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Effect of Combined Ethanol Leaf Extracts of Vernonia Amygdalina (Bitter Leave) and Gongronema Latifolium (Utazi) on Lead Induced Hepatoxicity in Wistar Rats

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ABSTRACT

This study evaluated the effect of leaf extract of Vernonia Amygdalina and Gongronema Latifolium on the liver of lead-induced toxicity wistar rats. A total of thirty (30) albino wistar rats were used for this study. Five (5) albino wistar rats were used for acute toxicity of the leave extracts of Vernonia Amygdalina and Gongronema latifolium; while the remaining 25 albino wistar rats were divided into 5 groups: Group 1 which served as normal control received only water. Group 2 (NC) negative control received only lead. Group 3 (PC) positive control received lead + Vit. C. (standard drug for lead) via oral administration. Group 4: animals received lead + ethanol fraction of Vernonia Amygdalina and Gongronema Latifolium through oral administration (200 mg/Kg)/. Group 5: animals received lead + ethanol fraction of Vernonia amygdalina and Gongronema Lafifolium via oral administration (400 Mg/Kg). The combined effect of Vernonia amygdalina and Gongronema Latifolium thus has protective and curative properties against lead-induced liver damage.

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Introduction

Africa is a continent known for its rich phylodiversity, with plant materials playing a significant role in improving human and animal health. It is estimated that 80% of the African population depends on medicinal plants to fulfill their healthcare needs. One such medicinal plant is the bitter leaf, scientifically called Vernonia Amygdalina, which is a shrub belonging to the family Asteraceae. This plant has been widely used in African traditional medicine for its numerous health benefits.

The bitter leaf, Vernonia Amygdalina, is a shrub that grows up to 2-5 meters in height. Its leaves are petiolate, with a diameter of about 6 centimeters and an elliptic shape [1]. The leaves are green in color and have a characteristic odor and bitter taste. This plant has been used for centuries in traditional African medicine due to its numerous health benefits [2].

The plant has been domesticated in many parts of Nigeria [3]. It is known by various names in different Nigerian languages, such as "Ewuro" in Yoruba, "Onugbu" in Igbo, "Oriwo" in Benin, "Ityuna" in Tiv, "Chusardoki or Fatefate" in Hausa, and "Etidot" in Cross River State. This plant grows under a range of ecological zones in Africa and is known for its drought tolerance [4].

The leaves of Vernonia amygdalina are widely used as soup condiments in Nigerian cuisine. Before consumption, the leaves are washed to remove their bitter taste. They are also used as vegetables in meals to stimulate the digestive system and are believed to have fever-treating properties [5].

The Vernonia amygdalina contains a wide array of phytochemicals. Studies have reported the presence of oxalate, phytates, and tannins in the plant [6-8]. Vernonia amygdalina extracts have also been shown to exhibit pharmacological properties viz antimalarial and antibacterial properties. Vernonia amygdalina (bitter leaf), though bitter contains essential vitamins such as Vitamin A, C, E, and Vitamin B1 and B2. Other important nutrients found in the bitter leaf are nutrient of fiber, proteins, and minerals such as manganese, Zinc, Iron, Calcium, Potassium, and Phosphorus in traceable amounts.

This plant contains complex active components that are pharmacologically useful. The roots and the leaves are used in medicine to treat fever, hiccups, kidney problems, and stomach discomfort. The stem and root divested of the bark are used as chew sticks in many West African countries like Cameroon, Ghana, and Nigeria. Remedies made from bitter leaf are used in treating 25 common ailments in sub-Saharan Africa, these include

common problems such as fever, and different kinds of intestine complaints, as well as parasite–induced diseases like malaria. Bitter leaf also helps to clean such vital organs of the body like the liver and the kidney. Bitter leaf is also used in the treatment of skin infections such as ringworm, rashes, and eczema.

However, bitter leaves and other vegetables contain both essential and toxic metals over a wide range of concentrations [9].

Gongronema Latifolium commonly known as Utazi in southeastern Nigeria, is a tropical rainforest plant primarily used as a spice and vegetable in traditional folk medicine This plant, belonging to the Asclepiadaceae family, is a shrub with simple and opposite leaves and has a bitter taste due to its phytochemical composition containing saponins [10].

In some African countries, it is used as a spice to support the pancreas. In southern Nigeria, where it is commonly grown, the Igbos call the plant Utazi, the Yoruba know it as arokeke, and the Efik and Ibibio call it Utasi. Both the fruits, seed, leave, root, and bark are all useful [11]. The leaves are believed to neutralize the intoxicating properties of alcohol and its harmful effects on the liver [12].

Polyherbal therapy which is the use of a combination of various agents from different plant sources for therapeutic purposes is a current pharmacological principle and has the advantage of producing maximum therapeutic efficacy with minimum side effects [13]. This enhanced efficacy is thought to derive from phytochemicals endowed in traditional medicinal plants.

Generally speaking, bitter–tasting herbs, such as a bitter leaf, Utazi, Phyllanthus, etc. are known to act as stimulating tonics for the digestive system. They also tend to contain "bitter principles "(a combination of alkaloids, glycosides, tannins, essential oils, etc). They stimulate the flow of bite and appetite for food and they enhance the activities of the pancreas, regulate blood sugar, and promote the detoxification actions of the liver [14].

The metabolism of drugs and other exogenous compounds mainly takes place in the liver. Since the liver is one of the major reservoirs of lead accumulation, lead poisoning can cause chronic liver injury.

The term lead toxicity is a particularly insidious hazard with the potential to cause irreversible health effects. Exposure to lead produces various deleterious effects on the hematopoietic, renal, reproductive, and central nervous systems, mainly through increased oxidative stress.

Lead is a cumulative toxicant that affects multiple body systems and is particularly harmful to young children. Lead in the body is distributed to the brain, liver, kidney and bones. It is stored in the teeth and bones, where it accumulates over time.

Lead toxicity is an important environmental disease and its effects on the human body are devastating. There is almost no function in the human body that is not affected by lead toxicity. Lead is known to cause liver damage by increasing oxidative stress, thus when the liver is exposed to this chemical, the liver could be damaged additively by the same mechanism of oxidative stress. Lead poisoning can be diagnosed through a lead blood test [15].

Vernonia Amygdalina and Gongronema Latifolium are generally used in Nigeria as a vegetable for cooking soups and other local delicacies; also, for medicinal purposes without the full ken of the phytochemical composition and also its effect on the liver. Thus, this study will analyze the effect of natural vegetables on the liver of Wistar rats with hepatoxicity.

Materials and Methods

A total of thirty (30) albino Wistar rats were used for this study and five (5) albino rats were used for acute toxicity (LD50) study. All the animals were purchased from the Animal House of David Umahi University of Health Sciences, Uburu, Ebonyi State. The rats were housed in wooden cages with wire gauze for adequate ventilation and with provision for feed and water, where bedding was replaced every day. The animals were fed with growers' marsh and water obtained from the tap. The guide for the care and use of laboratory animals' procedures was followed in this study (Indian Council of Medical Research 2001). They were acclimatized for two weeks before the commencement of the experiment [16].

Raw data obtained such as body weights, relative organ weights and hormonal results were analyzed using Statistical Package for Social Science (SPSS) version 20, and the results were expressed as mean +- standard deviation.

Ethical Statement

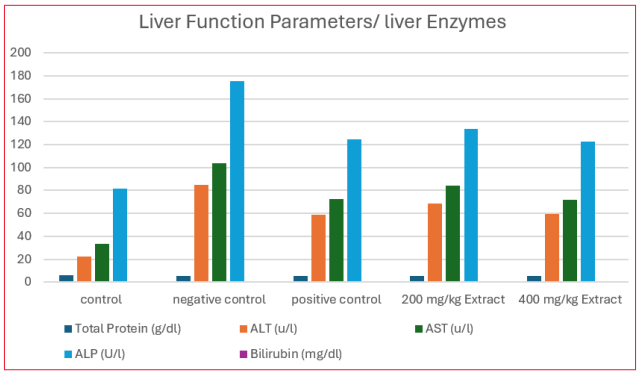
This research was sought and obtained from the Chairman Ethical Committee in the Anatomy Department Faculty of Basic Medical Sciences, David Umahi University of Health Sciences, Uburu Ebonyi State. The experimental procedure of this study complied with ARRIVE guidelines. National Institutes of health (NIH) guidelines, and National Health Research in Body Laboratory Animals.

Results and Discussion

The effect of combined ethanolic leaf extracts of (Vernonia Amygdalina) Bitter leave and Gongronema Latifolium (Utazi) on lead induced Hepatoxicity in Wistar Rats

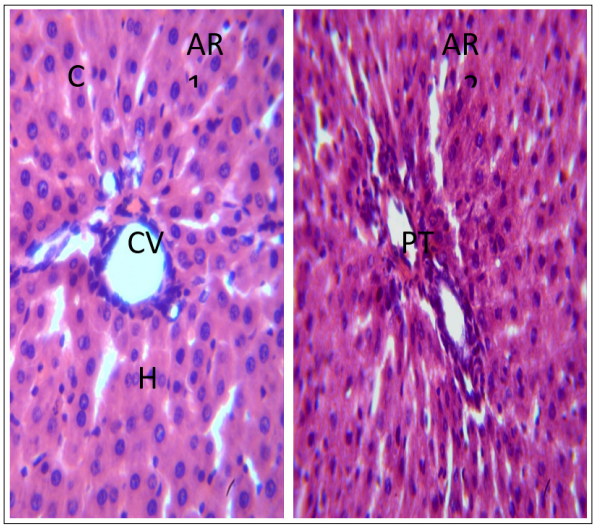
Table 1: Liver Function Parameters

Treatment Groups	Total Protein (g/dl)	ALT (u/l)	AST (u/l)	ALP (U/l)	Bilirubin (mg/dl)
Control	5.9 + 0.28^{d}	22.40 + 2.30^{a}	33.60 + 2.30^{a}	81.80+4.49^{a}	0.54+0.06^{a}
Negative Control	5.09+0.19^{a}	85.00+5.24^{d}	103.60+ 6.88^{d}	175.40+4.68^{d}	0.93+0.04^{d}
Positive Control	5.27+0.08$^{a, b}$	58.60+2.30^{b}	72.40+3.21^{b}	124.40+4.04^{bc}	0.73+0.3^{c}
200 mg/kg Extract	5.40+ 0.14^{bc}	68.40+2.70^{c}	84.20+5.12^{c}	133.80+6.94^{c}	0.73+0.06^{c}
400mg/kg Extract	5.53+0.0.10^{c}	59.60+4.72^{b}	71.80+4.72^{b}	122.60+4.72^{b}	0.63+0.03^{b}



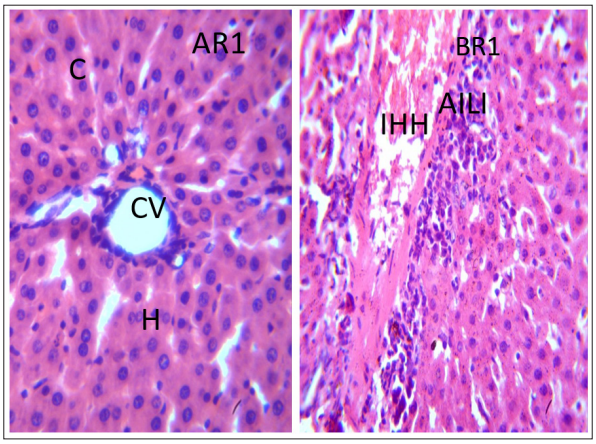
Values are presented as mean + standard deviation (n =4) and values with different superscripts are significantly ($p < 0.05$) different from any paired mean in each column.

Histological Findings



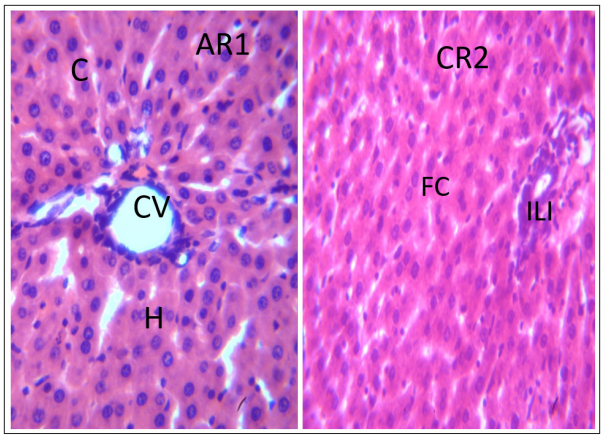
Control (x400) A Plate 1 (x400) B

Plate 1: Photomicrograph of Liver group A (control group) given only water and food showing normal hepatic architecture with portal traid, well perfused cytoplasm (C), hepatocyte (H), portal traid (PT) and central vein (CV) well represented in the micrograph (A & B) H & E stain: (x400)



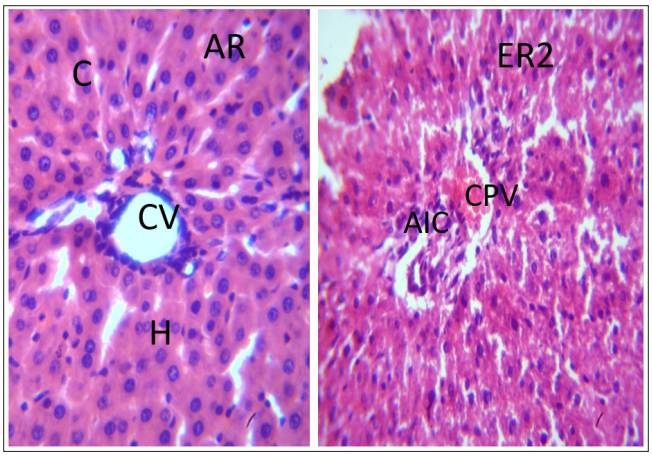
Control (x400) A Plate 2 (x400) B

Plate 2: Photomicrograph of Liver group B given lead only which served as negative control showing moderate to severe degermation with severe fatty change (FC), moderate intra hepatic Inflammation (IHI), and congestion of the central vein (CCV). (A & B) H & E stain: (x400)



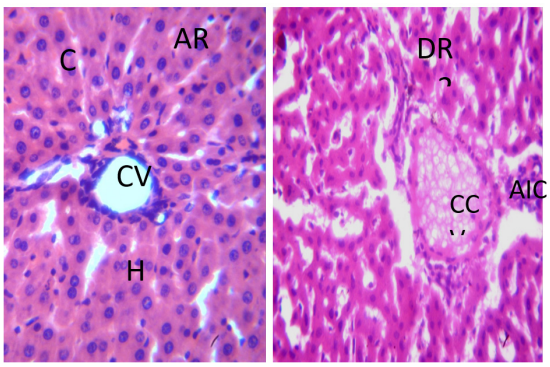
Control (x400) A Plate 3 B (x400)

Plate 3: Photomicrograph of group C given lead and Vitamin C for 28 days as positive control showing mild to moderate regeneration with moderate fatty change (FC) and mild portal inflammation (PI) (A & B) H & E stain: (x400)



Control (x400) A late 4 (x400) B

Plate 4: Photomicrograph of liver group D lead and 200 mg/kg extract of bitter leaf and Utazi for 28 days showing mild to moderate regeneration with moderate fatty change (FC) and mild portal inflammation (PI) (A & B) H & E stain: (x400).



Control (x400) A Plate 5 (x400) B

Plate 5: Photomicrograph of liver group E induced with 400 mg/kg extract of bitter leaf and Utazi for 28 days showing mild to

moderate regeneration with mild portal inflammation (PI) and mild congestion of the central vein (A & B) H & E stain: (x400).

Discussion and Conclusion

Generally, Vernonia amygdalina, also known as bitter leaf, and Gongronema latifolium, known as utazi, are two popular leafy vegetables consumed in Africa. These plants are valued not only for their culinary uses but also for their potential health benefits. Both plants are rich in various nutrients and bioactive compounds that contribute to their medicinal properties [17].

The Vernonia amygdalina and Gongronema latifolium are rich sources of vitamins, minerals, and phytochemicals. They contain essential nutrients such as vitamins A, C, and E, as well as minerals like calcium, potassium, and iron. Additionally, these plants are known to contain bioactive compounds including flavonoids, alkaloids, glycosides, tannins, and saponins. These compounds contribute to the bitter taste of the leaves and are believed to have various health-promoting effects [18].

The liver is considered one of the target organs affected by lead toxicity owing to its storage in the liver after lead exposure. Also, the liver being one of the main organs involved in the storage, biotransformation and detoxification of toxic substances, is of relevance in heavy metal poisoning [19]. Absorbed lead is stored in soft tissues mainly in the liver via the portal vein, so it is the first organ for the histological analysis can be used to examine the morphological changes that reflect possible lead effects on somatic cells [20,21]. Histopathological results used in agreement with the measured activities of serum liver enzymes are being provided supportive evidence for the biochemical analysis in this current study. The histopathological analysis of the liver tissue of the control group showed normal hepatic architecture with the portal trail, central vein and hepatocytes.

Histopathological analysis of the liver tissues of animals treated with lead showed moderate to severe degeneration with severe fatty change, moderate intrahepatic inflammation and congestion of the central vein. The degenerative changes observed in this study following lead treatment showed evidence of cell instability, inflammation and hypersensitivity to the toxicant used. To these findings l, Suradkar et al., and shalom, also observed cell infiltration, dilation of central veins, degeneration of hepatocytes as well and severe fatty change in their studies. These findings relate to high levels of serum liver enzyme activities found in the group treated with lead in the present result [22].

In this study, the photomicrograph of group 3 treated with vitamin C shows moderate regeneration with mild fatty change and mild portal inflammation.

From this study, Group 4 that were treated with 200mg/kg extract bitter leave and utazi showed mild to moderate regeneration with mild portal inflammation and mild congestion on the central vein. In this present study, animals that were treated with lead showed a significant rise in liver enzymes, when compared to the control group. These increasing levels of liver enzymes are mainly due to the leakage of these enzymes from the liver cytosol into the bloodstream. This is being compared to a work by Abdelkadar et al., following the administration of 1000ppm of lead acetate in water to Sprague Dawley rats for weeks. It can be deduced that high levels of these enzymes in the group treated with lead acetate is a sign of cell damage leading to liver dysfunction in treated rats.

Lead is a strong cumulative toxicant that affects multiple body systems. It causes destructive and deleterious effects on the body and tissues of the body generally. Based on observation during this study, the ethanol leaf extract of vernonia amygdalina and Gongronema latifolium possessed very effective hepato-protective potential against lead by reducing the concentration level of liver enzymes.

Thus, the ethanok leaf extract of bitter leaf and utazi has protective and curative properties against lead-induced liver damage.

Conclusion

In conclusion, based on the study's findings, it is recommended that individuals working in lead-based industries consider increasing their consumption of Utazi and Bitter leaf extracts due to the potential protective effects against lead-induced liver damage. However, further research is needed to validate these findings and to gain a deeper understanding of the specific benefits and mechanisms of action associated with these plant extracts.

Recommendation

Following the result of this study, I recommend that people who work in lead-based industries should consume more of these plant extracts (Utazi and Bitter leaf) due to the deleterious effect of lead on the liver and other systems of the body. Further studies of the effects of Utazi and Bitter leaf extracts on the human liver indicates a need for more comprehensive investigations into the potential benefits of these plant extracts. Human trials or in-depth laboratory studies may be necessary to fully understand the mechanisms by which these extracts exert their protective effects on the liver and to determine their overall safety and efficacy in human subjects.

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