocimum canum* on the heart was studied. According to the findings of this study, treatment of ethanol leaf extract of *Ocimum canum* resulted in a significant decrease in platelet counts, red blood cell counts, and haemoglobin levels in rats. A decrease in platelet count has been linked to a

negative impact on the oxygen carrying capacity of the blood as well as thrombopoietin [12]. The reduction in platelet counts seen in this study suggests that treatment of *Ocimum canum* may affect the blood's oxygen carrying ability. At large doses, *Ocimum canum* was found to affect hemoglobin synthesis. Many disorders cause hemoglobin failure, including iron deficiency anemia, thalassemia, and anemias caused by persistent infection or disease [13]. The extract had no effect on the levels of basophils, neutrophils, eosinophils, or lymphocytes, indicating that the plant may not interfere with immune system activities.

The effect of *Ocimum canum* on the lipid profile of rats was studied in this study. LDL, triglyceride, and cholesterol levels did not alter significantly, while HDL increased significantly. LDL cholesterol is commonly regarded as harmful cholesterol because it promotes fatty buildup in arteries (atherosclerosis). This disorder causes artery clogging and raises the risk of cardiovascular diseases such as heart attack, stroke, and peripheral artery disease [14]. HDL serves as a scavenger, taking LDL (bad) cholesterol from the arteries and transporting it to the liver, where it is broken down and excreted. However, HDL cholesterol does not entirely eliminate LDL cholesterol from blood arteries. HDL transports only one-third to one-fourth of blood cholesterol [15]. Triglyceride is a type of fat that retains excess energy. A high triglyceride level in conjunction with high LDL cholesterol or low HDL (good) cholesterol has been related to fatty buildups within the artery walls, which increases the risk of heart attack and stroke. A high amount of LDL cholesterol or a low level of HDL cholesterol is a critical risk factor for CVD. The link between low HDL cholesterol levels and an elevated risk of CVD has been clearly established in epidemiological and clinical studies [16]. The preventive actions of HDL cholesterol against CVD have been proposed to occur in a variety of ways [12,14]. HDL counteracts LDL oxidation, which contributes to its anti-atherogenic activity. According to recent research, HDL enhances the reverse cholesterol transport route by stimulating the elimination of excess cellular cholesterol, hence preventing the formation of an oxidative modified LDL [17]. Furthermore, HDL not only reduces LDL oxidation by transition metal ions, but it also prevents 12-lipoxygenase-mediated lipid hydroperoxide formation [18]. There was no change in the levels of LDL, cholesterol, and triglyceride after 28 days of oral administration of an ethanol extract of *Ocimum canum*, indicating that the plant has less tendency to induce atherosclerotic plaque, while a high level of HDL indicates that it may be useful in managing

cardiovascular diseases. Histological examination confirms biochemical indicators showing the plant does not have cardiotoxic properties.

Phytochemical analysis of *Ocimum canum* revealed Phenols, saponins, alkaloids, flavonoids, tanina, terpenoids, steroids and glycosides. The antioxidant capacity of plant molecules such as saponins, tannins and terpenoids can reduce tissue necrosis in studied [19,20]. Presence of these secondary metabolites may have been responsible for cellular protection of rat against other possible deleterious molecules in the plants. It may also be responsible improved level of HDL when compared to the group that did not receive extract. Additionally, *Ocimum canum* extract can improve wound healing through three main mechanisms: contraction, tissue matrix deposition, and epithelialization. Open wound healing by contraction; The interaction between cells and matrix causes tissue to migrate to the wound site.

#### 4. Conclusion

According to the findings of this study, *Ocimum canum* has no harmful effects on cholesterol levels or heart tissue. Result also suggests that extract of *Ocimum canum* may be useful in preventing and managing cardiovascular conditions due to its ability to improve the level of HDL. Finding from this study indicates that caution should be exercise when consuming this plant for a prolong period of time and at a particular dose as it may cause a reduction in the value of certain haematological parameters.

#### Conflict of interest

The authors declare no conflict of interest.

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