International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 8; Issue 05 (D); May 2019; Page No.18799-18803
DOI: http://dx.doi.org/10.24327/ijcar.2019.18803.3601

Research Article

TOXICITY STUDY OF ETHANOL LEAF EXTRACT OF Ocimum canum ON HEART AND LIPID PROFILE OF WISTER RATS

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A R T I C L E I N F O

Article History:
Received 15th February, 2019
Received in revised form 7th March, 2019
Accepted 13th April, 2019
Published online 28th May, 2019

Key words:
Ocimum canum, rats, heart

A B S T R A C T

Background/Aim: The human heart is an organ that pumps blood throughout the body via the circulatory system, supplying oxygen and nutrients to the tissues and removing carbon dioxide and other wastes. Several substances consumed has direct and indirect effect on the ability of the heart to carry out it constant responsibility. 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 effect of Ocimum canum on the liver of Wister rats after 30 days of oral administration of the extract.
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 cardiotoxic 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 cause significant ($P<0.05$) change in the level of cholesterol, LDL and triglyceride, although there was significant increase in the level of HDL. Histological study of the heart tissue also agrees with other parameter.
Conclusion: result revealed that the plant may have no cardiotoxic property. Further research may need to be done to establish it use in managing cardiovascular disease.

INTRODUCTION

The human heart is situated in the middle mediastinum, at the level of thoracic vertebrae T5-T8. A double-membraned sac called the pericardium surrounds the heart and attaches to the mediastinum[1]. The back surface of the heart lies near the vertebral column, and the front surface sits behind the sternum and rib cartilages[2]. The upper part of the heart is the attachment point for several large blood vessels—the venae cavae, aorta and pulmonary trunk. The upper part of the heart is located at the level of the third costal cartilage[3]. The lower tip of the heart, the apex, lies to the left of the sternum (8 to 9 cm from the midsternal line) between the junction of the fourth and fifth ribs near their articulation with the costal cartilages[4].

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Atherosclerosis is a condition that develops when a substance called plaque builds up in the walls of the arteries. This buildup narrows the arteries, making it harder for blood to flow through[5]. If a blood clot forms, it can block the blood flow. This can cause a heart attack or stroke[6]. Appropriate care of the patient with heart failure includes not only optimizing pharmacotherapy, as described in published national guidelines, but adhering to proper nonpharmacologic measures[7]. One vital strategy that must be followed in managing these patients is eliminating or minimizing the use of agents that can cause or exacerbate heart failure[8]. Drug-induced heart failure is relatively common, but it is difficult to precisely define its incidence because patients often have other risk factors that can potentially contribute to new-onset or exacerbated heart failure[9].

Ocimum canum, known as American basil or "hoary basil"[10], is an annual herb with white or lavender flowers. It is used for medicinal purposes[11]. Despite the misleading name, it is native to Africa, the Indian Subcontinent, China, Southeast Asia[12].

Ocimum canum Sims. (Hairy Basil) is a traditional medicinal plant distributes throughout sub-Saharan Africa and very well known in northern Nigeria[13]. 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[14]. The essential oils found in this species have strong fungicidal activity against certain plant pathogens[15]. 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[16]. 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[17].

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[18]. Essential oil from the leaves of *O. canum* possesses antibacterial and insecticidal properties[19]. In this study effect of *Ocimum canum* on heart was investigated 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.

Chempathology Analysis

Second portion of the blood was collected into plain bottle, allowed to clot and centrifuged at 300rpm for 10 minutes. The serum collected was used to estimate biochemical parameters such as cholesterol, triglyceride, high density lipopolysaccharide (HDL) and low density lipopolysaccharide (LDL)

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.

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

Effects of 28 days oral Administration of Ocimum canum on feed Consumption (g) in rats

The ethanol leaf extract of *Ocimum canum* significantly ($p<0.05$) decrease feed consumption at 100, 200 and 400 mg/kg dose level in the first week when compared with the control. The increase in the 2nd, 3rd and 4th by the extract was not significant when compared with the control (Table: 1).

Effect of 28 days oral Administration of Ocimum canum on Hematological Parameters in rats

Administration of Ocimum canum extract produced significant ($p<0.05$) decrease in WBC, RBC, HGB, HCT, PLT, MCV and significant ($p<0.05$) increase in MCHC in the rats at the dose level of 200 mg/kg compared to the control. LYM, NEUT, EOSI and BASO were however not significantly ($p<0.05$) affected by extract (Table 2).

Effect of 28 days oral Administration of Safi® herbal blood Purifier (SHBP) on Lipid Profile in Wistar rats

Significant ($p<0.05$) increases were observed in total cholesterol and HDL levels at 100 mg/kg dose level of SHBP when compared to the control. The extract did not produce significant changes in all other parameters (LDL, TRIG levels) studied when compared to the control (Table 4.6).

Effect of 28 days oral Administration of Ocimum canum on Histology of Heart in rats

Histopathological examination of heart showed slight necrosis of cardiac muscles at all doses and normal features at the

control (10 ml/kg). Study reveals normal elongated and rod shaped cells, striated muscles and blood vessels.

Table 3 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)	398.90±1.30	303.90±40.00	204.75±8.45	119.10±12.20
100	286.45±4.55*	293.75±58.65	363.45±8.45	224.40±55.50
200	260.70±14.70*	262.50±63.50	237.50±37.50	229.50±4.50
400	281.70±4.50*	355.65±18.85	370.60±59.30	260.05±11.45

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

Effect of 28 days oral Administration of Ocimum canum on Hematological Parameters in wistar rats

	Treatment (mg/kg)			
Hematological parameters	DW(10 ml/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. 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 4.6 Effect of 28 days oral administration of *Ocimum canum* on lipid profile in wistar rats

	Treatment (mg/kg)			
Lipid profiles	DW(10 ml/kg)	100	200	400
CHOL (mmol/L)	45.00±8.46	52.80±6.26	43.40±4.45	55.75±9.22
HDL (mmol/L)	38.20±3.18	55.00±3.11*	38.20±1.88	46.75±3.79
LDL (mmol/L)	7.40±1.81	7.40±1.81	8.400±4.25	4.50±2.18
TRIG (mmol/L	56.40±2.75	52.40±8.47	67.60±10.79	61.00±3.89

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.

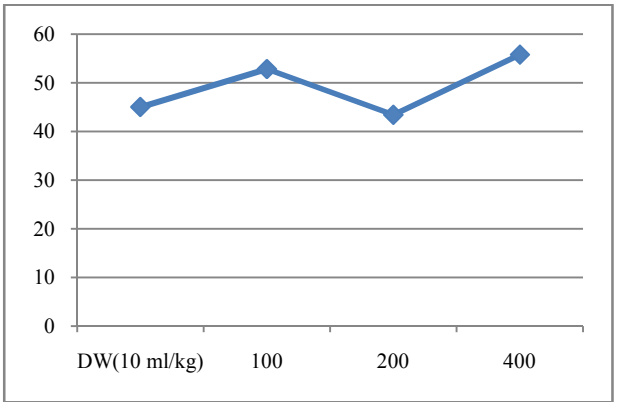

Figure 1 effect of *Ocimum canum* on cholesterol level in rat

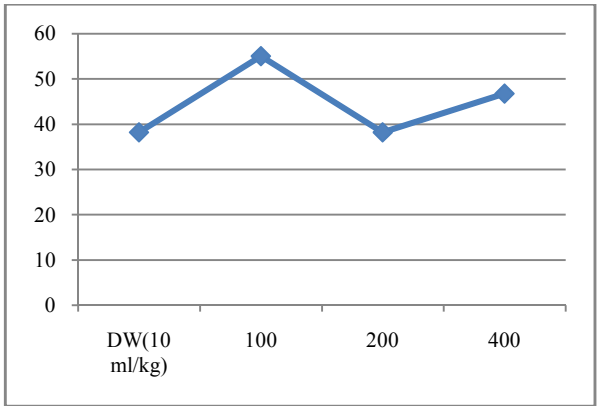

Figure 2 effect of Ocimum canum in the level of HDL in rat

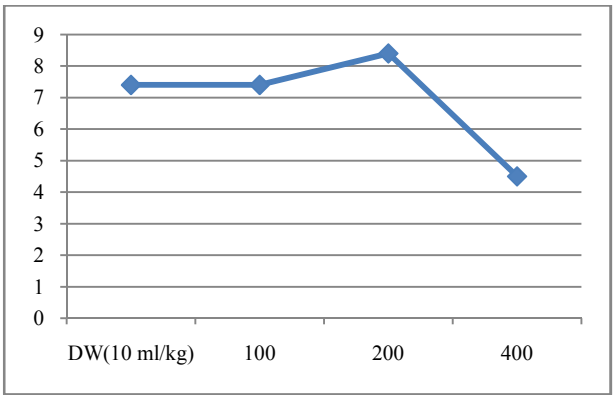

Figure 3 effect of Ocimum canum on LDL in rat

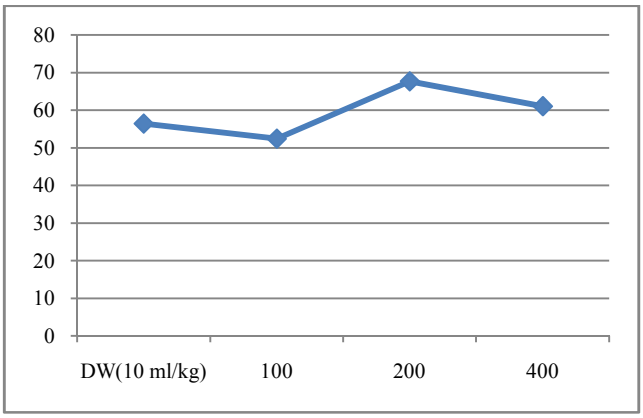

Figure 4 effect of Ocimum canum on triglyceride level in rat

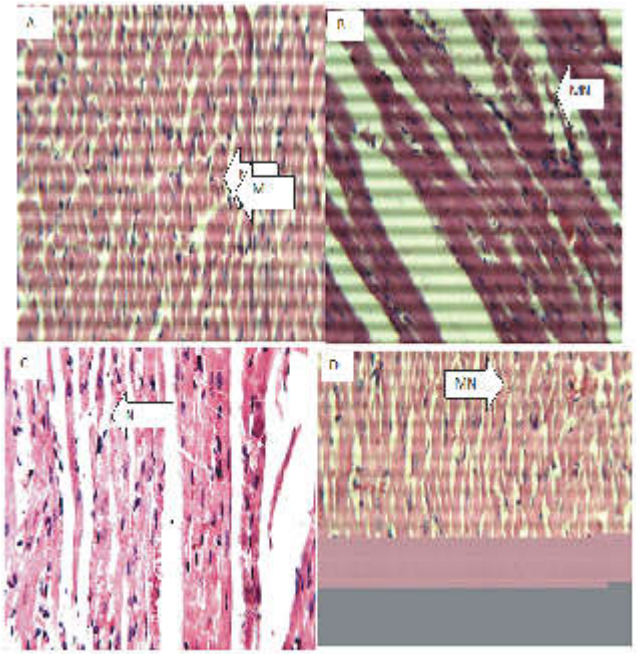

Figure 4 Figure of the heart (Hematoxylin and eosin. H and E ×100). (a) Control group, Shows normal myocardium (M). (b) 100 mg/kg, slight necrosis of myocardium (MN). (c) 200 mg/kg,moderate necrosis of myocardium (M). (d) 400 mg/kg, slight necrosis of myocardium (MN).

DISCUSSION

Toxicology is an important and integral part of science because every substance humans are exposed to, have a potential unintended consequence[20]. Medicinal plants are regularly consumed by locals, while there is recent improvement in formulating these herbs into standard pharmaceutical dose. There is therefore the need to investigate possible pharmacologic effects of plants on the body[21].

In this work, the effect of ethanol leaf extract of *Ocimum canum* on the heart was evaluated. From the results of this study, administration of ethanol leaf extract of *Ocimum canum* led to a significant reduction in the level of platelet counts, red blood cell and haemoglobin in rats. Reduction in platelets count has been reported to be indicative of adverse effect on the oxygen carrying capacity of the blood as well as thrombopoietin[22,23]. 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[24]. It was also observed that the level of basophiles, neutrophiles, eosinophils and lymphocytes were not affected by the extract, indicating that the plant may not interfere with the activity of the body immune.

In this work, the effect of Ocimum canum on the lipid profile of rat. There was no significant change in the level of LDL, triglyceride and cholesterol level while there was significant increase in HDL. LDL cholesterol is usually considered bad cholesterol, because it contributes to fatty buildups in arteries (atherosclerosis)[25]. This condition clogs the arteries and increases the risk for cardiovascular diseases, such as heart attack, stroke and peripheral artery disease[26]. HDL acts as a scavenger, removing LDL (bad) cholesterol away from the arteries and back to the liver, where it is broken down and passed from the body. But HDL cholesterol does not completely remove LDL cholesterol from the blood vessels. Only one-third to one-fourth of blood cholesterol is carried by HDL[27]. Triglyceride stores excess energy. A high triglyceride level combined with high LDL cholesterol or low HDL (good) cholesterol is linked with fatty buildups within the artery walls, which increases the risk of heart attack and stroke. The crucial risk factor for CVD includes a high level of LDL or low level of HDL-cholesterol. The association between a low level of HDL-cholesterol and an increased risk of CVD has been well established through epidemiological and clinical studies[28]. The protective roles of HDL cholesterol from CVD have been suggested to occur in various ways[29]. HDL exerts part of its anti-atherogenic action by counteracting LDL oxidation. Recent studies reveals that HDL promotes the reverse cholesterol transport pathway, by inducing removal of excess accumulated cellular cholesterol resulting in prevention of the generation of an oxidative modified LDL[30]. Furthermore, HDL inhibits the oxidation of LDL by transition metal ions, but also prevents 12-lipooxygenase-mediated formation of lipid hydroperoxides[31,32]. After oral administration of ethanol extract of *Ocimum canum* for a 28 days period, there was no change in the level of LDL, cholesterol and triglyceride suggesting that the plant has less tendency to induce atherosclerotic plague, while high level of HDL indicates that it may be useful in managing cardiovascular diseases. Histological evaluation agrees with biochemical parameters that the plant may not posses cardiotoxic property.

CONCLUSION

Result from this study suggests that Ocimum canum does not negatively affect the cholesterol level and heart tissue.

Acknowledgement

The authors want to appreciate everyone who has contributed to the success of this work

Reference

1. Moore, Keith L.; Dalley, Arthur F.; Agur, Anne M. R. (2009). "1". Clinically Oriented Anatomy. Wolters Kluwel Health/Lippincott Williams & Wilkins. pp. 127–173. ISBN 978-1-60547-652-0.
2. Starr, Cecie; Evers, Christine; Starr, Lisa (2016). Biology: Today and Tomorrow With Physiology. Cengage Learning. p. 422.
3. Reed, C. Roebuck; Brainerd, Lee Wherry; Lee, Rodney; Inc, the staff of Kaplan (2008). CSET : California Subject Examinations for Teachers (3rd ed.). New York: Kaplan Pub. p. 154.
4. *Dobson, Geoffrey P (August 2003). "On Being the Right Size: Heart Design, Mitochondrial Efficiency and Lifespan Potential". Clinical and Experimental Pharmacology and Physiology. 30 (8): 590–597*
5. Rombauer, Irma S.; Becker, Marion Rombauer; Becker, Ethan (1975). The Joy of Cooking. The Bobbs-Merrill Company. p. 508.
6. Schwabe, Calvin W. (1979) Unmentionable Cuisine, University of Virginia Press, ISBN 0-8139-1162-1, p. 96
7. Rombauer, Irma S. and Rombauer Becker, Marion (1975) The Joy of Cooking, p. 508

8. Hokche, O., Berry, P.E. & Huber, O. (eds.) (2008). Nuevo Catálogo de la Flora Vascular de Venezuela: 1-859. Fundación Instituto Botánico de Venezuela.
9. Figueiredo, E. & Smith, G.F. (2008). Plants of Angola. Strelitzia 22: 1-279. National Botanical Institute, Pretoria. P.67.
10. Shukla RP, Singh RK, Dwivedi RS. Toxicity of essential oils against Rhizoctonia solani Kuhn fungus causing sheath blight (ShB) in rice. International Rice Research Newsletter 1990; 15: 27
11. Dubey RC. Fungicidal effect of essential oils of three higher plants on sclerotia of Macrophomia phaseolina. Indian Phytopathology 1991; 44: 241–244
12. Weaver DK, Dunkel FV, Ntezurubanza L, Jackson LL, Stock DT. The effciency of linalool, a major component of freshly milled Ocimum canum Sims (Lamiaceae), for protection against post-harvest damage by certain stored product Coleoptera. Journal of Stored Products Research 1991; 27: 213–220 .
13. Grayer Renee J, Geoffrey C, Kite NC, Veitch MR, Eckert PD, Marin PS, Alan JP. Leaf flavonoid glycosides as chemosystematic characters in Ocimum. Biochemical Systematics and Ecology 2002; 30: 327–342.
14. Ngassoum MB, Ousmaila H, Ngamo LT, Maponmetsem PM, Jirovetz L, Buchbauer G. Aroma compounds of essential oils of two varieties of the spice plant Ocimum canum Sims. from northern. Cameroon J Food Comp Anal 2004; 17: 197–204
15. Bassole IHN, Guelbeogo WM, Nebie R, Costantini C, Sagnon NF, Kabore ZI, Traore SA. Ovicidal and larvicidal activity against Aedes aegypti and Anopheles gambiae complex mosquitoes of essential oils extracted from three spontaneous plants of Burkina Faso. Parasitology 2003; 45: 23–26.
16. Nkosi CZ, Opoku AR, Terblanche SE. Effect of pumpkin seed (Cucurbita pepo) protein isolate on the activity levels of certain plasma enzymes in CCl4-induced liver injury in low protein fed rats.Phytother Res. 2005;19:341–345.
17. Muriel P, Garcipiana T. Silymarin protects against paracetamol-induced lipid peroxidation and liver damage. J ApplToxicol. 1992;12:439–442
18. Sunmonu TO, Oloyede OB. Performance and haematological indices in rats exposed to monocrotophos contamination. Hum Exp Toxicol. 2010;29(10):845–850.
19. McLellan SA, McLellan DBL, Walsh TS. Anaemia and red blood cell transfusion in the critically ill patient. Blood Rev. 2003;17:195–208.
20. Assmann G, Gotto AM., Jr HDL cholesterol and protective factors in atherosclerosis. Circulation. 2004;109:III8–III14. [PubMed] [Google Scholar]
21. Fiske CH, SubbaRow Y. The colorimetric determination of phosphorus. J Biol Chem. 1925;66:375–83. [Google Scholar]
22. Friedewald WT, Levy RI, Fredrickson DS, *et al*. Estimation of the concentration of LDL cholesterol in plasma, without use of the preparative ultra- centrifuge. Clin Chem. 1972;18:499–502. [PubMed] [Google Scholar]
23. Fungwe TV, Cagen LM, Cook GA, *et al*. Dietary cholesterol stimulated hepatic biosynthesis of triglyceride and reduces oxidation of fatty acids in the rat. J Lipid Res. 1993;34:933–41. [PubMed] [Google Scholar]
24. Oduola T, Adeniyi FAA, Adenaike FA, Ogunyemi EO, Bello IS, Idowu TO. Haematological response to intake of unripe Carica papaya aqueous extract. IJMR. 2009;(1):20–
25. Iatropoulos MJ. Endocrine considerations in toxicologic pathology. Exp Toxicol Pathol. 1994;45:391–410.
26. Avwioro OG. Histochemistry and Tissue Pathology: Principles and techniques. 1st ed. Claverianum Centre; 2002.
27. Hu SH, Liang ZC, Chia YC, *et al*. Antihyperlipidemic and antioxidant effects of extracts from *Pleurotus citrinopileatus*. J Agric Food Chem. 2006;22:2103–10.
28. Igile GO, Oleszek W, Jurzysta M, *et al*. Flavonoids from *Vernonia amygdalina* and their antioxidant activities. J Agric Food Chem. 1994;42:2445–8. [Google Scholar]
29. Oyepata Simeon Joseph, Jude e Okokon, Opeyemi tosin Joseph (2018). Hepatoprotective activity of extract of Homalium Letestui stem against carbon tetrachloride-induced liver injury. Advanced Herbal Medicine. 3(4): 1-11.
30. Iwalokun BA, Efedede BU, Alabi-Sofunde JA, *et al*. Hepatoprotective and antioxidant activities of *Vernonia amygdalina* on acetaminophen-induced hepatic damage in mice. J Med Food. 2006;9:524–30. [PubMed] [Google Scholar]
31. Izevbigie EB. Discovery of water-soluble anticancer agents (edotides) from a vegetable found in Benin City, Nigeria. Exp Biol Med. 2003;228:293–8. [PubMed] [Google Scholar]

How to cite this article:

Joseph O.S *et al* (2019) 'Toxicity Study of Ethanol Leaf Extract of Ocimum Canum on Heart and Lipd Profile of Wister Rats', *International Journal of Current Advanced Research,* 08(05), pp. 18799-18803.
DOI: http://dx.doi.org/10.24327/ijcar.2019.18803.3601

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