Evaluation of Liver Toxicity of Three Different Herbal Bitters (Confam, G. Winco and 1960 Roots) on Wister Albino Rats

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Abstract: This study evaluates the liver toxicity of three different herbal bitters (G. Winco, 1960 roots and Confam) on Wister albino rats. A total of 40 rats were randomly divided into 4 groups labeled A, B, C and D and kept in a well ventilated room. Group A served as control and these rats were treated with distilled water. Wister albino rats in the groups B, C and D were treated with 3 different doses of the bitters (20, 30 and 40mL/Kgbw) respectively. The drugs were administered once daily for 10 and 21 days consecutively. Animals were sacrificed 24 hours after the last treatment. Blood samples were collected into heparinized sample bottles for analysis. There was no significant difference in the results obtained. Alkaline phosphatase and aspartate transaminase was decreased in a non-dose dependent manner in this study. Alanine transaminase was elevated in a dose dependent manner. Histopathological changes were seen in all doses and duration of administration of confam. 1960 roots showed these changes with increasing duration of use and doses. These results further suggest that these bitters cause some degree of hepatic damage and should be used with care, in moderate amounts and with proper monitoring of liver function indices.

Keywords: Herbal Bitters, Hepatic Damage, Wister Albino Rats, Histopathological Changes

1. Introduction

Traditional medicine practice which uses locally sourced and prepared herbs (medical plant) have in recent times in Nigeria, been gaining much recognition and publicity for their solution to different conditions which seem elusive to modern medicine. It is believed by most of the populace that there are little differences in terms of efficacy and safety of modern and traditional medicine. Unfortunately, there is limited scientific evidence to back up this. The rationale for their utilization has rested largely on long-term clinical experience.

The therapeutic use of herbal medicine started about 5000 years ago by the Indian, Egyptians, Greek, Chinese, Roman and Syrian [1]. These products were made from fermentation of honey (meads), fruits and berries (wines) and those from cereals were the first beers [2, 3]. Plants serves as a basic for medical treatment and their different parts have been a source of herbal medicine which has been shown to be effective in about 80% of the population [4, 5]. Herbal medicines are believed to be benign because they are sourced from different plant parts. The problem with their production and use is the lack of regulation by government agencies and proper scientific research guiding the processes involved. The liver is the metabolic powerhouse of the body and is primarily involved in breakdown, storage and providing the building blocks needed by the body [6].

Liver dysfunction usually manifests as inflammation, congestion and increased abnormal fat deposition. The liver is the main site of drug metabolism hence it is necessary to access its integrity in rats that are exposed to these herbal medicines which are not standardized. This research is thus done with the intent of evaluating the toxicity level of these
herbal preparations at different doses and different lengths of exposure.

2. Materials and Methods

Confam bitter used in this study was obtained herbal store in Mile 3 market, Diobu Port Harcourt G. Winco bitter used in this study was got from a herbal store in Mile 3 market, Diobu Port Harcourt. 1960 roots bitter used in this study was gotten from a herbal store in Mile 3 market, Diobu Port Harcourt. Specimen (animal) used for the experiment: forty (40) albino rats were purchased from animal house of the Department of Biochemistry, University of Port Harcourt, Choba Park.

The animals were fed with rat pellets, water and libitum. Chemicals and reagents: all chemicals and reagents used in this study were obtained from Randox Laboratories UK. Preparation of Drug solution for administration: 20ml/kg, 30ml/kg and 40 ml/kg of the preparation was given to the rats each day after weighing depending on their respective groups. Experimental procedure: a total forty (40) albino rats of weight range (124-194g/BW) were randomly divided into four groups labeled A, B, C, D and E where group A served as control and albino Wisterrats (n=2rats/dose) were treated with distilled water.

Rats in groups B, C and D (n=2 rats/dose) were orally treated with 3 different doses of Confam (20, 30 and 40ml/kgBW), G. Winco (20, 30 and 40ml/kgBW) and 1960 (20, 30 and 40ml/kgBW) roots for 10 and 21 days respectively. Animals were sacrificed twenty four (24) hours after last treatment.

2.1. Collection of Blood and Preparation of Serum

The rats were withdrawn from the cages in each of the group twenty four (24) hours after the last administration of the drugs for 10 and 21 days and placed in a desiccator containing cotton wool soaked in chloroform to anaesthetize the rats. The blood samples were obtained by cutting the jugular vein of the rat on the neck by means of surgical blade and put in anticoagulant sample bottles smeared with lithium-heparin and fluoride oxalate. The blood samples were spun at 5000rpm using MSE Centrifuge to obtain plasma. The animal was dissected and only the liver was collected for pathological studies.

2.2. Measurement of AST (SGOT) and ALT (SGPT)

The activities of glutamic pyruvate transaminase and glutamic –oxaloacetate transaminase was analysed according to the method specified by Reitman and Frankel (1957). Measurement of ALP: Plasma alkaline phosphatase activity was measured by the method of Rec (1972).

2.3. Histological Procedures and Analysis

The liver was cut on slabs about 0.5cm thick and fixed in 10% normal saline for a day after which they were transferred to 70% alcohol for dehydration. The tissues were passed through 90% alcohol and chloroform for different durations before they were transferred into two changes of molten paraffin wax for 20mins each in an oven at 57%.

Several sections of the 5µm thick were obtained from a solid block of tissue and were stained with hematoxylin and eosin staining after which they were passed through a mixture of equal concentration of xylene and alcohols, following clearance of xylene, the tissues were oven dried. Photomicrographs were taken with a JVC colour video digital camera (JVC China) mounted on an Olympus light microscope (Olympus UK Ltd Essex, UK) to demonstrate cyto architecture of the liver.

3. Results and Discussion

Results from Figure 1 indicated that aspartate aminotransferase (AST) at 10 days showed variable changes in mean plasma activity levels for animals on confam, significant progressive decrease with increasing drug administration for animals on 1960 roots and no significant change amongst those on G. Winco when compared to the control levels of 36±2.90 IU/L. At 21 days of administration, confam showed a decreasing trend, 1960 roots showed a slight increase with increasing dose and G. Winco showed a decreasing trend.

![Figure 1. Effect of three local bitters (Confam, 1960 roots and G. Winco) on aspartate aminotransferase (IU/L).](image-url)
Figure 2 shows that alanine aminotransferase (ALT) at 10 days of administration showed a similar trend as that seen in AST at 10 days of administration as compared to control of 17IU/L. At 21 days of administration, there was a significant increase at 40ml/kg of confam and a slight increase with increasing dose of administration for both 1960 roots and G. Winco bitters.

Results gotten from Figure 3 indicated that alkaline phosphatase (ALP) at 10 days of administration showed no significant difference for any of the bitters. At 21 days of exposure, variable changes were gotten for confam and G. Winco bitters and a significant decrease for 1960 roots. Values are expressed in means ± standard error of the mean (Figures 1-3).

Result for day 0 (control) represented the control with normal morphology (Figure 4), indicating photomicrograph of liver tissue without herbal bitters. Figures 2-10 shows the photomicrographs of liver tissue treated with different concentrations of herbal bitters (G. Winco, 1960 roots and Confam) on Wister albino rats.

At 10 days of administration confam was the only bitters to show microvesicularsteatosis (intracellular fat deposition) at all doses (20ml/kg, 30ml/kg and 40 ml/kg).
Figure 5. (40-42): Photomicrographs of liver tissue treated with the different herbal bitters on day 10: 40: Photomicrograph of normal liver tissue treated with confam 20ml/kg, 41: confam 30ml/kg and 42: with confam 40ml/kg. 40-liver tissue treated with confam 20ml/kg showed hepatocytes, central vein and microvesicular steatosis (shown by arrows). 41-liver tissue treated with confam 30ml/kg showed hepatocytes, central vein and microvesicular steatosis (shown by arrows). 42-liver tissue treated with confam 40ml/kg showed hepatocytes, central vein and microvesicular steatosis (shown by arrows).

Figure 6. (44-46): Photomicrographs of liver tissue treated with the different herbal bitters on day 10. From left to right: 44: Photomicrograph of liver tissue treated with distilled water, 45: Photomicrograph of liver tissue treated with 1960 roots 20ml/kg, 46: Photomicrograph of normal liver tissue treated with 1960 roots 30ml/kg. 44-liver tissue treated with distilled water showed a sinusoid, central vein and hepatocytes arranged in cords and radiating away from the central vein. 45- liver tissue treated with 1960 roots 20ml/kg showed normal tissue with central vein and hepatocytes arranged in cords and radiating away from the central vein. 46- liver tissue treated with 1960 roots 30ml/kg showed a sinusoid, central vein and cords of hepatocytes.

Figure 7. (47-49): Photomicrographs of normal liver tissue treated with the different herbal bitters on day 10. From left to right: 47: Photomicrograph of normal liver tissue treated with 1960 roots 40ml/kg, 48: Photomicrograph of normal liver tissue treated G. Winco 20ml/kg, 49: Photomicrograph of normal liver tissue treated with G. Winco 30ml/kg. 47- histologically normal liver tissue treated with 1960 roots 40ml/kg showed a sinusoid, central vein and cords of hepatocytes. 48- photomicrograph of histologically normal liver tissue treated with G. Winco 20ml/kg showed a sinusoid, central vein and cords of hepatocytes. 49- photomicrograph of histologically normal liver tissue treated with G. Winco 30ml/kg showed a sinusoid, central vein and cords of hepatocytes.

At 21 days of administration, confam bitters had microvesicular steatosis at all doses, 1960 roots had microvesicular steatosis at 30ml/kg and 40 ml/kg and G. Winco had normal liver cytoarchitecture (Figures 8-10).

Figure 8. (81-83): Photomicrographs of normal liver tissue treated with the different herbal bitters on day 21. From left to right: 81: Photomicrograph of liver tissue treated with G. Winco 40ml/kg, 82: photomicrograph of histologically normal liver tissue treated with confam 20mg/kg and 83: photomicrograph of histologically normal liver tissue treated with confam 30ml/kg. 81- liver tissue treated with G. Winco 40ml/kg showed a sinusoid, central vein and cords of hepatocytes. 82- liver tissue treated with confam 20mg/kg showed mildly distorted tissue, hepatocytes, central vein and microvesicular steatosis. 83- liver tissue treated with confam 30ml/kg showed mildly distorted tissue, hepatocytes, central vein and microvesicular steatosis (shown by arrows).
4. Discussion

From this study, significant results were gotten at 21 days of administration of the bitters. There was no significant difference in the results gotten. However, alkaline phosphatase was decreased in a non-dose dependent manner in this study. This is similar to what was reported by Aniagu et al. [7] who had a significant decrease in ALP. Ogechi and Ibioku [8], on the other hand had elevated ALP readings.

Alanine aminotransferase was elevated in a dose dependent manner in this study. This is same as what was reported by Aniagu et al. [7]. Aspartate aminotransferase was decreased in a non-significant, non-dose dependent manner in this study. Aniagu et al. [7] however reported variable changes but no significant responses. Histopathological changes were seen in all doses and durations of confam bitter administration and increasing concentrations and durations of 1960 roots. ALT is present in higher concentrations in the liver and to a lesser extent in the kidneys, heart, skeletal muscle, pancreas, spleen and lung. Increased levels of ALT are generally as a result of liver disease linked to hepatic necrosis such as cirrhosis, carcinoma, viral or toxic hepatitis and obstructive jaundice.

Typically ALT is generally higher than AST in acute viral or toxic hepatitis, whereas for most patients with chronic hepatic disease, ALT levels are generally lower than AST levels [8-14]. Elevated ALT levels have also been found in extensive trauma, muscle disease, hypoxia, myocardial infarction, circulatory failure and also shock and haemolytic disease. Usually, about 80% of AST is found in the mitochondria whereas ALT is a purely cytosolic enzyme [15].

Therefore, AST appears in higher concentrations in a number of tissues (liver, kidneys, heart and pancreas) and is released slowly in comparison to ALT. But since ALT is localized primarily in the cytosol of hepatocytes, this enzyme is considered a more sensitive marker of hepatocellular damage than AST and is therefore a better marker for liver integrity [16]. This implies that whatever herbal bitters being used, the liver which is the main metabolic hub of the body is affected to some extent and should be appropriately monitored.

5. Conclusion

In all three herbal bitters used in this study, the degree of hepatocellular damage to be expected, as evidenced by the elevated alanine aminotransferase levels and histopathological changes, can be said to be based on the type of bitters used, the dosage of the bitters and the duration of administration of the bitters. The production of these bitters in the Nigerian market should be left to pharmaceutical companies where proper management and monitoring of finished products and effects can be done.
References


