

HISTOLOGICAL EFFECT OF GRADED DOSES OF *VERNONIA AMYGDALINA* ON THE OVARY OF FEMALE WISTAR RATS

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ABSTRACT

Introduction: Medicinal plants or herbal medicine is the oldest form of health care available to mankind. This study was aimed at evaluating the effect of ethanolic leaf extract of *Vernonia amygdalina* on the ovary of adult female wistar rat. **Materials and methods:** A total of 20 female albino rats weighing 150g – 200g were used in this study and were randomly divided into 4 groups of 5 animals each. Group A served as the control group, group B received 200 mg/kg of ethanolic leaf extract of *Vernonia amygdalina*, group C received 400 mg/kg of ethanolic leaf extract of *Vernonia amygdalina* and group D received 800 mg/kg of ethanolic leaf extract of *Vernonia amygdalina*. The extracts were given orally and the treatment lasted for 28 days. At the end of the study the animals were sacrificed and their ovaries harvested for histological examination. Data analysis was done using One way ANOVA and results were considered significant at $P < 0.05$. **Results:** Findings from this study showed a significant decrease in the body weight of all treated groups when compared to the control. There was a significant increase in the relative weight of the ovary in the treated groups when compared to the control. Histopathological findings showed mild follicular development and moderate degeneration of corpus luteum which occurred at a different doses. **Conclusion:** The extract of *Vernonia amygdalina* leaf could cause damage to the ovaries especially at high doses.

KEYWORDS: *Vernonia amygdalina*, Ovary, Wistar Rats.

INTRODUCTION

Vernonia amygdalina is a genus with numerous species and are members of the family Asteraceae. The leaves are elliptical and up to 20 cm (7.9 in) long.^[1] These herbs or shrubs are predominantly grown in tropical Africa especially Nigeria, Zimbabwe, and South Africa.^[2,3] It is popularly called bitter leaf due to its bitter taste and mostly used as vegetables in making soups. In Nigeria, it is known by several local names such as “Ewuro” in Yoruba, “Onugbu” in Igbo, “Oriwo” in Bini, “Ityuna” in Tiv, “Chusar doki or fatefate” in Hausa and “Etidot” in Ibibio.^[4] The roots and leaves of *V. amygdalina* are used in ethnomedicine to treat hiccups, diarrhoea, fevers, kidney problems, hepatitis and discomfort of the stomach.^[5,6] In addition, the plants extract have been reported to be used as tonic, in the control of tick and treatment of hypertension in some herbal home in Nigeria.

V. amygdalina have been reported to possess several pharmacological properties like antimicrobial,^[7] antimalarial,^[8] antioxidant,^[9] anti-diabetic,^[10] anti-allergic,^[11] antibacterial,^[12] etc. These pharmacological

activities of *V. amygdalina* have been attributable to the presence of complex bioactive compounds such as flavonoids, saponins, alkaloids, tannins, phenolics, terpenes, steroidal glycosides, triterpenoids.^[13,14] This investigation is geared towards finding out if the consumption of graded doses of *V. amygdalina* has an effect on the ovary which is one of the major reproductive organs in females.

MATERIALS AND METHODS

Breeding of animals

A total of twenty [20] female albino rats weighing between 150-200 g were purchased from the animal house of Anatomy Department and were housed in standard cages under normal temperature with cross ventilation. The animals were acclimatized for a period of two weeks before commencement of treatment and were fed with rat chow and water *ad libitum*. The study design was approved by the ethical committee of the College for animal care and use in compliance with the National regulation for animal research.

Extract preparation

Fresh leaves of *V. amygdalina* were procured from farmlands in Anambra State. They were identified at the herbarium units of the Department of Botany, Nnamdi Azikiwe University Awka, Anambra State. The fresh leaves were washed in running tap water to remove dirt and dried under ambient temperature. The dried leaves were ground to a coarse powdery form using laboratory blender. 400 g of the powder was macerated in 1000 ml (1L) of ethanol and allowed for 48 hours inside mechanical shaker. After 48 hours, the mixture was sieved using a porcelain cloth and further filtered using filter paper into a clean beaker. The filtrate was later concentrated using rotary evaporator into a jelly-like/paste-like form and stored in refrigerator for future use.

Experimental Design

The twenty female rats were weighed and randomly allocated into four (4) groups of five (5) animals. The groups were designated as a group A, B, C, and D. Group A served as the control group and was administered 2 ml/kg body weight of distilled water. The experimental groups B, C, and D were administered with 200 mg/kg, 400 mg/kg and 800 mg/kg body weight of the extract of *V. amygdalina* respectively. Administration of the extract was orally between the hours of 1-2pm daily and lasted for twenty eight (28) days. The animals

were then anaesthetized by chloroform inhalation and dissected 24 hours after the last dose. Their ovaries were harvested and fixed in 10% formal saline for histological examination.

Histopathological Examination

The tissues of the ovaries were processed by passing them through histochemical methods of fixation, dehydration, clearing, impregnation, embedding, sectioning, mounting and staining. Fixation was carried out in 10% formal saline and dehydration was carried out in ascending grades of alcohol (50%, 70%, 80%, 95% and absolute alcohol) and then cleared in xylene after which embedding in paraffin wax was carried out. Sections of about 3-5µm was obtained using a rotary microtome. The sections were later deparaffinised, hydrated and stained using haematoxylin and eosin (H&E) dye. They were later mounted using neutral dibutylphthalate xylene (DPX) medium for microscopic examination at x150 magnification.

Statistical Analysis

Data were analysed using Analysis of Variance (ANOVA) test followed by multiple comparisons using Least Significant Difference (LSD). The levels of significance were considered at $P < 0.05$ and data was expressed as Mean \pm SEM.

RESULTS

Table 1: Effect of ethanolic extract of *V. amygdalina* leaf on body weight.

		MEAN	\pm SEM	P-VALUE	T-VALUE
Group A	Initial	180.00	\pm 0.00		
	Final	227.50	\pm 10.30	0.019*	4.608
Group B	Initial	225.00	\pm 10.40		
	Final	180.00	\pm 4.08	0.032*	3.781
Group C	Initial	222.50	\pm 11.08		
	Final	190.00	\pm 4.08	0.023*	4.333
Group D	Initial	217.50	\pm 11.81		
	Final	187.50	\pm 4.78	0.046*	3.286

Table 1 above shows a significant increase in the body weight of animals in group A when compared to their initial weight. Groups B, C and D show a significant

decrease in body weights when compared to their initial weights.

Table 2: Effect of ethanolic extract of *V. amygdalina* leaf on Relative Organ weight.

		MEAN	\pm SEM	P-VALUE	F-VALUE
Relative Ovary weight (g)	GroupA (Control)	0.26	\pm 0.01		
	Group B	0.34	\pm 0.02	0.015*	21.300
	Group C	0.32	\pm 0.03	0.047*	
	Group D	0.47	\pm 0.01	0.000**	

* $P < 0.005$, ** $P < 0.001$ when compared to the control

Table 2 shows a significant increase in the relative organ weights of the treated groups when compared to the control with group D having a more significant increase.

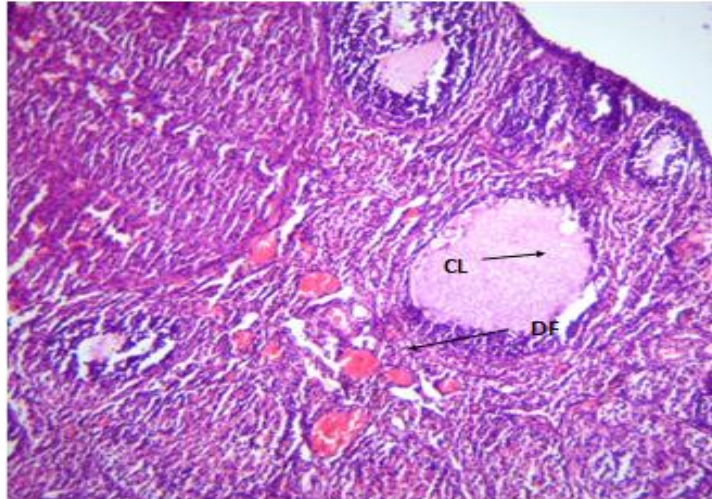
Histopathological findings

Figure 1: Photomicrograph section of the ovary in group A treated with 2 ml/kg body weight of distilled water showing normal ovarian tissue with large corpus luteum (CL) and well developing follicles (DF).

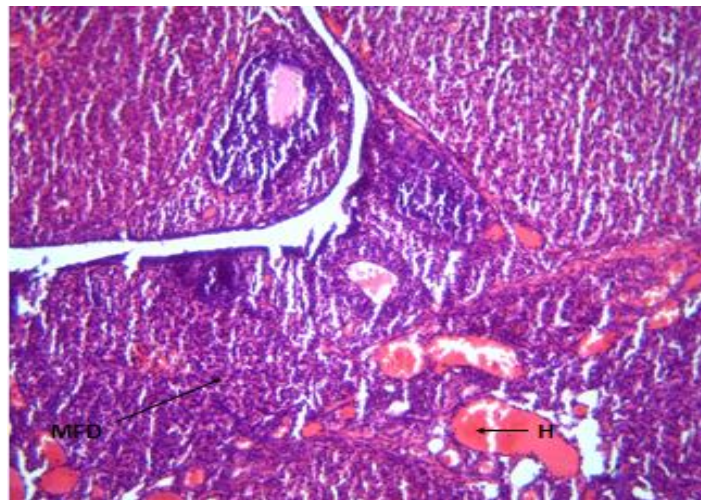


Figure 2: Photomicrograph section of the ovary in group B treated with 200 mg/kg body weight of *V. amygdalina* showing mild increase in the follicular development (MFD) and moderate areas of haemorrhage (H).

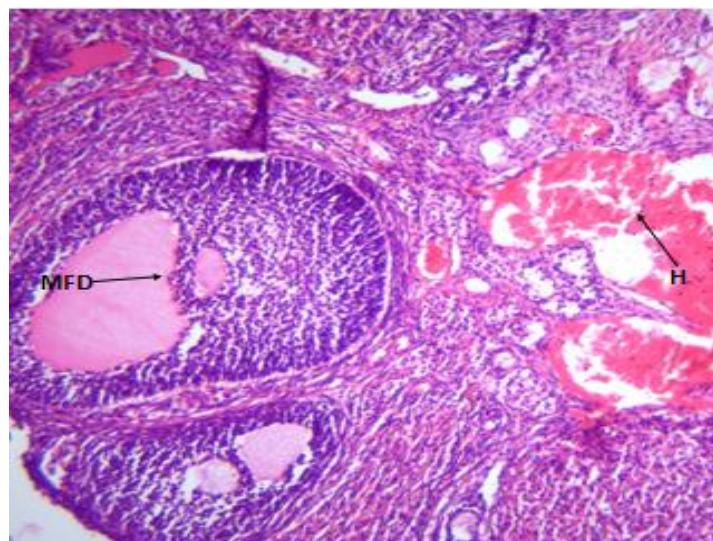


Figure 3: Photomicrograph section of the ovary in group C treated with 400 mg/kg body weight of *V. amygdalina* showing moderate increase in the follicular development (MFD) and moderate area of haemorrhage (H).

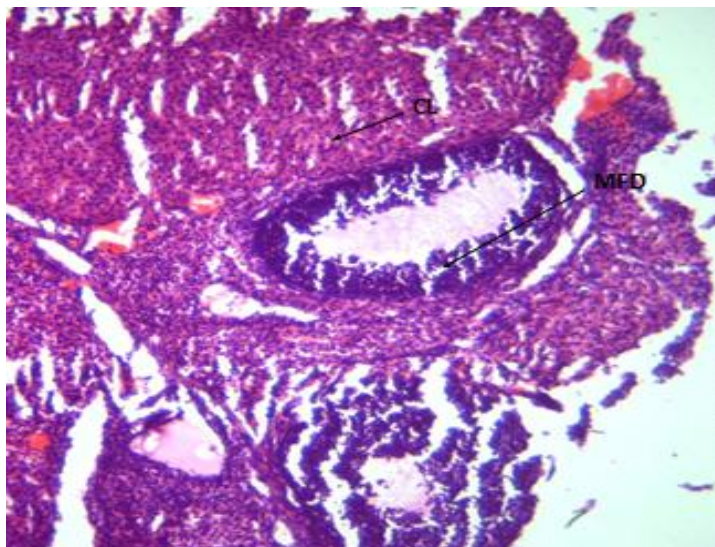


Figure 4: Photomicrograph section of the ovary in group D treated with 800 mg/kg body weight of *V. amygdalina* showing moderate increase in the follicular development (MFD) and moderate degeneration of the corpus luteum (CL).

DISCUSSION

Medicinal plants precursors for the synthesis of drugs and thousands of plants have been known to be used for medicinal purposes especially in Africa with very few being studied.^[15] The knowledge of its use and side effects provide a great contribution to health.

From the study, there was significant difference in the body weight of animals in all groups. However, while there was a significant increase in body weight of animals in group A, there was a significant decrease in body weight of the treated groups. The increase in group A could be physiological as they were not exposed to the extract and the decrease in the treated groups could be due to loss of appetite. This is in line with the study of Chike *et al.*^[16] who reported that there was a significant decrease in mean body weight of rats on day 28 for *Vernonia amygdalina* 300 mg/kg when compared to day 0. It is also in accordance with the reports of Mebratu *et al.*^[17] which showed an increase in body weight of mice treated with methanol leaf extract of *Vernonia bipontini* at a dose 800 mg/kg compared to the control.

The relative ovary weight showed a significant increase in the treated groups when compared to the control with the highest dose having a more significant increase. This could be as a result of increase in follicular cell development or a sign of toxicity.

Histopathological findings revealed distortions in the ovary cytoarchitecture with the treated groups having degenerating corpus luteum, increase in follicular development and some areas of haemorrhage. The histopathological changes might be due to the presence of bioactive compounds (saponins, alkaloids, tannins, oxalate).^[18,19] The presence of oxalate in food has been reported to be associated with toxicity and acidity.^[19]

CONCLUSION

From this investigation, it can be deduced that consumption of ethanolic extract of *V. amygdalina* leaf at high doses consecutively might damage the ovaries. Therefore its consumption should be minimized

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CONFLICT OF INTEREST

There was no conflict of interest during the research

REFERENCES

1. Johri RK, Singh C. Medicinal uses of Vernonia species. *Journal of Medicinal and Aromatic Plant Science*, 1997; 19: 744–752.
2. Farombi EO. African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents. *Afr. J. Biotech*, 2003; 2: 662–671.
3. Erasto P, Grierson DS, Afolayan, AJ. Bioactive Sesquiterpene Lactones from the Leaves of *Vernonia amygdalina*. *Journal of Ethnopharmacology*, 2006; 106: 117–120.
4. Igile GO, Wieslaw O, Jurzysta M, Stanislaw B, Fasanmade A. Flavonoids from *Vernonia amygdalina* and their Antioxidant Activities. *Journal of Agricultural and Food Chemistry*, 1994; 42: 2445–2448.
5. Burkill, H.M. The useful plants of West Tropical Africa. 2nd ed. Volume 1, Families A–D. Royal Botanic Gardens, Kew, Richmond: United Kingdom, 1985; 960.
6. Hamowia AM, Safran AM. Pharmacological Studies on *Vernonia amygdalina* (Del) and *Tithonia*

- Diversifolia (Gray). *J.Vet. Medicine*, 1994; 42: 91-97.
7. Anibijuwon II, Oladejo BO, Adetitun DO, Kolawole OM. Antimicrobial Activities of *Vernonia amygdalina* against Oral Microbes. *Global Journal of Pharmacology*, 2012; 6(3): 178–185.
 8. Egharevba C, Osayemwenre E, Imieje V, Ahomafor J, et al. Significance of Bitter Leaf (*Vernonia amygdalina*) In Tropical Diseases and Beyond: A Review. *Malaria Chemotherapy Control and Elimination*, 2014; 3(1): 1–10.
 9. Ayoola G, Coker H, Adesegun S, Adepoju-Bello A, Obaweya K, et al. Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria Therapy in South western Nigeria. *Tropical Journal of Pharmaceutical Research*, 2008; 7(3): 1019–1024.
 10. Letchuman GR, Nazaimoon WMW, Mohamad WBW, Chandran LR, et al. Prevalence of Diabetes in the Malaysian National Health Morbidity Survey III 2006. *Medical Journal of Malaysia*, 2010; 65(3): 173–179.
 11. Ngatu NR, Okajima MK, Yokogawa M, Hirota R, Takaishi M, et al. Anti-Allergic Effects of *Vernonia amygdalina* Leaf Extracts in Hapten-Induced Atopic Dermatitis-Like Disease in Mice. *Allergology International*, 2012; 61(4): 597–607.
 12. Alo MN, Anyim C, Igwe JC, Elom M, Uchenna DS. Antibacterial Activity of Water, Ethanol and Methanol Extracts of *Ocimum gratissimum*, *Vernonia amygdalina* and *Aframomum melegueta*. *Pelagia Research Library*, 2012; 3(2): 844–848.
 13. Jisaka M, Ohigashi H, Takegawa K, Hirota M, Irie R et al. Steroid glucosides from *Vernonia amygdalina*, a possible chimpanzee medicinal plant. *Phytochemistry*, 1993; 34: 409–413.
 14. Kiplimo JJ, Koorbanally NA, Chenia H. Triterpenoids from *Vernonia auriculifera* Hiern Exhibit Antimicrobial Activity. *African Journal of Pharmacy and Pharmacology*, 2011; 5(8): 1150–1156.
 15. Taylor JL, Rabe T, McGraw LJ, Jager AK, Staden J. Towards the scientific validation of traditional medicinal plants. *Journal Plant Growth Regulation*, 2001; 34: 23-37.
 16. Chike CPR, Njoku B, Green K, Akpojotor PI, et al. Effect of ethanolic leaf extract of *Vernonia amygdalina* on some haematological parameters in wistar rat., 2018; 5(1): 1-7.
 17. Mebratu A, Yamrot K, Eyasu M, Yonas B, and Keibesia U. Toxicological effect of aqueous leaf extracts of *V. biontini* vatke on blood, liver and kidney tissues of mice. *Monona Ethiopian Journal of Science*, 2013; 5: 15-31.
 18. Nwanjo HU. Efficacy of aqueous leaf extract of *Vernonia amygdalina* on plasma lipoprotein and oxidative status in diabetic rat models. *Journal of Physiological Sciences*, 2005; 20: 39-42.
 19. Eleyinmi A, Adebowale Y, Oluwalana I, Ajisafe O, Akintomide T. Effect of dietary inclusion of *Garcinia kola*, *Gongronema latifolium* and *Vernonia amygdalina* on the nutritional quality of a complementary diet. *Journal of Biological Sciences*, 2006; 1: 43-49.