
EVALUATION OF AQUEOUS EXTRACT OF VERNONIA AMYGDALINA ON THE HISTOPATHOLOGY OF THE LIVER OF WISTAR RATS

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Abstract: Bitter leaf (*Vernonia Amygdalina Delile*) (VA), family of Asteraceae is a plant that is consumed locally as food and serves important ethno-medicinal uses. It grows throughout tropical Africa to a height of about 1-5 meters and it is indigenous to many West African countries. This study was designed to evaluate the effect of aqueous extract of *Vernonia amygdalina* in Wistar rats. Twelve's Adult Wistar rats weighing 89-106g were divided into three groups of four rats each. Groups 2 and 3 were administered orally with *Vernonia Amygdalina* aqueous extract at 0.2mg/kgBW and 0.4mg/kgBW respectively for 15 days. Group 1 was control and received 0.4ml of normal saline. The effects of aqueous extract of *Vernonia Amygdalina* on the body weight, liver enzyme were evaluated as well as liver architecture. After the end of administration (day 15), the weight were taken before sacrificed. After sacrifice, liver were excised and fixed in 10% formal saline, then processed for rapid routine paraffin embedding. In this research, it was observed that oral administration of aqueous extract of *Vernonia Amygdalina* resulted in significant increase ($P < 0.05$) in body weight. Serum chemistry revealed significant increase ($P < 0.05$) in ALT (alanin aminotransferase), ALP (Alkaline Phosphate) and AST (Aspartate Amino Transferase) in animals treated with 0.2mg and 0.4mg of the extract relative to the control. From the result of this study, it may be concluded that the administration of aqueous extract of *Vernonia Amygdalina* is not toxic to wistar rats at the dose administered.

Keywords: Bitter Leaf, Liver, Wistar Rat, Liver Enzymes, AST

INTRODUCTION

Herbal nutraceuticals and pharmaceuticals are becoming increasingly in acceptance worldwide (Ezekwesili *et al* 2011). Plants, as gift of nature are the ancient form of health-care and have been known and used by mankind throughout history. Plants are generally important to man as they serve great functions as foods, cloths and medicines to sustain life. Plants that are generally effective in the inhibition or control of micro-organisms are mainly used in the traditional medicines and are likely to yield pharmaceutical active compounds

(Hostteman *et al* 1996, Ali- Shayeh and Abu-Ghideib, 1999). *Vernoniaamygdalina* is a valuable medicinal plant that is widespread in West Africa, it is known as bitter leaf due to its characteristic bitter taste and flavour, and can be used as an active anticancer, antibacterial, anti-malarial and anti-parasitic agent. This plant contains complex active components that are useful pharmacologically. In ethno medicine, the roots and the leaves are used to treat fever, hiccups, kidney problems and stomach discomfort. Many West African countries like Cameroon, Ghana and Nigeria use the stem and root as chewing sticks (Challand and Willcox, 2009). *Vernoniaamygdalina* can be commonly found along drainage lines and in natural forests or at home and commercial plantations (Alem and Woldemariam, 2009). VA is a common homestead farming vegetable and fodder tree in Nigeria (Ndaeyo, 2007) and has been used as an ingredient to prepare Nigerian (Ogbono soup) or Cameroon (Ndole) dish after removal of its bitter taste through soaking in several changes of water or by boiling (Abosi *et al* 2003). The widespread use of bitter leaf both as food and medicine in the rural communities call for urgent attention in order to effectively exploit the maximum benefits of the plant and to avoid/ameliorate the adverse side effects that may emanate from such indiscriminate use of the plant. VA has a variety of names in various languages. In English, it is referred to as bitter leaf (Okokon & Onah 2004), in Yoruba, it is known as “Ewuro”; it is called “Etidot”, in Efik, Ijaw and Ibibio. The Igbos call it “Onugbo” or “Olubu”, it is referred to as “Ityna” in Tiv, “Oriwo” in Edo and “Chusa-doki” in Hausa (Egedigwe 2010). The plant grows throughout tropical Africa. It is drought-resistant and thrives in humid environments (Ijeh & Ejike 2011).

It is grown commonly in Benin, Nigeria, Cameroun, Gabon and DR Congo, and to a lesser extent in their neighboring countries. It is a unique plant, so unique that every part of it has an economic importance. Its leaves are macerated and used in cooking soup, while the extracts are used as tonic for prevention of certain illness. *Vernoniaamygdalina* have been shown to be valuable nutritionally. It contains significant quantities of lipids (Ejoh *et al.* 2007), proteins with essential amino acids (Igile *et al.* 1994). It also contains carbohydrates (Eleyinmi *et al.* 2008) and carotenoids, though not in large quantities (Udensi *et al.* 2002). Also contained in this plant are essential elements such as calcium, iron, protein, potassium, phosphorus, manganese, copper and cobalt (Bonsi *et al.* 1995). *Vernoniaamygdalina* also finds applications in the treatment of various ailments. It is a medicinal herb used popularly by traditional practitioners especially in villages. The plant has been shown to be anti-helminths, blood purifier, anti-laxative and anti-malarial. It is also used by scientists in curing joint pains associated with AIDS, diabetes, persistent headache, fever reduction and a host of others. The roots are used for treatment of gastro-intestinal problems, malaria, toothache and fertility problems. It is also used as digestive tonic, appetizer and

febrifuge and for topical treatment of wounds as a substitute for iodine (Momoh et.al). *V. amygdalina* can also be used as a control agent against diseases in plants. The aqueous leaf extract in combination with *Azadirachta indica* leaf extract is the best cure for type 2 diabetes in Nigeria (Eyong *et al.* 2007). Its stem is used as chewing stick. A lot of researches have been done on its leaf extract but the reverse is the case with the roots. The liver develops from an endodermal bud that arises from the ventral aspect of the gut, at the point of junction between foregut and mid-gut. This bud grows into the ventral mesogastrium and passes through it into the septum transversum. It enlarges and soon shows a division into a larger cranial part called the pars hepatica, and a smaller caudal portion called the pars cystic. (Singh, 2012)

The liver is generally and structurally known to be divided into the left and right lobes. The falciform ligamentum teres and ligamentum venosum accounts for this division. However, a more important division of the liver based on its blood supply has been advanced. This division puts the liver in the right and left hemilevers which are further divided into a total of eight segments in line with the hepatic and portal vein. Smaller units made up a central vein, radiating sinusoids separated by a single hepatocyte plate and peripheral portal tracts now form multiple lobules that contribute each segment (Sadler 2010). The liver performs many different functions yet is also a discrete organ and many of its functions interrelate with one another. This becomes especially evident in the abnormalities of the liver because of its functions are disturbed simultaneously. Its functions are vast ranging from metabolism to immune response. The liver regulates most chemical levels in the blood and excretes a product called bile which helps to breakdown fats, preparing them for further digestion and absorption (Sembulingam 2013). The study therefore was designed to find out the effects of aqueous root extract of *V. amygdalina* on the histopathology of Wistar rats.

MATERIALS AND METHOD

Plant Material

Fresh leaves of *Vernonia amygdalina* were harvested in June 2016 from Calabar, Cross River State, Nigeria. The leaves were washed properly and air dried for 14 days, Mashed into powder with mortar and pestle. They were verified by a taxonomist before the commencement of administration.

Experimental Animals

15 adult male Wistar rats were used, weighing between 120g-180g bred in the Animal House of Anatomy Department. They were housed under standard laboratory conditions with a 12 hours daylight cycle and had free access to feed

and water; they were acclimatized to laboratory conditions for three weeks before the commencement of the experiments.

Extract Preparation

The *Vernoniaamygdalina* powder was dispensed 100gm in 1,200ml of distilled water in a plastic rubber after been weighed. The mixture was vigorously stirred intermittently with a magnetic stirred and then allowed to stand for 48 hours, and then filtered through a whatman filter paper tined funnel into a conical flask. The filtrate was evaporated at 37°C with a water bath to obtain the crude solid extract, for 2 weeks. The extract obtained was stored in a refrigerator until required for use.

Experimental Procedure

The rats were all weighed before administration. They were divided into 3 three group; high dose, low dose and control, with 5 rats per cage. 2g of the extract was dissolved in 10ml of normal saline. High dose and low dose group were administered with *Vernoniaamygdalina*, while the control group were administer with normal saline for 14 days. The high dose was given 0.5mg/kgBW of the extract, low dose 0.2mg/kgBW of the extract, and control 0.4ml of normal saline. At the end of the two weeks period, animals in all the groups were sacrificed the day after the end of administration under chloroform anesthesia. Blood was collected through cardiac puncture from the left ventricle into labeled specimen bottles. Serum was separated by centrifugation for 5 minutes at 1000 rpm and used for assay to determine the serum liver enzymes: sodium. The liver of each animal were removed and washed with normal saline. The liver of these animals were also removed, evaluated to ascertain the effect of the extract administered in the liver enzymes which includes Alanine Aminotransferase (ALT), Asparate Amino Transfrase (AST) and Alkaline Phosphates (ALP). Part of these tissues was processed through paraffin sections for Heamatoxylin and Eosin (H[∞] E) and Paraffin acid Schiff methods to stain for glycogen.

RESULT

Effect of Aqueous Extract of *Vernoniaamygdalina* on Body Weight

At the end of the research work, the mean body weight of the animals in the control groups (A) was 107± 0.29g as against its initial weight of 132+ 4.5g, whereas the mean body weight of the treatment groups (B) and (C) were 150+ 3.1g and 148+ 9.4g as against 132+ 1.0g and 145+ 0.8g respectively. The present study shows a significant difference between group (A, B, C **** denote P < 0.05 (figure 1).

Effect of Treatment on the Cytoarchitecture of the Liver

The microscopic examination of section of the liver from the control group which received 0.3ml of distilled water revealed normal cytoarchitecture of the liver with polygonal hepatocytes (h) radiating from the central vein (V). The sinusoids (S) run in between the cords of the liver cells (Plate 1). The photomicrograph of a section of the liver tissue from animals treated with 0.2mg of (Va) leaf extract showed an ephased architectural pattern with degenerated atrophic hepatocytes (H) Portal Traia (PT), and vein (V) (Plate 2). Plate 3: shows the photomicrograph of a section of the liver from the animals treated with 0.5mg of the leaf extract of (Va). It also shows degenerated hepatocytes (H).

Biochemical Analysis

Liver Serum Enzyme Alkaline Phosphate (ALP)

Group A animals showed a normal level of ALP (205.9 ± 3.1). Group B and C animals showed significantly reduced values compared to the control group. (173.6 ± 6.3 u/L) and (129.0 ± 2.1 u/l) respectively (figure 2)

Alanine Aminotransferase (ALT) concentration

Group A animals showed normal level of ATP (12.24 ± 0.57 u/l). Group C animals showed increase concentration which is significant ($P < 0.05$) compared to control group (2.63 ± 0.19 u/l). Group B animals showed ALT concentration significantly increase ($P < 0.05$) compared to control group (8.82 ± 0.58 u/l) (figure 3).

Aspartate aminotransferase (AST) concentration

The effects of treatment on the Aspartate aminotransferase (AST) revealed that the groups that received 0.2ml and 0.5ml of leaf extract showed their concentrations as 22.48 ± 0.55 u/l and 18.72 ± 0.43 u/l. while the control is 12.31 ± 1.53 respectively. This shows that the results from the experimental groups were higher than that of the control group and significant (Figure 4).

DISCUSSION

The present study assessed the biochemical and histopathological effects of aqueous vernoniaamygdalina on the micro-anatomy of the liver and liver enzymes, and to determine the effect on the morphology of the liver, and also evaluating the effect on the microanatomy of the liver on male Wistar rats. The study shows a significant increased ($p < 0.05$) in all the groups on body weight gained. The present study also shows the microscopic examination of section of the liver from the control group which received 0.3ml of distilled water revealed normal cytoarchitecture of the liver with polygonal hepatocytes (h) radiating from the central vein (V). The sinusoids (S) run in between the cords of the liver cells. It was also observed that the photomicrograph of a section of the liver tissue from animals treated with 0.2m b of (Va) leaf extract showed an ephased architectural

pattern with degenerated atrophic hepatocytes (H) Portal Traia (PT), and vein (V). And also shows the photomicrograph of a section of the liver from the animals treated with 0.5ml of the leaf extract of (Va). It also shows degenerated hepatocytes (H). However, the findings of this study agrees with that of several researchers that demonstrated that V. Amygdalina extracts have an antibacterial activity against several species of bacteria and even fungi. Therefore, the efficacy of these extracts as reported by Ogundare, (2011) may be due to the age of the plant, solvent extraction, extraction method and the period of harvest of plant materials. Serum AST and ALT are sensitive indicators of liver damage or injury. The ratio of AST to ALT can be useful in differentiating between the causes of liver damage and elevated levels of AST are not specific for liver damage (Giboney, 2005). In this research work, it was observed that there was marked and significant increase in the activities of ALT. ALT is a cytoplasmic enzyme and increase in plasma, is an indication of mild injuries caused by chemicals to the liver (Orlu and Oblor 2014). AST is a mitochondria enzyme whose increased activity in plasma reflects severe tissue injuries (Ogbuleka, 2016). ALP comes mainly from the cells lining bile ducts but also in bones. It is an enzyme that transports metabolites across cell membrane (Giannini, 2005). In the treated animals, this indicates that the extract may have an adverse effect on the liver enzymes as reported by Palmar (2004) who stated that below normal values of liver enzymes may suggest liver dysfunction or insufficient protein intake. This work is in agreement with the research study of Awe and Kolawale (2013) and who reported significant elevation in the activities of serum ALT and ALP and insignificant changes in plasma AST implicating the actions of cyanogenic glucosides, linamarin and lotaustralin the toxic component of *Manihotesculenta* extract.

CONCLUSION

From the results, it can be deduced that the leaf extract caused no noticeable effect on the liver of rats evaluated compared to the control. Consequently, these plant leaf extracts possessed high index of safety and the continual use are advocated among the rural and urban population.

REFERENCES

- Ali-Shayeh, M.S and Abu-Ghideib, S.I (1999). Antifungal activity of plant extracts against Dermatophytes. *Mycoses* 42:665-672.
- Bonsai, M. L., Osuji, P. O., Tuah, A. K. & Umunna, M. N. (1995), "Vernonia amygdalina as a supplement of teff straw (*Eragrostis tef*) fed to Ethiopian Menz sheep", *Agroforestry Systems* 31(3), 229-241.

- Challand S, Willcox M (2009) A clinical trial of the traditional medicine Vernoniaamygdalina in the treatment of uncomplicated malaria. *J Altern Complement Med* 15: 1231-1237.
- Egedigwe, C. A. (2010), "Effects of Dietary Incorporation of Vernoniaamygdalina and Vernoniacolorata on Blood Lipid Profile and Relative Organ Weights in Albino Rats", M.Sc. Dissertaton, Department of Biochemistry, Michael Opara University of Agriculture Umudike, Nigeria.
- Ejoh, R. A., Nkonga, D. V., Innocent, G. & Moses, M. C. (2007), "Nutritional components of some nonconventionalleafy vegetables consumed in Cameroun", *Pakistan Journal of Nutrition* 6, 712-714.
- Ejoh, RA; Nkonga, DV; Inocent, G; Moses, MC, (2007). Nutritional Components of Some Non Conventional Leafy Vegetables Consumed in Cameroon. *Pak J Nutr*, 6(6): 12-717.
- Eleyinmi, A. F., Sporns, P. & Bressler, D. C. (2008), "Nutritional composition of Gongronemalatifolium and Veronica amygdalina", *Nutrition and Food Science* 38, 99-109.
- Eyong E.U., Attangwho I.J, Ebong P.E., Eteng M.U., and Obi A.U., (2007). Effect of Vernonia Amygdalina Del on kidney function of Diabetic Rats. *International Journal of Pharmacology*, 3:143-143
- Ezekwesili, Chinwe Nonyelum, Obidoa, Onyechi, Nwodo, Okwesili Fred Chiletugo, Ezekwesili-ofili, Josephine Ozioma (2011). Toxicity of AcelyphaTorta (Muell) Leaf Ethanolic Extract in mice and rat. *Animal Research International*, 8(1): 1315-1322.
- Giannin E G *et al*: liver Enzyme Alteratio; A Guide for clinicians: (MA) 172 (3): 367-379
- Giannini E. G., Roberto T. and Vincenzo S. (2005). Liver enzyme alteration: a guide for clinicians. *Canadian Medical Association Journal* 172(3):10-15
- Giboney PT (2005) "Mildly elevated liver transaminase levels in the asymptomatic patient". *America Family Physician* 71(6) 1105-10.
- Hostteman, J.L., Wolfender, S., Rodriguez, S. and Marston A (1996). Strategy in the search for bioactive plant constituents in chemistry, biological and pharmacological properties of African medicinal plants University of Zimbabwe pubt. Zimbabwe Pp 21-42:
- Igile, G. O., Oleszek, W. I., Jurysta, M. R., Burda, S. I., Farifunso, M. E. & Fansanmade, A. A. (1994), "Flavonoids from Vernoniaamygdalina and

Obeten, K.E., Ujong G. O, & Adoga M. O.

their antioxidant activities”, *Journal of Agricultural and Food Chemistry* 42, 2445-2448.

Ijeh, I. I. & Ejike, C. C. (2011), “Current perspectives on the medicinal potentials of Vernonia amygdalina”, *Journal of Medicinal Plant Research* 5(7), 1051-1057.

Inderbir Singh G P Pal (2012). Human Embryology (9th ed). 176-177 Medical publisher, New Delhi.

Kolawole S, Awa E (2013) Phytochemical Composition and Antifungal Actions of aqueous and Ethanolic Extracts of the Peels of 2 Yam Varieties. *Med Aromat Plants* 2:128

Ogundare A. O. (2011). Antibacterial properties of the leaf of *Vernonia amygdalina*, *Ocimum gratissimum*, *Corchorus olitorius* and *Manihot palmate*. *Journal of Microbiology and Antimicrobials* 3(4):78-80

Palmer M. (2004). Guide to Hepatitis and Liver disease: A practical guide to understanding, treating and living with hepatitis and Liver. Revised Edition. Pp 23

Sadler T.W (2010) Medical Embryology (11th ed) 221-223

Sembulingam, K., and Sdembulingam. P. (2003). Essential of medical physiology. Jaypee Brother Medical publisher, New Delhi.

Udensi, E., Ijeh, I. & Ogbonna, U. (2002), “Effect of traditional processing on the phytochemical and nutrient composition of some local Nigerian leafy vegetables”, *Journal of Science and Technology* 8, 37-40.

Figure 1: Comparison of initial and final mean body weight in the different experimental groups. Values are mean \pm SEM

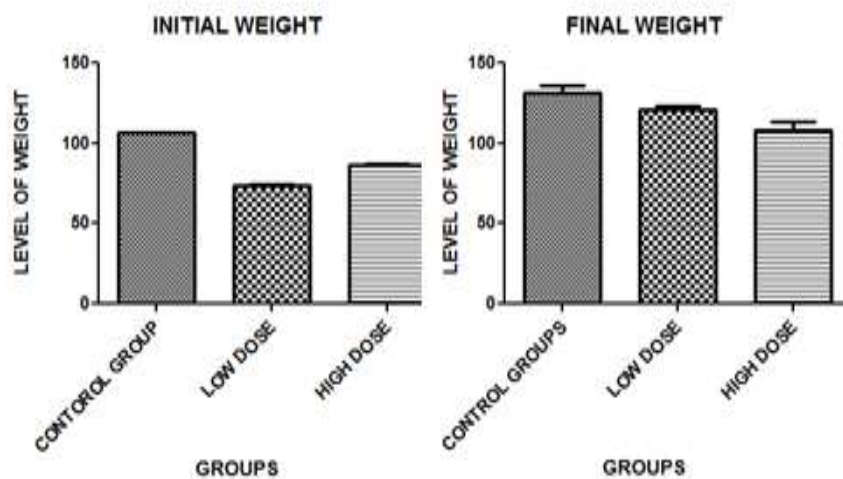


Figure 2: Comparison of serum alkaline phosphatase levels in control and test groups. Values are mean + SEM. * significantly different from control at $p > 0.05$

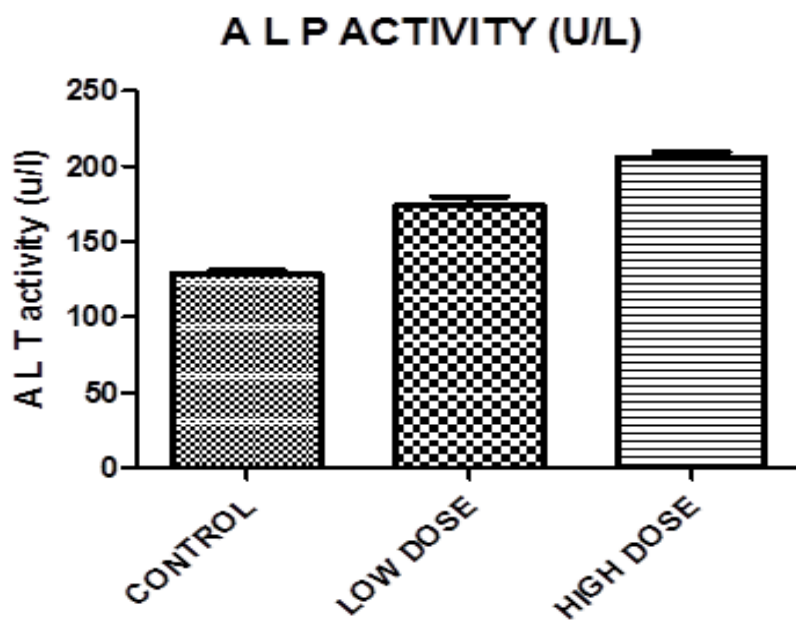


Figure 3: Comparison of serum Alanine Aminotransferase levels in control and test groups. Values are mean + SEM. *significantly different from control at $p > 0.05$

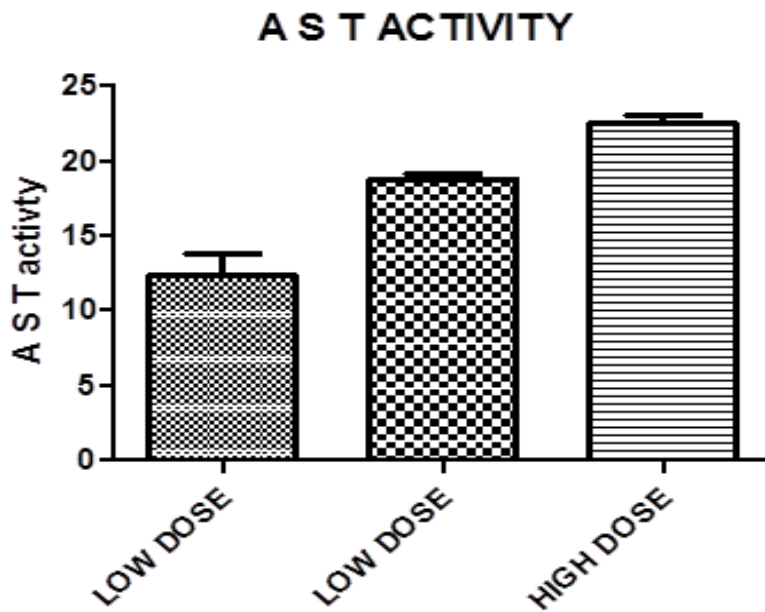
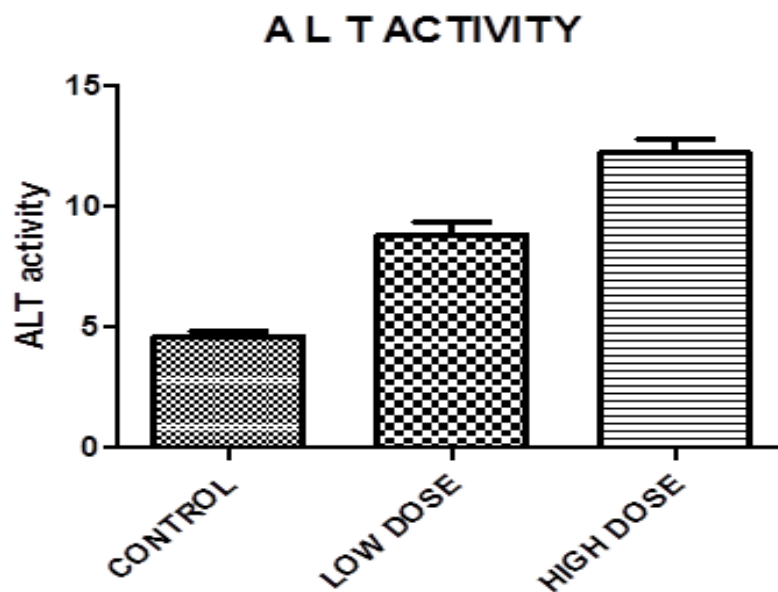


Figure 4: Comparison of serum Aspartate Aminotransferase levels in control and test groups. Values are mean + SEM. *significantly different from control at $p < 0.05$



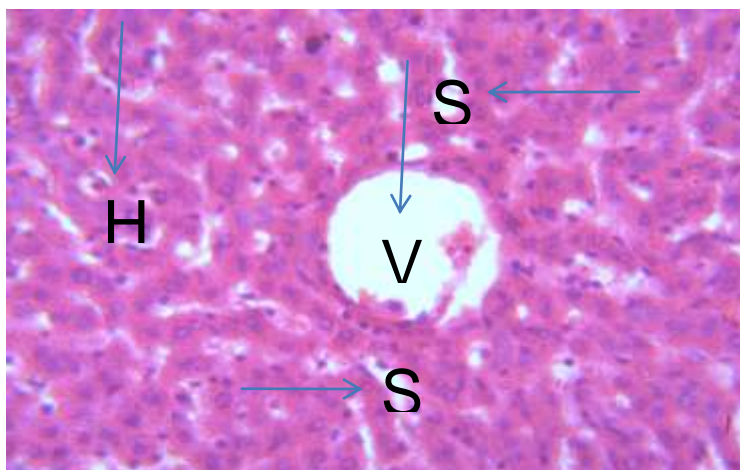


Plate 1: Photomicrograph of the liver from the control group (A) administered with distilled water for 14 days using H & E×400stain shows well defined hepatic vein (V), Hepatocytes (h) and sinusoids(S). (Control group).

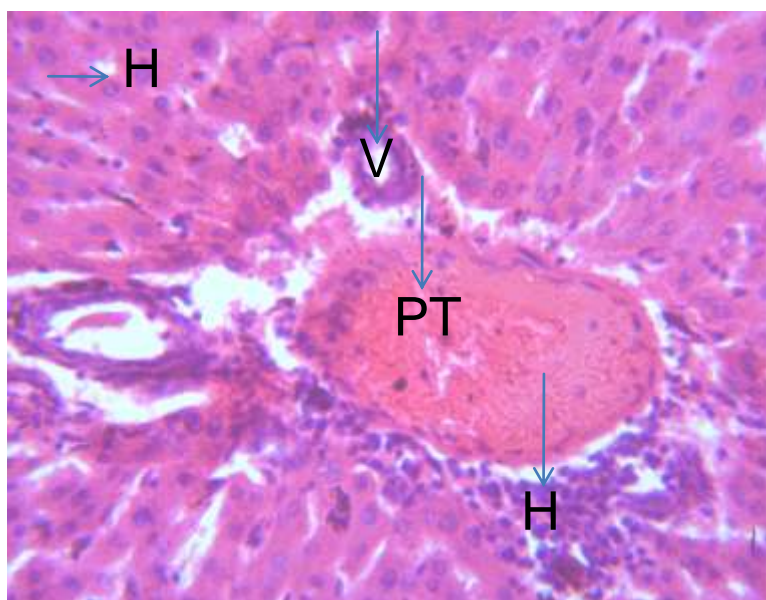


Plate 2: Photomicrograph section of the liver from the group (B) animals treated with 0.2mg of leaf extract for 14 days shows Ephased Architectural and Degenerating Atrophic Hepatocytes (H) using H & E stain (X400)

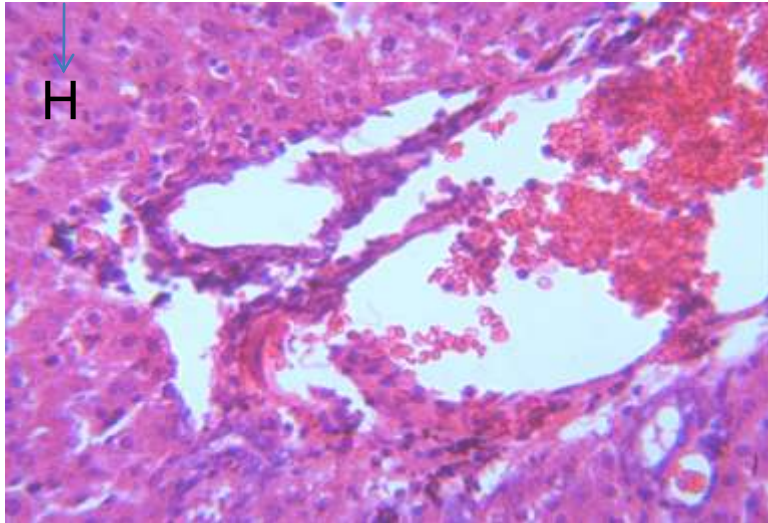


Plate 3: Photomicrograph section of liver from group (C) animals administered with 0.5mg of the extract for 14days showing Atrophic Hepatocytes with Pyknoticnuclei and Ephased Architectural Pattern. Stain H & E (×400)

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