

Full Length Research Paper

Is *Vernonia amygdalina* hepatotoxic or hepatoprotective? Response from biochemical and toxicity studies in rats

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The effects of various concentrations of aqueous extract of *Vernonia amygdalina* leaves on some biochemical indices of liver function were investigated in albino Wistar rats. Acute toxicity tests of the extract gave an LD₅₀ of 500 mg/kg. Phytochemical analysis of the plant material showed that anthracene glycosides, steroids, flavonoids, proteins, carbohydrates, reducing sugars, saponins and tannins were present. Liver function tests revealed that the activity of aspartate aminotransferase (AST) increased significantly ($p < 0.05$) for all the concentrations administered. There was no significant ($p > 0.05$) increase in both alanine aminotransferase and alkaline phosphatase activities for all the concentrations administered. Also the increase in mean values of conjugated and unconjugated bilirubin for all the concentrations administered were not statistically significant ($p > 0.05$). The results, therefore, strongly suggest that *V. amygdalina* leaf extract is not hepatotoxic in rats. The findings are of nutritional, clinical and veterinary relevance considering the diverse applications of the plant in almost all African populations.

Key words: *Vernonia amygdalina*, liver function tests, LD₅₀.

INTRODUCTION

Vernonia amygdalina Del. popularly known as bitter leaf is a shrub of 2-5 m tall with petiolate green leaves of about 6 mm diameter. The leaves are characteristically bitter but the bitterness can be abated by boiling or by soaking in several changes of clean water (Burkill, 1985). The stem and root divested of the bark are used as chew-sticks in Nigeria. More importantly, the leaves are a very popular soup vegetable and have even been reported to be consumed by goats in some parts of Nigeria (Aregheore et al., 1998).

All parts of the plant are pharmacologically useful. The roots and the leaves are used in ethnomedicine to treat fever, hiccups, kidney problems and stomach discomfort among several other uses (Burkill, 1985; Hamowia and

Saffaf, 1994). Both aqueous and alcoholic extracts of the stem, bark, roots and leaves are reported to be extensively used as a purgative, antimalarial and in the treatment of eczema (Kupcham, 1971). The plant has acquired special relevance recently, having been proved in human medicine to possess potent antimalarial and antihelminthic properties (Abosi and Raseroka, 2003) as well as antitumorigenic properties (Izevbogie et al., 2004) with an amazing antiparasitic efficacy in zoopharmacognosy as it is easily recognized and used for self-medication by parasitized chimpanzees (Huffman, 2003). Pharmacological studies have also shown that the leaf extract has both hypoglycaemic and hypolipidaemic properties in experimental animals and so could be used in managing diabetes mellitus (Akah, 1992). The active components of the plant have been shown to be mainly sesquiterpene lactones like vernodalin and vernoamygdalin and steroid glycosides like vernonioside B1 and vernoniol B1 (Kupcham et al., 1969).

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Nutritionally, *V. amygdalina* is used mainly in soup making in the tropics and also as an appetizer and febrifuge (Ijeh et al., 1996; Iwu, 1996) and has proven to be a successful supplement in weaning foods (Eleyinmi, 2005). In Nigeria, as in other tropical countries of Africa where the daily diet is dominated by starchy staple foods, vegetables are the cheapest and most readily available sources of important proteins, vitamins, minerals and essential amino acids (Okafor 1983). The importance of *V. amygdalina* in animal nutrition in Nigeria has also been well documented (Onwuka et al., 1989; Aregheore et al., 1998).

Despite these varied uses of the plant, there has been insufficient information on its exact toxicological potentials on the animal system. There have been reports of the presence of toxic phytochemicals (Aregheore et al., 1997; Bonsi et al., 1995). There are also reports of actual hepatotoxicity in mice (Igile et al., 1995), yet there is a report that extracts from the plant are able to inhibit and even reverse carbon tetrachloride-induced hepatotoxicity in rats (Babalola et al., 2001). This study was therefore designed primarily to examine the actual effect of an aqueous extract of *V. amygdalina* leaves on some biochemical parameters of liver integrity in rats and also to examine the response of the animals to acute toxicity tests using extracts of the plant.

MATERIALS AND METHODS

Preparation, crude extract administration and bioassay

Pesticide-free fresh leaves of *V. amygdalina* were collected from within Owerri, Imo State, Nigeria and the botanical identity kindly confirmed by Dr. S. E. Okeke of the Department of Plant Biology and Biotechnology, Imo State University, Owerri, Nigeria where voucher samples were kept for reference. Healthy fresh leaves were sorted, washed to remove debris and dust particles without squeezing and then sun-dried for four days before the final drying in an oven at 65°C for 24 h. The dried leaves were milled into a coarse powder from which 25 g was soaked with 250 ml of distilled water in a beaker and the mixture shaken on the laboratory bench for 24 h before filtering. The filtrate was evaporated using a rotary evaporator to obtain a solid residue (2.4 g) called the aqueous extract. Appropriate weights of the residue were prepared in distilled water to obtain the various concentrations used for the tests.

Acute toxicity tests

The acute toxicity of the extract was tested using 30 Wistar albino rats divided into 5 groups of 6 rats each, with each group receiving a different dose of the extract administered intraperitoneally as described by Miller and Tainter (1944). The number of deaths in each group within 24 h was recorded. The LD₅₀ was estimated from the graph of percentage (%) mortality (converted to probit) against log-dose of the extract, probit 5 being 50%.

Liver function tests

Eighteen albino Wistar strain rats were randomly assigned into 3 groups of six rats each and housed in stainless steel cages and

kept in a room where a 12-h light/dark cycle was maintained. They were allowed access to water and feed diet (product of Pfizer Nigeria Ltd.) *ad libitum* throughout the period of the experiment. In addition, Group I (the high dose group) received 100 mg extract/kg body weight while Group II (the low dose group) received 50 mg/kg body weight administered through the intraperitoneal (i.p) route twice daily for four days. Group III (the control group) was maintained on feed diet and water only.

24 h after the last injection all rats were weighed and sacrificed under chloroform anesthesia and with a sterile syringe and needle, 8 ml of blood was collected from each animal by cardiac puncture and transferred into a centrifuge tube and allowed for 30 min to clot before centrifuging using Wisperfuge Model 1384 centrifuge (Tamson, Holland) for 5 min and the resulting supernatant used for the assessment of liver integrity.

Total and conjugated bilirubins were determined according to the method of Malloy and Evelyn (1937) as modified by Tietz (1976). Total bilirubin was determined in the presence of caffeine benzoate which releases albumin-bound bilirubin. Bilirubin then reacts with diazotized sulphanilic acid to form pink-coloured azobilirubin which in the presence of alkaline tartarate is converted to a green colour. Aspartate and alanine aminotransferases were assayed by the method of Reitman and Frankel (1957) while alkaline phosphatase activity was assayed using disodium phenol phosphate as substrate in a phosphate buffer of pH 10.0 (Bassey et al., 1947).

Statistical analysis

Data collected were summarized as mean \pm SD. Differences between individual groups were assessed by student's t-test. A P-value \leq 0.05 was considered significant.

RESULTS AND DISCUSSION

The acute toxicity studies produced an LD₅₀ of 500 mg/kg body weight of experimental animal. Table 1 shows the results of serum bilirubin levels for the animals. The differences in the mean values of total, conjugated and unconjugated bilirubin concentrations in groups I and II were not significant when compared with the control group or with one another ($P > 0.05$). Table 2 shows the result of the administration of aqueous leaf extract of *V. amygdalina* on some liver function diagnostic enzymes.

The results show no significant increase in total, conjugated and unconjugated bilirubin levels between the various groups. Also, the activities of both alanine aminotransferase and alkaline phosphatase in the presence of *V. amygdalina* leaves extract increased slightly in a dose-dependent manner when compared with the control but none of these observed slight increases was statistically significant ($P > 0.05$) when compared to the control or when compared within doses. The result showed, however, that the aspartate aminotransferase activity significantly ($P < 0.05$) increased in both the low-dose and high-dose groups when compared with the control group. There was also a significant dose-dependent difference in the activities of the high-dose and the low-dose groups.

The elevations in liver function parameters as found in this work are not such that should discourage the indica-

Table 1. Concentration of serum bilirubin in rats fed *V. amygdalina* leaves extract.

Parameter	High dose	Low dose	Control
Total bilirubin (mg/100ml)	1.14±0.15	1.08±0.11	0.88±0.08
Conjugated bilirulim (mg/100ml)	0.62±0.08	0.53±0.07	0.4±0.18
Unconjugated bilirulim (mg/100ml)	0.52±0.06	0.55±0.3	0.48±0.10

Table 2. Activities of serum liver diagnostic enzymes in rats fed *V. amygdalina* leaves extract.

Enzyme (IU/L)	High dose	Low dose	Control
Alanine aminotransferase	22.4±1.84	20.0±1.64	18.6±1.52
Aspartate aminotransferase	44.4±1.1.68**	30.4±1.96*	10.6±1.34
Alkaline phosphatase	104.15±5.36	101.6±5.2	88.4±4.26

*Significantly different from control group (P<0.05)

**Significantly different from low dose group

tes its nonhepatotoxic effect. Alanine aminotransferase is a more reliable marker of liver integrity than aspartate aminotransferase. The observed significant increase in the activity of aspartate aminotransferase alone may therefore be of extrahepatic origin. We are currently testing this extrahepatic hypothesis.

Furthermore, the acute toxicity studies show an LD₅₀ of 500 mg/kg body weight. This indicates that the vegetable when consumed in high quantities may, indeed, elicit hepatotoxicity as observed in small animals like mice by Igile and co-workers (1995). Such very large quantities of the vegetable are, however, not consumed at the same time. It is therefore, unlikely that consumption at any time will attain toxic levels. Even at this, the toxic value in our present work was obtained for an unprocessed aqueous extract of the vegetable. Traditionally, however, the vegetable is macerated and the dilute extract administered for therapeutic purposes while in nutritional applications, the leaves are soaked and washed severally in clean water prior to its use in soup making. Some times, the washed vegetables are sun-dried and then used installmentally. Such processing methods have been shown to detoxify several leafy vegetables that are ordinarily very toxic in their raw state, including the more popular vegetable *Telfaria occidentalis* (fluted pumpkin).

This side of the logic may then explain why even though antinutritional factors were shown to be present in *V. amygdalina* (Eleyinmi et al., 2005), nutritional studies on the same vegetable after the traditional processing methods gave very commendable results (Eleyinmi et al.). This may also explain why processed extracts from the plant were able to inhibit and even reverse carbon tetrachloride-induced hepatotoxicity in rats as observed by Babalola and his co-workers (2001).

Our findings confirm that *V. amygdalina* leaves may be toxic (just like several other vegetables) if consumed in very large quantities but the potential danger is not higher than has been observed for other common vegetables

that are routinely consumed in Africa in even larger quantities. Our results do not confirm hepatotoxic effects of the vegetable, at least, in rats. The several useful applications of the vegetable in both human and veterinary nutrition and medicare should, however, take cognizance of this fact of possible toxicity in very high doses.

REFERENCES

- Abosi AO, Raseroka BH (2003) *In vivo* antimalarial activity of *Vernonia amygdalina*. Br. J. Biomedical Sci. 60(2): 89-91
- Akah PA, Okafor CI (1992). Hypoglycaemic effect of *Vernonia amygdalina* Del, in experimental rabbits. Plant Med. Res. 1: 6-10
- Aregheore EMK, Makkar HPS, Becker K (1998). Feed value of some browse plants from the central zone of Delta State, Nig. Trop. Sci. 38(2): 97-104.
- Babalola OO Anetor JI, Adeniyi FA (2001). Amelioration of carbon tetrachloride-induced hepatotoxicity by terpenoid extract from leaves of *Vernonia amygdalina*. Afr. J. Med. Sci. 30 (1-2): 91-3.
- Bassey OA, Lowry OH, Brock MJ (1947). Measurement of plasma alkaline phosphatase activity. J. Biol. chem. 164: 321-322
- Gupta SA, Seth CB (1962). Effect of *Momordica charantia* on glucose tolerance on albino rats. Indian J. Physiol. Pharmacol. 8: 240-244.
- Hamowia AM, Saffaf AM (1994). Pharmacological studies on *Vernonia amygdalina* (Del) and *Tithonia diversifolia* (Gray). Vet. Med. J. Giza 2: 91-97.
- Huffman MA (2003) Animal self-medication and ethno-medicine: exploration and exploitation of the medicinal properties of plants. Proceedings of the Nutr. Society. 62(2):371-81.
- Ijeh 1, Nwugo VO, Obidoa O (1996). Comparative studies on the nutritive Phyto-chemical and antimicrobial properties of two varieties of *Vernonia amygdalina*. Plant Pros. Res. Comm. 1: 71-75.
- Izevbigie EB, Bryant JL, Walker A (2004). A novel natural inhibitor of extracellular signal-regulated kinases and human breast cancer cell growth. Experimental Biol. Med. (Maywood) 229(2):163-169.
- Iwu MM, Okunji E, Akah PA, Tempesta MS, Carley DG (1996). Dioscoretine: The hypoglycaemic principles of *Dioscorea dumetorium*. Plant Med. 56: 119-126
- Katsuri M, Manivannan B, Ahmed RN, Shaikh PD, Nathan RM (1995). Changes in epididymis structure and function of albino rats treated *A. Indica* leaves. Ind. J. Expt. Biol. 33: 725-729.
- Kupcham SM, Hernichway RJ, Karim A, Wermer D (1969). Tumor inhibitors. XLVII. Vernodalin and vernomygdin, two new cytotoxic

- sesquiterpene lactones from *Vernonia amygdalina* Del. J. Org. Chem. Dec. 34 (12):3908-11.
- Kupcham SM, Hernichway RJ, Wermer D (1969). Vernodaline and Vernomygdin. Two new sesquiterpene lactones from *Vernonia amygdalina* del. J. Org. Chem. 3: 3908-3909
- Kupcham SM (1971). Drugs from Natural products. Plant source in drugs discovery, science and development. Am. Chem. Soc. 6: 311-318.
- Masaba SC (2000). The antimalarial activity of *Vernonia amygdalina* Del (Compositae). Trans R Soc Trop Med Hyg. 94 (6):694-5.
- Malloy HT, Evelyn KA (1937). Measurement of plasma bilirubin (modified). J.Biol.Chem. 2: 119-481.
- Miller LC, Tainter ML (1944). Estimation of EC_{50} and its error by means of log-probit graph paper. Proc. Soc. Exp. Biol. Med. 57: 261-269.
- Okafor JC (1983). Horticulturally promising indigenous wild plant species of the Nigerian forest zone. Acta Hort. 123:165-176.
- Onwuka CFI, Akinsoyinu AO, Tewe OO (1989). Feed value of some Nigerian browse plants: chemical composition and *in vitro* digestibility. East African Agriculture and Forestry J. 54: 157 – 163.
- Reitman SN, Frankel S (1957). A colorimetric method for the determination of SGPT and SGPT. Am.J. Clinical Pathol. 25: 56-62.
- Rodwell VK, Gramner DK, Ma'yer PA, Murray RR (1990). Cholesterol biosynthesis and transport. Harpers Biochemistry (22nd Ed) Prentice Hall Inter. Inc. pp.240-250.
- Tietz NW (1976). Clinical Guide to Laboratory Tests. W.B. Saunders Company, Philadelphia. p. 17.
- Trease GE, Evans MC (1983). Textbook of pharmacognosy. 12th edition Bailliere, Tindall, London, pp 343-383.