The effects of aqueous and ethanolic leaf extracts of *Vernonia amygdalina* on some vital organs in adult Wistar rats

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Received: 15 February 2011; Accepted: 20 June 2011

**Abstract**

The aim of this study was to comparatively determine the effects of aqueous and ethanolic extracts of *Vernonia amygdalina* (Asteraceae) leaves, adopting histological procedures on the liver, stomach and kidney in adult wistar rats. There were four groups of animals: A, B, C and D. Groups A, B and C served as the treated animals while group D served as the control animals. Each of the groups was further subdivided into two i.e A1 & A2; B1 & B2; C1 & C2 and D1 & D2. Animals in groups A1, B1 and C1 were administered orally with aqueous extract of *V. amygdalina* 100, 200 and 300 mg/kg respectively while animals in groups A2, B2 and C2 were administered orally with ethanolic extract of *V. amygdalina* 100, 200 and 300 mg/kg respectively. The control groups, D1 and D2, received equal volume of normal saline. There were no significant derangement in the cytoarchitecture of the stomach, liver and kidney. Rather, the cytoarchitecture of the animals treated with both the ethanolic and aqueous extracts (300mg/kg) of *V. amygdalina* were better organized when compared with the control. It was therefore concluded that both ethanolic and aqueous leaf extracts of *V. amygdalina* are non-toxic and may possess cytoprotective potential.

**Keywords:** *Vernonia amygdalina*, histological procedures, liver, stomach, kidney

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**Vernonia amygdalina** yaprağının sulu ve etanolik özütlerinin erişkin Wistar sıçanlarının bazı hayati organlarına etkileri

**Özet**

Çalışmanın amacı, erişkin Wistar sıçanlarında *Vernonia amygdalina* (Asteraceae) yapraklarının sulu ve etanolik özütlerinin karaciğer, mide ve böbrek üzerindeki etkilerini histolojik yöntemlerle karşılaştırmak olarak belirlemektir. Çalışmada dört grup hayvan bulunmaktadır: A, B, C ve D. D grubu kontrol hayvanları olarak belirlenirken; A, B ve C grupları muamele edilmiş hayvanları göstermektedir. Her bir grup ayıracı iki alt gruba bölünmüştür; A1 & A2; B1 & B2; C1 & C2, D1 & D2. A1, B1 ve C1 gruplarındaki hayvanlara sırasıyla 100, 200 ve 300 mg/kg *V.amygdalina* sulu özütü oral olarak uygulanırken A2, B2 ve C2’yı ise sırasıyla 100, 200 ve 300 mg/kg *V.amygdalina* etanolik özütü oral olarak uygulanmıştır. Kontrol grupları olan D1 ve D2 eşit hacimde normal tuz almışlardır. Mide, karaciğer ve böbreğin hücresel yapısında belirgin bir bozulma bulunmamıştır. Bilakis hem sulu, hem de etanolik *V. amygdalina* (300mg/kg) özütü uygulanan
hayvanların hücresel yapıları kontrolle karşılaştırıldığında daha iyi organizedir. Bu nedenle suyu ve etanolik V. amygdalina yaprak özütlerinin her ikisinin de toksik olmadığını ve hücre koruyucu potansiyele sahip olabileceğini sonucuna varılmıştır.

Anahtar Sözcükler: Vernonia amygdalina, histolojik süreçler, karaciğer, mide, böbrek.

Introduction

Vernonia amygdalina (Del. Asteraceae) commonly known as “bitter leaf” is a valuable shrub that is widespread in East and West Africa. It is 2-5 m tall with petiolate green leaves of about 6 mm diameter (Ojiako and Nwanjo, 2006). In Nigeria the stem is used as chew-sticks, while the leaves are being used as a popular vegetable for soups particularly among the Igbos of Southern Nigeria, Africa (Ojiako and Nwanjo, 2006). Its medicinal values for fever, laxative, pile (haemorrhoids) and gastro-intestinal troubles have been investigated and reported by the following authors: (Oliver, 1960; Ainslie, 1973; Kupcham, 1971; Akah and Okafor, 1992; Abosi and Raseroka, 2003; Huffman, 2003; Izevbigie et al., 2004), In fact all parts of the plant have been known to be pharmacologically useful. Oral administration of the aqueous leaf extract of the plant was found to relieve pain (Tekobo et al., 2002) and to lower body temperature (Tekobo et al., 2002). Atangwho et al. (2010) reported instantaneous reduction of blood glucose and a variation in blood glucose similar to that of insulin-treated rats in animals administered with ethanol extract of V. amygdalina.

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, Ojiako and Nwanjo, 2006) and has proven to be a successful supplement in weaning foods (Ojiako and Nwanjo, 2006). 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; Ojiako and Nwanjo, 2006). The importance of V. amygdalina in animal nutrition in Nigeria has also been well documented (Onwuka et al., 1989; Aregheore et al., 1998; Ojiako and Nwanjo 2006). Despite these beneficial uses of the plant, there has been conflicting reports on its exact toxicological potentials on some visceral organs.

For instance Aregheore et al., (1998) reported the presence of toxic phytochemicals. There are also reports of actual hepatotoxicity in mice (Igile et al., 1995), also, there was a report on hepatoprotective effects in rats (Babalola et al., 2001). Ojiako and Nwanjo (2006) reported 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.

In view of these conflicting reports, we therefore set to comparatively determine the effects of aqueous and ethanolic leaf extracts of V. amygdalina, adopting histological procedures on the stomach, liver and kidney in adult wistar rats.

Materials and methods

Experimental Site

This research was conducted in the Department of Biochemistry, School of Basic Medical Sciences, Igbinedion University, Nigeria.

Experimental Animals

A total of forty healthy wistar rats obtained from a private farm in Benin City were used for the experiment. These animals were acclimatized for two weeks before the commencement of the study. The animals were treated in accordance with the “Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH, 1985).

Experimental Design

There were four groups of animals: A, B, C and D. Groups A, B and C served as the treated animals while group D served as the control animals. Each of the groups (n=10) was further subdivided into two i.e A: A1 & A2; B: B1 & B2;
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C: C1 & C2 and D: D1 & D2. Animals in groups A1, B1 and C1 were administered orally with aqueous extract of *V. amygdalina* 100, 200 and 300 mg/kg respectively while animals in groups A2, B2 and C2 were administered orally with ethanolic extract of *V. amygdalina* 100, 200 and 300 mg/kg respectively. The control groups D1 and D2 received equal volume of normal saline.

**Collection of plant materials**

Fresh but matured leaves of *Vernonia amygdalina* was procured from a local market in Okada, Edo-state. They were authenticated in the Botany Department, Igbinedion University, Nigeria. The leaves were rinsed severally with clean tap water to remove dust particles and debris and thereafter allowed to completely drain.

**Preparation of plant extracts**

Plant materials were separately chopped into bits with a knife on a chopping board. The leaves were then air dried and one kilogram (1kg) *V. amygdalina* was reduced to powder with an electric blender. The powder was divided into two portions. One of the portions was percolated with 80% ethanol while the other was percolated with distilled water. The mixtures were allowed for 48 h in the refrigerator at 4°C for thorough extraction of the plants active components. These were then filtered with cheesecloth and later with Whatman No. 1 filter paper to obtain a homogenous filtrate. These filtrates were then concentrated in vacuo at low temperature (37- 40°C) to about one tenth the original volume using a rotary evaporator. The concentrates were allowed open in a water bath (40°C) for complete dryness for both ethanol and aqueous extracts of *V. amygdalina*. The extracts (26.7% yield) were then refrigerated at 2- 8°C until use.

**Histological Procedure**

Histological study was carried out using the method of Carleton (1967). These procedures involved dehydration of the liver, kidney and spleen tissues with graded ethanol concentrations (50%, 70%, 90% and 100%, respectively), clearing in xylene, followed by infiltration in paraffin wax for 2 h at 56 °C and embedding in paraffin wax for 48 h. Sections (5 μm thick) were then obtained, using a rotary microtome, subjected to haematoxylin and eosin (H & E) staining procedure and examined under a light microscope. Permanent photomicrographs of the observations were taken, using an Olympus Research Microscope (model BX51).

**Results**

There were no significant derangement in the cytoarchitecture of the stomach, liver and kidney. Rather, the cytoarchitecture of the animals treated with both the ethanolic and aqueous extract (300mg/kg) of *V. amygdalina* were better organized when compared with the control (Figures 1-3). In the stomachs of treated animals, there were no disruptions of surface epithelium and no presence of submucosal edema as well as leucocytes infiltration as compared with control groups (Figure 1). There were no hypertrophy of the liver and no necrosis of the hepatocytes in the treated animals as compared with control groups (Figure 2). Also, the kidneys of animals in the treated groups presented well preserved renal corpuscles and Bowman’s spaces as compared with control groups (Figure 3). Animals in both the treated and control groups showed no physical changes in their appearances. The present study demonstrated that oral administration of both ethanolic and aqueous extracts (300 mg kg⁻¹) preserved the hepatic, renal and gastric integrity as compared to animals administered with lesser dose (200 and 100 mg kg⁻¹) and only distilled water (Figures 1-3). There were no significant differences between the cytoprotective abilities of the animals treated with ethanolic or aqueous extracts compared to the animals treated with control.
Figure 1. Histological section of gastric mucosa treated with A: aqueous extract *V. amygdalina* (A1-100, B1-200, C1-300mg/kg); B: ethanolic extract of *V. amygdalina* (A2-100, B2-200, C2-300mg/kg). Note that there were no disruptions of surface epithelium. No presence of submucosal edema and leucocytes infiltration as compared with control groups D1 and D2 (H and E stain, 100x).
Figure 2. Histological section of hepatic parenchyma treated with A: aqueous extract *V. amygdalina* (A1-100, B1-200, C1-300mg/kg); B: ethanolic extract of *V. amygdalina* (A2-100, B2-200, C2-300mg/kg). Note that there were no hypertrophy of the liver and no necrosis of the hepatocytes as compared with control groups D1 and D2 (H and E stain, 100x).
Figure 3. Histological section of renal parenchyma treated with A: aqueous extract V. amygdalina (A1-100, B1-200, C1-300mg/kg); B: ethanolic extract of V. amygdalina (A2-100, B2-200, C2-300mg/kg). Note that the renal corpuscles and Bowman’s spaces were well preserved. Also, there were no evidence of cellular necrosis as compared with control groups D1 and D2 (H and E stain, 100x).

Discussion

The histopathological changes observed in the liver, kidney, stomach of aqueous and ethanolic extracts of V. amygdalina on rats showed no evidence of lesions in the treated animals when compared with control. Our histological findings further revealed that the cyto-architecture of the liver parenchyma was better organized in the groups treated with 300mg/kg of both ethanolic and aqueous extracts of V. amygdalina. The functions of the liver which includes detoxification, metabolic activities, synthesis of plasma protein and destruction of spent red blood cells among other functions will be better enhanced based on
histological evidences from our study. This shows that administration of ethanolic and aqueous extracts of *V. amygdalina* provides a protective role for the liver which is the first organ susceptible to any injurious substances in case of toxicity. This is in line with previous work (Ofusori *et al.* 2008) who investigated the effect of *Croton zambesicus* (euphorbiaceae) on the liver. The renal parenchyma showed no evidence of vacuolations or distortion of any kind rather, renal corpuscles and Bowman’s spaces were well preserved as compared with control groups in all the experimental groups. It was evident on the photomicrograph that the tubules constitute the bulk of the renal parenchyma with different shapes, diameters and staining intensities. This points to the fact that *V. amygdalina* may have a vital role to play in osmoregulation and excretion. Also, the gastric mucosa of the treated groups were better organized when compared with the control (Figure 1). There was no evidence of ulceration in the surface epithelium, no presence of submucosal edema and leucocytes infiltration. Studies have shown that anti-oxidants significantly strengthen the gastric walls and protect tissue from oxidative damage (Marins, 1996).

All these evidences revealed that *V. amygdalina* is a very important plant that can be exploited for cyto-protective purposes. The cyto-protective mechanism may be by mopping up free radical there by protecting the cells of the organs from any type of assaults. Several other studies have shown that the aqueous and ethanolic leaf extracts of *V. amygdalina* is non-hazardous, even when taken for some time. The potency of these extracts may have been amplified on account of increased concentration or build up of specific phytochemicals, although the chemistry is yet unknown. Several findings showed that *V. amygdalina* has strong antioxidant activity corresponding to mitigation of the generation of hydroxyl radicals. Yeh *et al.* (2003) and Battell *et al.* (1999) postulated that this antioxidant activity may provide possible rationale for the observed therapeutic effects of *V. amygdalina*.

Based on the results obtained, it can be concluded that ethanolic and aqueous leaf extracts of *V. amygdalina* are non toxic and may possess cytoprotective potential.

**References**


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