

A Study of the Serum Concentrations of Some Hepatic Enzymes in Doses of Aqueous Leaf Extract of *Vernonia amygdalina* in Rabbits

¹E.B. Ezenwanne and ²R.E. Ucheya

¹Departments of Physiology,

²Departments of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria

Abstract: Healthy adult female rabbits were used to assess possible alterations in the serum levels of some of the enzymes associated with metabolic functions of the liver, in doses of aqueous leaf extract of *Vernonia amygdalina*. The study was carried out in standard laboratory diet and clean water. Twenty rabbits were placed into four groups of five animals per cage. Three test groups of the rabbits received daily oral doses of 400, 200 and 100 mg/kg of body weight, of the liquid leaf extract of *Vernonia amygdalina*, respectively, for a total period of three weeks. The control group of five animals were placed on normal laboratory feeds and clean water, and received no treatments with *Vernonia amygdalina* extract for the same period of three weeks. Weekly assessments of serum levels of two of the key enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), were compared with those of the age and sex matched controls. Evaluation analysis of the collected data was by the students' t-test taking the value, $p \leq 0.05$ as statistically significant. The results revealed a steady dose-dependent increase ($p \leq 0.05$) in serum levels of both ALT and AST enzymes, compared with those of the baseline controls values. The observed dose-dependent alterations in the serum levels of these hepatic enzymes were noted as indicative that *Vernonia amygdalina* may possess some clearly definable influences or modulating effects on the metabolic functions of the liver in rabbit.

Key words: Hepatic enzymes, increase, rabbit, serum level, *Vernonia amygdalina* extract

INTRODUCTION

Vernonia amygdalina is the botanical name of the shrub commonly known as bitter leaf in local parlance in most communities in West Africa, Asia and Middle East. The shrub belongs to the plant family *compositae*, and is widely distributed in tropical Africa, Asia, China, the Middle East and South America, where phyto-medicine is upheld and the consumption of a wide variety of herbs is employed in the treatment of several illnesses, in most cases, the medicinal preparations have been noted as polyherbal (Okafor and Anichie, 1983). In addition, many communities especially in Africa and Asia hold on to *Vernonia amygdalina* as important vegetable, a delicacy, hence is widely consumed in staple food. Furthermore, the plant is also believed to be available as one of the important and cheapest source of vitamins and minerals.

The roots and leaves of bitter leaf have been shown to be effective in the treatment of a number of illnesses. For example, in Western Uganda it is applied to induce labour and in the management of retained placenta, post partum bleeding, induced abortion, malaria, bacterial and fungal infections, infertility, colic pain, irregular and painful menstruation etc. (Sade, 2008). In other countries such as Nigeria, the leaves of *Vernonia amygdalina* are

crushed for its bitter taste, and rubbed on the breasts for weaning infants. *Vernonia amygdalina* is also used extensively for other medicinal preparations, like the treatment of diabetes, fevers etc. The leaf decoction of *Vernonia amygdalina* is taken as laxative by some communities. There has also been research that evaluated the use of *Vernonia amygdalina* in its application as important worm expeller in intestinal parasitic infections (Aregheore *et al.*, 1998). These and several other reports from cell culture research and animal model studies, revealed that bitter leaf formula supplement may improve health conditions of immune system of AIDS patients and symptoms such as wasting, nausea, vomiting etc. In Nigeria, these observations have led to the commencement of a multi-center human clinical trials of a similar drug, at the Institute for Pharmacological Research and Development (NIPRD) (WWW. ONLIN ENIGARIA.COM Daily News).

It is obvious from these accounts, that *Vernonia amygdalina* is a plant with a wide range of applications and medicinal uses attributed to it. Not long ago, it was reported that *Vernonia amygdalina* extract is taken in large quantities as a tonic without dose considerations (Sade, 2008) and the shrub is steadily gaining increasing popularity for its many other uses, especially among

indigenous populations of Africa, Asia, China, and many other parts of the world.

The liver is a major internal organ saddled with several metabolic functions, including detoxification of drugs and ingested food substances. Other roles include responsibilities such as synthesis, secretions and excretions etc. Like the drugs, *Vernonia amygdalina* and indeed every other plant material or food substances consumed orally, passes through the intestine, is absorbed into the blood, and is ultimately metabolized by the liver. For all its many and diverse roles, the liver receives large blood supply from the intestine, and the fact that it also contains many enzymes, places on it a wide variety of functions as already mentioned above. It was the aim of this study to assess any alterations in the serum level of two key enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), both of which are sensitive indicators of the functional integrity of the liver, in doses of aqueous leaf extract of *Vernonia amygdalina*.

MATERIALS AND METHODS

The leaves of *Vernonia amygdalina* obtained from private farms in Benin City, Edo state, Nigeria, were sundried for about 5 days after washing and cleaning with water. Care was taken to ensure that no particles of dust or any dirt remained on the leaves before and after the drying. The carefully dried leaves were crushed into powder in a mortar. The powder was soaked in 1000mls of water for 24 h to allow for extraction of the constituents. The solution was then filtered and the filtrate placed in an evaporator at 60°C for 48 h. The complete evaporation of water molecules leaves a sticky and dark greenish extract weighing about 600g. The extract was dissolved in 500 mls of pure water and dose calculations were made based on mg/kg body weight of the animal.

Administration of *Vernonia amygdalina* extract was done orally using an orogastric tube, to ensure that all animals in each test group received the same amount of extract daily.

Preparation of animals: Twenty female rabbits of comparable age, size and weight, were procured from both local farms and the Zoological garden, Department of Zoology, Faculty of Life Sciences of the University of Benin, Benin city Nigeria. The animals were allowed to acclimatize in the laboratory for a period of one week. The baseline parameters (including weight, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), levels) were measured and recorded. The animals were fed with standard livestock feeds, edible grasses and clean water. The animals were organized in cages into 4 groups of 5 rabbits per group. In each group, animals received the dose of the extract of *Vernonia amygdalina* in mg/kg of body weight, for a period of three weeks, as follows: Group A received 400 mg, Group B received 200 mg, and Group C received 100 mg, while the Control group received no treatment with the extract for the same

Table 1: The weekly mean serum aspartate aminotransferase (iu/L) in various groups of rabbits

	Baseline	1 st Week	2 nd Week	3 rd Week
Group A	14.9±1.10	39.4±1.30*	44.0±1.4*	50.7±1.70*
Group B	15.9±1.50	34.2±1.50*	42.9±1.30*	39.8±0.50*
Group C	13.8±1.40	31.4±3.70	35.6±1.30*	39.7±0.50*
Control	14.2±1.40	14.7±1.40	14.3±1.60	13.2±1.50

*: Significant values ($p \leq 0.05$) Mean±SEM are versus baseline control

Table 2: Weekly estimations of serum alanine aminotransferase (mg/dL) in the various groups of rabbits

	Baseline	1 st Week	2 nd Week	3 rd Week
Group A	16.1±0.90	23.8±1.80	29.3±1.80*	45.5±3.90*
Group B	13.6±0.70	24.6±1.60*	32.5±2.10*	36.6±1.40*
Group C	13.2±0.70	21.2±0.70	30.7±1.90*	36.5±1.50*
Control	12.5±0.80	11.7±0.80	13.3±1.20	13.1±1.10

*: Significant levels ($p \leq 0.05$) are Mean± SEM compared with the baseline control

period. All the experiments were carried out between the months of August, September and October, in the secure environment of the laboratory and the animal house of the Department of Physiology, University of Benin, Nigeria.

At the end of each week, blood samples were collected from the marginal ear artery, using hypodermic syringes, needles. Samples were stored in sample containers and taken to the laboratory for analysis of serum levels of alanine aminotransferase and aspartate aminotransferase, using automated spectrophotometric analyzer.

At the end of the experiments, sections of liver tissue from each of the animal groups were fixed in formalin, prepared into slides, and were observed microscopically for any morphological alterations.

RESULTS

The weekly estimations of the serum values of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes, revealed a significantly steady increase in rabbits of Groups A, B and C, compared to the baseline controls as shown in Table 1 and 2, respectively. The observed increase in the serum levels of aspartate aminotransferase (AST) appeared to be more pronounced in rabbits of group A, receiving the bigger dose of 400 mg of *Vernonia amygdalina* (Fig. 1). The magnitude of the observed alterations in the plasma values of these enzymes appeared to be slightly less in respect of alanine aminotransferase (ALT) and this was consistently seen in all the groups of the test rabbits (Table 2).

DISCUSSION

The results of this study clearly indicate definite alterations in the serum levels of the two enzymes during the treatment with varying doses of *Vernonia amygdalina* in the rabbits. The serum levels of the hepatic enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), significantly increased steadily in the rabbits ($p \leq 0.05$) throughout the period of the

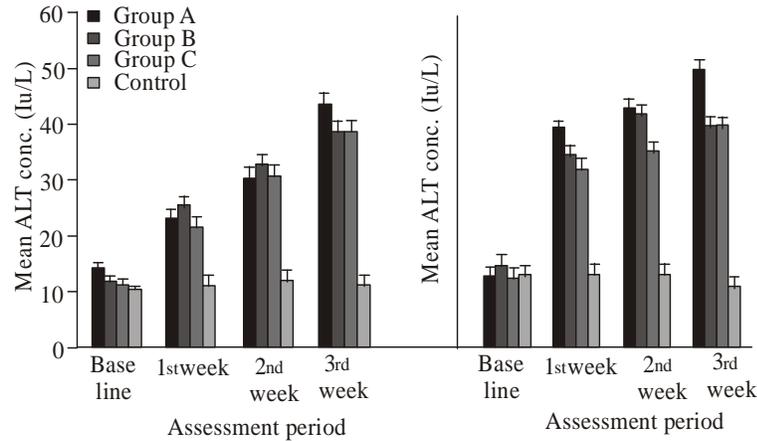


Fig. 1: Comparative chart of the Weekly serum ALT and AST levels during the period of experiments in various groups of test animals

administrations with the doses, and this was seen in all the rabbit groups (Table 1 and 2). Secondly, the observed increase in the plasma levels was in a dose-dependent manner, and this correlates well with the reports of Ojiako and Nwanjo (2006), in a research which earlier speculated on the possible hepatotoxic properties of *Vernonia amygdalina* in rats.

As can easily be deduced from Fig. 1, there is little doubt that the observed rise in the plasma levels of these enzymes was probably of hepatic origin, since these weekly estimations was based on comparison with those of the age and sex matched baseline control. Enzymes are known to be organic catalysts produced by living cells to facilitate the rates of biochemical reactions. Assessment determination of the activity of certain enzymes is an important aid in ascertaining the functional status of the liver. These two enzymes, aspartate aminotransferase and alanine aminotransferase, are known to find use very often by workers, in the assessment study of the functional integrity of the liver.

It is possible that the increases in the plasma levels of these enzymes seen in this study, is probably indicative of some clearly definable modulating effect on the metabolic functions of the liver. On the other hand, it could be indicative of damage to the cytoplasm and or mitochondrial membranes of the hepatocytes, as is known that such damage could be drug induced. Thus, the dose-dependent alterations in the level of alanine and aspartate aminotransferase enzymes seen in this study is most likely indicative of some level of hepatotoxicity of *Vernonia amygdalina*, and this can be attributed to the flavonoids and polyphenols which are known constituents of pure extract of *Vernonia amygdalina* (Atangwho *et al.*, 2009). Such hepatotoxicity could either be by direct action or by hypersensitivity reactions. It has however, been noted that in the case of hypersensitivity reactions, damage to the liver is usually not dose-dependent (Michael, 2003).

Constitutional analysis of *Vernonia amygdalina* has shown that it contains sesquiterpene lactones such as vernodalin, vernodiol and vernomygdin, glycosides like saponin and amygdalin, and flavonoids to which most of its physiologic effects is attributed (Erasto *et al.*, 2006). There are reports that saponins form unstable interaction molecules with proteins, phospholipids and cholesterol on erythrocyte membranes, resulting in membrane fragility and ultimate haemolysis (Assa *et al.*, 1973).

However, the histological study examination of the liver tissues carried out at the end of the experiments in this study, showed no visible indication of fibrosis or alterations in the histology of the cells of the liver, in any of the groups of the test rabbits. Nevertheless, results have shown that ingestion of *Vernonia amygdalina* in high doses and for prolonged periods may result in some toxicity. In other words, it is possible that *Vernonia amygdalina* may possess hepatotoxic properties in large doses, since all drugs and indeed all compounds of plant origin ingested orally for food, are potentially hepatotoxic, as they ultimately undergo metabolism by the liver. There is limited knowledge about the constituents of plants employed in herbal medicines and their effects in humans, especially in poly herbal formulations which are even more in use. Since the use of *Vernonia amygdalina* as vegetable in most staple food is increasingly becoming even more popular, especially amongst rural populations in countries like Nigeria and many parts of Africa, Asia and South America, perhaps further studies may be needed to fully ascertain a clearer picture on the specific constituents of *Vernonia amygdalina*, and the exact mechanism of action, for the present observations. Nonetheless, the present observation of dose-dependent increase in the serum concentrations of these hepatic enzymes is noted in this study as very significant finding and original, since a rise in plasma

aminotransferase activity is a sensitive indicator of damage to the cytoplasm and mitochondrial membranes of the hepatocytes, thus, this observation could represent a reliable pointer to the suspected possible hepatotoxic properties of *Vernonia amygdalina* in earlier reports.

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