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Hepatorenal Histopathological Morphology Effects of Vernonia Amygdalina Leaf Extracts in Wistar Rats Models

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Abstract
Vernonia amygdalina with local name ewuro (bitter leaf) is a small shrub member of the Asterceae family and is a widely used local plant in Nigeria for both therapeutic and nutritional purposes. Bitter leaf is a natural herb that is useful in taking care of the kidney and the liver. Diabetic patients can use bitter-leaf as one of their medications to prevent high sugar levels in the blood and to repair impaired pancreas. An experimental and observational study was carried out to determine the effects of *Vernonia amygdalina* leaf extract on the kidney and liver of Wistar rats. Twenty (20) rats weighing between 150 to 250g each were randomly distributed into different groups of four rates, both male and female. The first group served as negative control and were given only water and food. 0.5ml, 1.0ml, 1.5ml, and 2.0ml of the extract was given twice daily to group two, three, four, and five respectively. The histological effect of the bitter leaf extract on the liver of the control group showed normal hepatic structure, no inflammatory cell, no significant portal or lobular inflammation nor was there any hepatocyte damage. Groups 2, 3, 4, and 5 showed mononuclear cell infiltrates of the portal cell tracts with absent, minimal, moderate, and severe lobular activity respectively. The histological effect of the bitter leaf extract on the kidney of the control group showed normal renal structure free of inflammatory cells. Groups 2, 3, 4, and 5 showed mild, moderate, severe, and severe infiltration of the interstitium by mononuclear cells. All groups showed normal glomeruli except for Group 5 which showed 25% glomeruli sclerosis.

Keywords: *Vernonia amygdalina,* bitter leaf, rats, Wistar, H&E

Introduction

Vernonia is a genus of about 1000 species of forbs and shrub in the family Asteracea (Wargovich *et al.,* 2001). Some species are also known as ironweed. Some are edible and of economic value. They are known of having intense purple flowers. The genus was named after the English botanist William Vernon. *Vernonia amygdalina* is commonly called bitter leaf in English because of its bitter taste (Erastos *et al*., 2007), which is due to anti-nutritional factors such as alkaloids, saponins, tannins and glycosides (Butter and Bailey, 1973). It typically grows to a height of 2-5m. The leaves are elliptical and up to 20cm long.

The consumption of a variety of local herbs and vegetables by man is believed to contribute significantly to the improvement of human health, in terms of prevention, and or cure of diseases because plants have long served as a useful and rational source of therapeutic agents (Kokwaro *et. al*., 2009). Bitter leaf (*Vernonia amygdalina)* is a bitter plant whose leaves extracts stems, and barks are used for culinary, medicinal and curative purposes, and it is widely used in Nigeria for these purposes (Ijeh *et al.,* 2001).

Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs of the pharmaceutical industry. *Vernonia amygdalina* possesses several bioactive compounds and is used in traditional medicines.

Vernonia amygdalina extracts and isolated chemical constituents have been studied for their potential pharmacological effects, including:

1. Induction of apoptosis as determined in cell culture and animal studies.
2. Enhanced chemotherapy sensitivity - *V. amygdalina* extracts may render cancerous cells to be more sensitive to chemotherapy
3. Inhibition of the growth or growth signals of cancerous cells.
4. Suppression of metastasis of cancerous cells in the body by the inhibition of NFκB is an anti-apoptotic transcription factors as demonstrated in animal studies.
5. Reduction of estrogen level in the body by the suppression of aromatase activity. The involvement of blood estrogen level in the etiology of estrogen receptor (ER) positive breast cancer has been widely reported. Additional source of estrogen production in humans besides the ovary and adrenal gland is the conversion of testosterone to estrogen in a reaction catalyzed by aromatase.
6. Antioxidants - *V. amygdalina* may provide antioxidant benefits.
7. Enhancement of the immune system - Many studies have shown that *V. amygdalina* extracts may strengthen the immune system through many cytokines (including NFκB, pro inflammatory molecule) regulation.
8. Studies conducted using streptozotocin-induced diabetic laboratory animals showed that *V. amygdalina* administration decreased blood glucose by 50% compared to untreated diabetic animals.
9. Extracts of *V. amygdalina* possess *in vitro* anthelminthic anti-parasitic properties (Philipson *et al.,* 1993).

Teas containing bitter leaf (*Vernonia amygdalina*) are also used throughout West Africa for the management of diabetes and other metabolic diseases associated with the liver and kidney.

Phytochemicals in Bitter Leaf

The chemical constituents in bitter leaf include tannin, saponnins, flavanoid and anthraquinone. Physical adsorption mechanism has been proposed from the value of some of the thermodynamics parameters obtained**.**

The tannis are water-soluble polyphenols that are present in many plant foods. The anticarcinogenic and antimutagenic potentials of tannins may be related to their antioxidative property, which is important in protecting cellular oxidative damage, including lipid peroxidation.

Saponins are phytochemicals which can be found in most vegetables, beans and herbs, the beneficial effects on blood cholesterol levels, cancer, immunity booster, reduce bone loss, bone health, antioxidant, and stimulation of the immune system.

The aim of this study was to determine the effect of *Vernonia amygdalina* leaf extract on the kidney and liver of Wistar rats.

Materials and Methods

Study Area and Sampling

The study was carried out at the Medical Laboratory Science Department, Achievers University, Owo, Ondo state, Nigeria. The study population included a random sampling of twenty albino rats of both sexes weighing between 150-250g housed in five groups of four rats each. The research was an experimental and observational study designed for one month.

Sample Collection

The rats were allowed to acclimatize for 2 weeks and fed with commercially formulated rat feed *ad libitum*. 0.5ml, 1.0ml, 1.5ml, and 2.0ml of the extract was given twice daily to group two, three, four, and five respectively. At the end of the treatment, the animals were euthanized under chloroform vapour. They were sacrificed and the kidney and the liver were surgically removed and kept in a deep jar containing 10% buffered formalin solution and used for histological examination.

Preparation of Vernonia amygdalina extract

The leaves of *Vernonia amygdalina* was collected from Owo market in Ondo State and was identified at the Department of Plant Science and Biotechnology, Achiever University, Owo, Ondo state Nigeria.

Fresh leaves of *V. amygdalina* were cut into pieces and air dried for three weeks in the laboratory. The dried pieces were reduced to

powder using a laboratory grinder. Sixty grans (60g) of the dried powdered form of the plant materials were weighed on electronic weighing machine and extracted with distilled water in a Soxhlet apparatus for 72 hours. Another 60g powder of the plant was weighed on electronic weighing machine and extracted with methanol in Soxhlet extractor for 48 hours. All the extracts were concentrated to dryness on a steam bath and weighed. The extracts were then stored in well-closed containers and kept in a refrigerator at 4 ºC to protect from light and moisture till used. The marc from the aqueous extraction was filtered with muslin cloth. The mixtures will be allowed for 48h in the refrigerator at 4^0C for thorough extraction of the plant's active components. These will then be filtered with cheesecloth and later with Whatman No. 1 filter paper to obtain a homogenous filtrate. All the extracts were concentrated to dryness on a steam bath. The extract obtained was weighed prior to further analysis. The powder was divided into two portions. One of the portions was percolated with 80% ethanol while the other was percolated with distilled water (Sukhdey, 2008).

Histopathological investigation

The rats were sacrificed, and the organs removed and immersed in 10% neutral buffered formalin solution for two hours. The organs were removed from the 10% Neutral buffered formalin (NBF) to freshly prepared 10% neutral buffered formalin for a period of one week, for proper fixation and transfer to cut-up room. In the cut-up room, the tissue was cut to the desired size and placed in tissue cassette with proper identification number and transferred to freshly prepared 10% neutral buffered formalin in ratio 1:10 for 1-hour 30minutes.

The organs were fixed (kidney and liver) and processed using Automatic tissue processer machine (Leica, Frankfurt, Germany). The processed tissues were embedded, trimmed at 0µ and sectioned at 5µ using rotary microtome. The sections will be stained with Haematoxylin and Eosin. Stained sections were photomicrographed for report. The specimens were grossed and processed in a 12 hours 30 minutes processing schedule on Histokinette as follows.

Dehydration: Dehydration was carried out majorly in beaker 2-4 on the Histokinette using three beakers of 50% alcohol which are hydrophilic and attract water molecules for 30minutes each from one beaker to another making a total time of 1 hour 30 minutes for dehydration.

Dehydration: This was carried out in beaker 5-7 on the Histokinette using three changes of 80% Ethanol and 20 % IPA (isopropanol) combination for a period of 2 hours 30 minutes.

Clearing: Alcohol was removed from tissues in beaker 8-10 with three changes of IPA for a period of 3 hours which also increases the refractive index of tissues.

Impregnation: IPA was removed from tissues in 11 and 12 with two changes of molten paraffin wax for a period of two hours each which subsequently fill the cavity and harden the tissues.

Embedding: Molten paraffin wax was poured into the moulds using manual method and sections orient in position making the surface to be cut to face downward. The labeled cassettes will stick to the solidifying wax and rapidly cool by placing on ice-block.

Sectioning: The blocks were placed on the block holder of the microtome making sure that the top, bottom and face of the block are parallel to the edge. The blocks will be firmly clamped in position and cutting knife will be inserted tightly into the knife. The section will be trimmed using 10u gauge and ribbons were cut at 5um thickness.

Floating: Cut sections were lifted onto slide with 20% alcohol and gently placed on water bath pre-heated to 45^oc and will be picked from the water bath with the aid of clean grease free slide and tissue section will be oriented at the center of slide. The section will be labelled with the aid of a diamond pencil and be placed on hot plate at 5^oC above the melting point of the paraffin wax for 20 minutes and later arrange in rack for staining using the Haematoxylin and Eosin staining technique (Avwioro, 2014).

Results

The result of Haematoxylin and Eosin staining (H&E) shows that administration of bitter leaf extract caused severe infiltration of the interstitium by mononuclear cells in the tubule of the liver and the kidney. There is a gradation in the degree of damage from severe to mild. Histologically the liver sections of rats administered high doses (3.0ml-4.0ml) showed mononuclear cell infiltrates of the portal tracts with severe lobular activity. Similarly, the kidney sections of the rats administered with high dose (3.0ml-4.0ml) showed severe infiltration of the interstitium by mononuclear cells with about 25% of the glomeruli sclerosed. Also seen are the tubules with their intact lining cells.

Plate 1A and 2A, shows group 1 control liver section shows hepatic lobules composed of hepatocytes arranged in one- cell- thick plates with sinusoids on sides, a centrally located terminal hepatic venule (central vein, long arrow), and peripherally distributed portal tracts (short arrow) which contain hepatic artery, portal vein, and bile duct. No inflammatory cell is seen Shows no significant portal or lobular. inflammation, there is no hepatocyte damage. The control kidney shows normal glomerulus (long arrow), tubule (short arrow) and the interstitium that is free of inflammatory cells. Shows normal glomeruli, tubules with their intact lining cells and blood vessels. The interstitium is free of inflammatory cells, respectively.

Plate 1B and 2B, shows group 2 liver section showing mononuclear cell infiltrates that are limited to the portal tracts (arrow). No lobular activity. Kidney sections show mild infiltration of the interstitium by mononuclear cells. Group 2 kidney also shows a normal glomeruli and the tubules with their intact lining cells.

Plate 1C and 2C, shows group 3 liver section showing mononuclear cell infiltrates of the portal tracts, with minimal lobular activity Kidney section shows moderate infiltration of the interstitium by mononuclear cells. Group 3 kidney section also shows normal glomeruli and the tubules with their intact lining cells.

Plate 1D and 2D, shows group 4 liver section showing mononuclear cell infiltrates of the portal tracts, with moderate lobular activity Kidney section shows severe infiltration of the interstitium by mononuclear cells. Group 4 kidney section also show are normal glomeruli and the tubules with their intact lining cells.

Plate 1E and 2E, shows group 5 liver section showing mononuclear cell infiltrates of the portal tracts (short arrow), with severe lobular activity (long arrow). Kidney section also shows severe infiltration of the interstitium by mononuclear cells with about 25% of the glomeruli sclerosed. Also seen are the tubules with their intact lining cells.

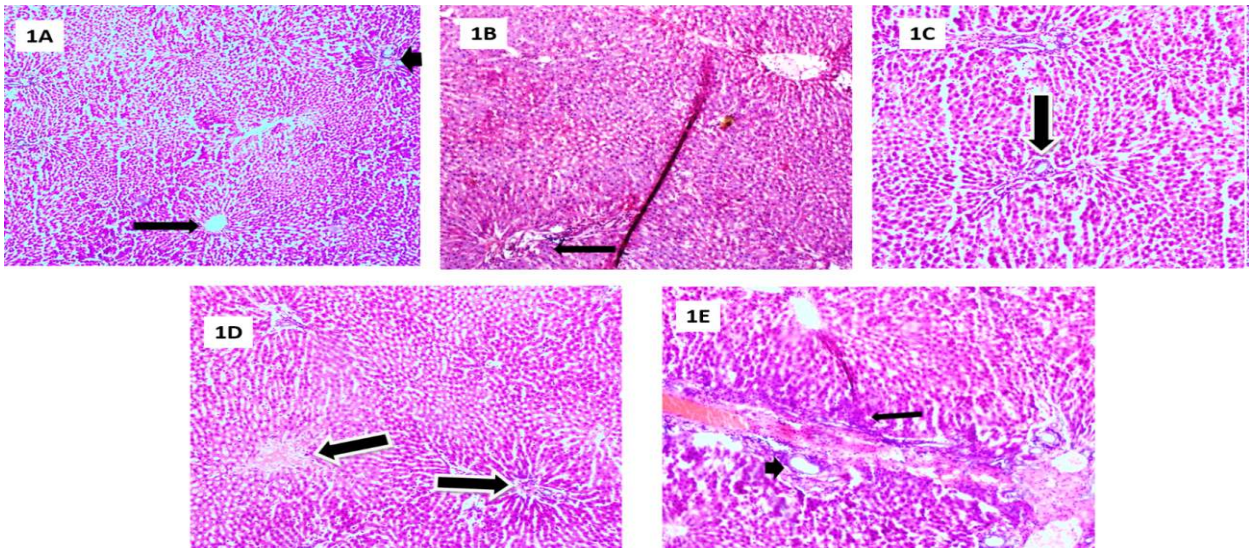

Figure 1: Photomicrographs of Liver sections of the control group and Bitter leaf treated groups

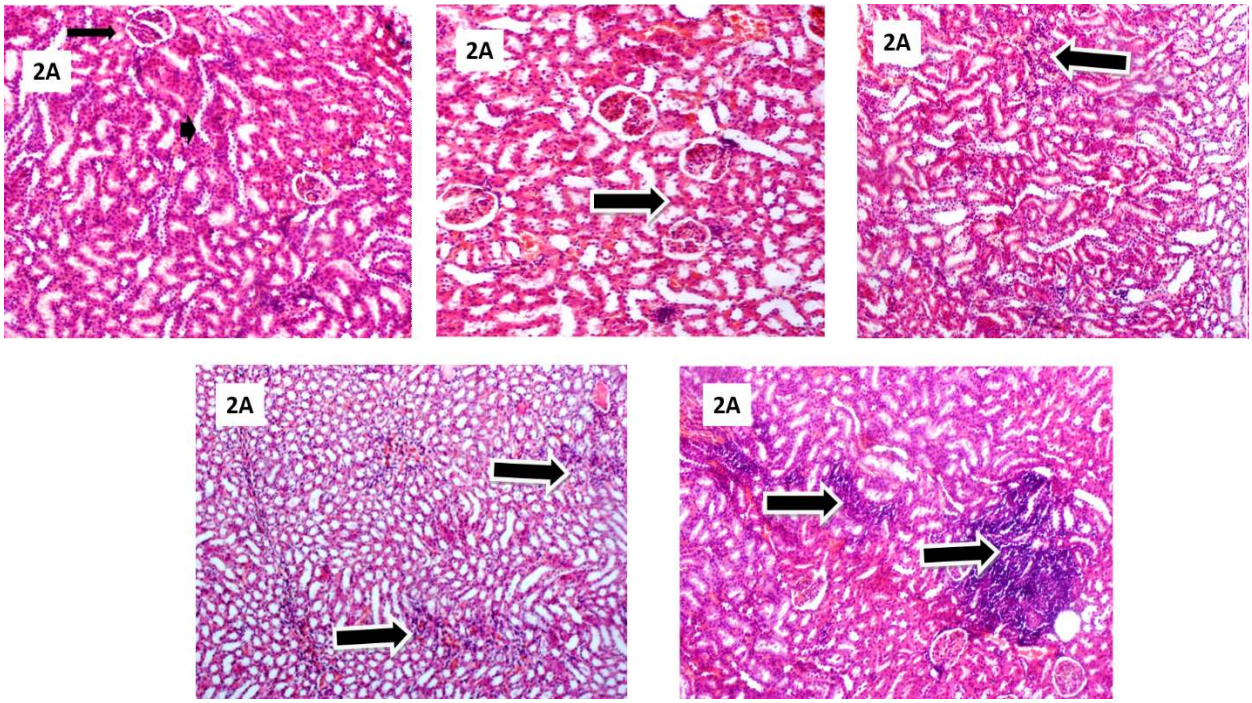

Figure 2: Photomicrographs of Kidney sections of the control group and Bitter leaf treated groups

Discussion

The Liver and kidneys are vital organs of the body in which metabolism and excretion of drugs takes place. This study elucidated any histological changes in liver and kidney tissue of albino rats treated with different concentrations of bitter leaf tonic (1.0 to 4mls) for group two to five while the control (group one) were given pellet and water only. During the study, there was an observable change (anorexia) in the test groups 4 and 5 that was given high concentration of bitter leaf tonic, and no death was recorded.

The rats in group two to three had little or no microscopic architectural changes like those in control group. However the rats in group four and five which were given 3mls and 4mls respectively showed marked microscopic architectural changes in both liver and kidney. These hepatotoxicity and renotoxicity noted with 3.0 and 4mls occurred probably because the therapeutic range (safe consumption range) for bitter leaf tonic was exceeded. However at a dose of 1.0 to 2.0mls it was renoprotective and hepatoprotective probably this was the therapeutic range (safe consumption range). This agrees with a study done by Amole *et al.,* (2006) who reported the effect of 42 days oral administration of *V. amygdalina* to experimental albino rats which did not show significant morphological changes. This is also in consonance with a study done by Ibrahim *et al. (*2000) in which the 65 days chronic feeding of *Vernonia amygdalina* did not produced significant change.

Conclusion

The therapeutic range (safe consumption dosage) of bitter leaf tonic is needed to be identified and it should be emphasized that patient with hepatorenal impairment should avoid bitter leaf tonic or take with caution if needed.

Limitation of the study and recommendations: A further study with a larger sample size is advised.

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