

Histological Studies on the Effects of Chronic Feeding of *Vernonia Amygdalina* Del. Leaves on Rats

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ABSTRACT

Vernonia amygdalina (bitter-leaf tree) is a tropical plant belonging to the family *Compositae* and is used widely as vegetable and medicinal plant. The study was conducted to evaluate the relative safety of chronic consumption of *V. amygdalina* in an experimental model (Rat) based on clinical and possible pathological observations. Four groups of male (Wistar) rats, aged 4-6 weeks, with average weight of 33.27g received, for 65 days, 25%(w/w), 50%(w/w), and 75%(w/w) powdered leaves of *V. amygdalina* Del. orally. The animals in the control group were fed on normal feed. All the animals were observed for feed and water intake daily and it was found that the difference in body weight for the treatment groups was significantly lower ($P < 0.05$) compared to the control group. After the treatment with *V. amygdalina*, the skin of the rats was found to be remarkably light coloured. Microscopically, the kidney, hepatic and testicular architectures were found to be normal (*Nig J Surg Res* 2000; 2:68- 74)

KEY WORDS: *Vernonia amygdalina*, Wistar rat, Water, Feed, Body weight, Tissue, Safe

Introduction

The plant *Vernonia amygdalina* Del. (Shuwaka, Bitter-leaf tree) of the family *Compositae* is a small tree that grows throughout tropical Africa. The green leaves of the plant are used as vegetables, in soup and as a tonic. The aerial parts are used as antihelminthic, laxative, and as remedy for infertility.^{1, 2} It is used as antimalarial agent against *Plasmodium falciparum* *in vitro*.³ Wild chimpanzees have been observed to eat

the plant more frequently during sickness.^{1, 2}

Phytochemical screenings of the plant revealed the presence of stigmastane -type steroidal saponins such as veroniosides A₁ and B₁,⁴ A₁ A₂ and A₃, B₁ B₂B₃ and A₄ and C, which were isolated from the leaves.⁵⁻⁷

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Flavonoids were identified from the leaves of *V. amygdalina* Del. as luteolin, luteolin 7 - 0 - β - glucuronoside and luteolin 7 - 0 - β - glucoside which were found to be antioxidants.⁸ Vernodalinalin and Vernomygdin isolated from the leaves have been found to antitumor activity.

It has been reported that the leaves contain 18% protein and 8.5% fibre in dry weight. Feeding 2 weeks old mice with the leaves of *V. amygdalina* for 14 days resulted in reduction in body weight and increased urinary and faecal output. However, their feeding performance was not affected.^{1,2}

In view of the importance of the plant as a medicinal plant and food item, this communication reports the potential of the plant to remedy infertility as it stimulates spermatogenesis.

Materials and Method

Plant collection and identification

The plant *V. amygdalina* Del. was collected around Zaria identified and authenticated by the Herbarium keepers of the Department of Biological Sciences, Ahmadu Bello University, Zaria. Its voucher specimen's number is 675.

Preparation of the plant materials

The leaves of the plant were air dried and ground to powdered form. This was subsequently referred to as the plant materials. The powdered leaves were added to standard grower mash to make up 25%(w/w), 50%(w/w) and 75%(w/w) of the plant materials in the formulated diet, while 0%(w/w) was the normal feed without plant materials.

Animals

Sixteen adult male rats (Wistar strain) weighing 33.27g averagely were used for the experiments. The animals were allowed to rest for 7 days prior to experiment. They were screened for pre-existing diseases during the period. The rats were divided into four groups; Group A was fed with 25%(w/w), B with 50%(w/w), and C with 75%(w/w) of the plant materials respectively, while the control group received only the standard feed. Water was given *ad libitum*. The animals were fed for 65 days, while their feed consumption, body weights, and water intakes were recorded.

The rats were subsequently sacrificed, necropsied, and various tissues were observed. Sections from the liver, lung, kidney, testis, intestine, spleen, brain, skin and various tissues were prepared and fixed in 10% buffered formalin for 48 hours. The tissues were processed for histopathological observations. The slides were stained with Haematoxylin and Eosin stains and examined microscopically. The histological features used in assessing the results were based on cellular morphological and architectural changes of the organs. The data obtained were recorded and analysed using student's t-test where applicable.

Results

The results of the study showed that rats in group A gained weight from 39.71 \pm 1.05g pre-treatment to 80.17 \pm 10.77g post-treatment. The rats in group B had a fairly constant weight of 107.94 \pm 1.51g pre-treatment compared to 107.34 \pm 2.64g post-treatment. However, the rats in group C lost weight from 143.63 \pm 1.64g pre-treatment to

118.35 ± 7.02g post-treatment (Table 1).

Microscopically, the observations made were similar in the various groups treated with the powdered leaves. The epidermis and dermis of the skin, melanocytes, melanin, collagenous fibres and hair follicles turned pink. The blood vessels of the organs were slightly congested. There were no significant changes in the organs of the rats in the control group. The kidneys had well organised renal tubular epithelium with abundant chromatin materials in their nuclei. The liver had organised hepatic cords with

good cellular morphology of the hepatocytes and Kupffer cells. Spermatogonia, spermatids and spermatocytes and sertoli cells in the testis were well organized within the seminiferous tubules. The nuclei of the cells had abundant chromatin materials and were actively involved in spermatogenesis (Fig. 1, 2, 3 and 4). The nuclei of the neurones and glial cells in the brain had abundant chromatin material and the nucleoli were prominent. The spleen had active germinal centre with dense proliferation of lymphocytes and active erythropoiesis.

Table 1: Pre-treatment and post-treatment mean values of water-intake feed and body weight per group per day.

PARAMETER/GROUP		A	B	C	D
WATER-INTAKE (ml) (Mn ± S.E)	Pre-treatment	14.31 ± 0.56	15.28 ± 0.41	16.62 ± 0.46	14.93 ± 0.62
	Post-treatment	14.60 ± 0.35	17.32 ± 2.04	17.01 ± 0.73	16.54 ± 1.98
FEED (g) (Mn ± S.E)	Pre-treatment	6.29 ± 0.43	7.02 ± 0.21	7.33 ± 0.26	6.67 ± 0.25
	Post-treatment	8.69 ± 0.76	9.38 ± 0.84	8.31 ± 0.79	9.41 ± 0.61
BODY WEIGHT (g) (Mn ± S.E)	Pre-treatment	39.71 ± 1.05	107.94 ± 1.51	143.63 ± 1.64	105.92 ± 1.63
	Post-treatment	80.17 ± 10.77*	107.34 ± 2.64*	118.35 ± 7.02**	148.21 ± 10.16

n = 4

Mn = Mean

S.E = Standard Error

*P < 0.05

**P < 0.01

% *V. amygdalina* leaves;

A = 25, B = 50

C = 75, D = 0

Figure 1:Section from testis of control rat (fed 0% (w/w) V. amygdalina Del. Leaves). Note the normally arranged spermatogenic cells in the seminiferous tubules (H&E X400).

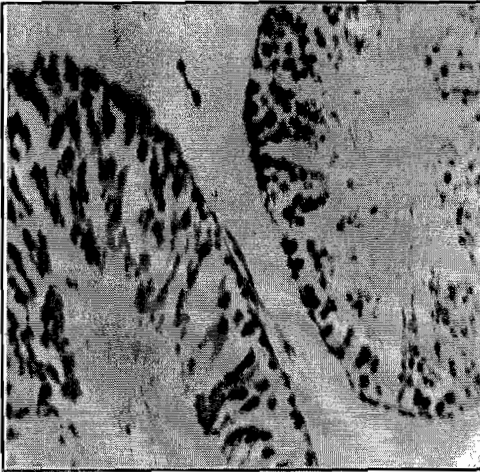


Figure 3:Section from testis of rat (fed 50%(w/w) V. amygdalina Del. Leaves). Note the activity of the spermatogenic cells in the seminiferous tubules (H&E X400).

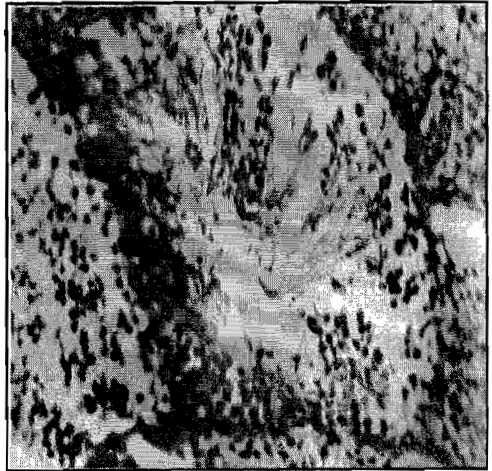


Figure 2:Section from testis of rat (fed 25% (w/w) V. amygdalina Del. Leaves). Note the active spermatogenic cells in the seminiferous tubules (H&E X 400).

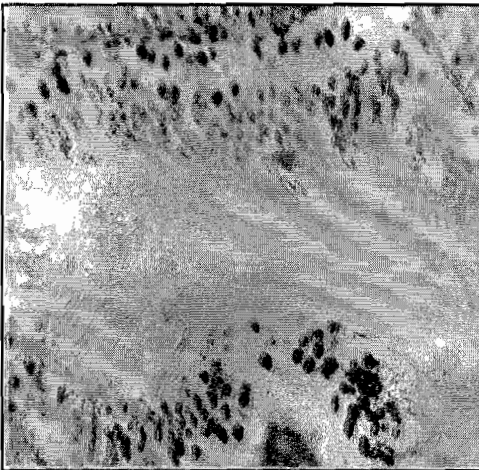


Figure 4:Section from testis of rat (fed 75%(w/w) V. amygdalina Del. Leaves). Note the abundance of the spermatogenic cells in the seminiferous tubules (H & E X 250).

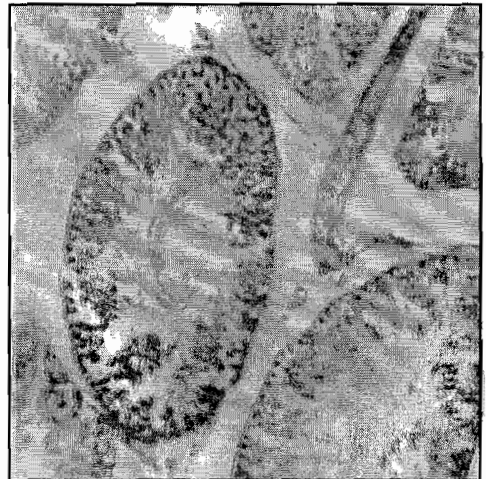


Figure 5: Section from skin of control rat (fed 0%(w/w) V. amygdalina Del. Leaves). Note the appearance of the colour of the skin (H & E X 400)



Figure 7: Section from skin of rat (fed 50%(w/w) V. amygdalina Del. Leaves). Note discolouration of the cells of the epidermis and dermis to pink (H & E X 400)

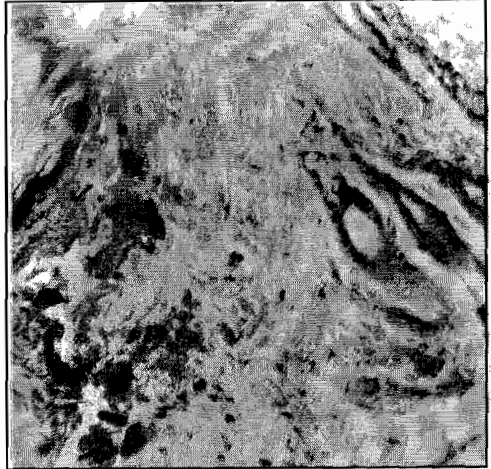
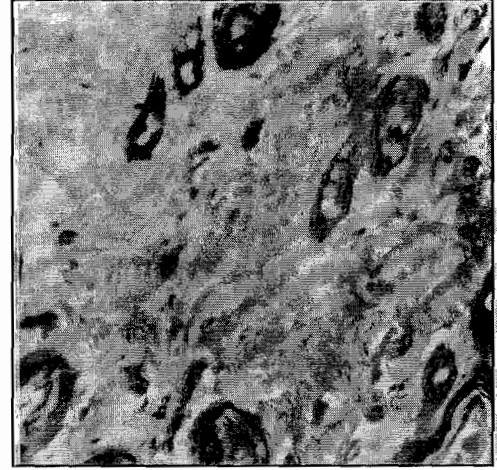


Figure 6: Section from skin of rat (fed 25%(w/w) V. amygdalina Del. Leaves). Note discolouration of the cells of the epidermis and dermis to pink (H & E X 400)



Figure 8: Section from skin of rat (fed 75%(w/w) V. amygdalina Del. Leaves). Note discolouration of the cells of the epidermis and dermis to pink (H & E X 400)



Discussion

The results after treatment with the various amended diets indicated that the rats in group A had added weight, those in group B had a fairly constant body weight, whereas those in group C had lost weight, when compared with the control group D ($P < 0.05$, $P < 0.01$, student's t-test). Throughout the period of this study, the water intake and feed intake were increasing. This may indicate a possible malabsorption of nutrients along the gastrointestinal tract at 75%(w/w) concentration. It was reported earlier, that, the small intestine of mice fed with *V. amygdalina* Del. leaves were enlarged, larger than those in the control group.^{1, 2} These observations have shown that consumption of *V. amygdalina* Del leaves, especially at very high concentration requires caution.

The microscopic appearance of the organs indicated that the efficiency of the kidney to excrete toxic substances and to concentrate urine and the detoxification capacity of the liver were possibly enhanced. Active spermatogenesis and erythropoiesis may lead to improved reproductive capacity and amelioration of anaemia by using the leaves of the plant. This, probably accounts for the documented role of the plant in the treatment of infertility.^{1,2} Apart from possible therapeutic effect of the plant on malaria,³ this study has shown that there was activation of the haemopoietic system. The skin discolouration observed in the present study may be as a result of some influence of *V. amygdalina* on biosynthesis of melanin, or its influence on tyrosinase. Tyrosinase is responsible for hydrolysis of tyrosine, dihydroxy phenylalanine and phenylalanine during biosynthesis of melanin.⁹ This opens a new area for further research. An earlier

study⁸ has shown that chloroform extract of *V. amygdalina* had significant tumour inhibitory activity *in vitro* against neoplastic cells derived from human carcinoma of nasopharynx in tissue culture. This implies that further studies need to be carried out on the plant for possible therapeutic use in the management of skin cancers such as malignant melanoma and squamous cell carcinoma. Apparently, information on the pathological studies on the plant is scanty. These results indicate that *V amygdalina* Del. can be considered to be relatively safe as a medicinal plant and for both human and animal consumption.

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