



## Hepatotoxic Effect of Aqueous Leaf Extract of *Millettia Aboensis* on Wistar Rats

Onyegeme-Okerenta Blessing M.\*

Department of Biochemistry, Faculty of Sciences, University of Port Harcourt, PMB 5323, Port Harcourt, Rivers State, Nigeria

Onyeike Eugene N.

Department of Biochemistry, Faculty of Sciences, University of Port Harcourt, PMB 5323, Port Harcourt, Rivers State, Nigeria

**Abstract:** The effect of aqueous leaf extract of *Millettia aboensis* on the liver of Wistar rats was investigated. The experimental animals were divided into six groups based on their body weight. Groups 1 - 5 were administered with 1000, 2000, 3000, 4000 and 5000mg/kg body weight of the extract respectively while the control group was given normal feed and water. The administration of the extract was for 7 days and thereafter, the rats were sacrificed and the effects of the plant extract on the liver investigated. Liver function tests and histological examinations of the liver were carried out. Results for liver enzyme assay showed that aspartate aminotransferase (AST) activity significantly increased ( $p < 0.05$ ) in the different groups as compared to the control group ( $200.1 \pm 3.12$  IU/L). It was observed that enzyme activity increased with increase in the concentration of the extract. Activities of alanine aminotransferase (ALT) for Groups 1 - 5 ranged from  $53.13 \pm 2.12$  IU/L to  $80.67 \pm 0.68$  IU/L. For alkaline phosphatase (ALP), activities of the enzyme for Groups 1 - 5 ranged from  $150.5 \pm 3.00$  IU/L to  $483.2 \pm 1.68$  IU/L. Photomicrograph of rats in Groups 1 and 2 showed normal liver architecture with preserved central vein and normal cytoplasm when compared to the control group. However, photomicrographs of the liver of rats in Group 3 showed moderate distortion and mild degeneration of the hepatocyte. Group 4 showed severe vacuolar degeneration while group 5 showed ballooning and vacuolar changes in the hepatocytes. The results of this study suggest that aqueous leaf extract of *M. aboensis* was hepatotoxic to Wistar rats, and toxicity was dose-dependent.

**Keywords:** Hepatotoxicity; *Millettia aboensis*; Leaf extract; Wistar rats; Photomicrographs.

### 1. Introduction

Traditional herbal medicines are naturally occurring, plant-derived substances with minimal or no industrial processing and used to treat illness within local or regional healing practices. Herbal medicines are generally regarded as safe based on their long-standing use in various cultures [1]. However, there are case reports of serious adverse events after administration of the herbal products [2]. Some of the plants used in herbal medicines can also be toxic when higher concentrations are consumed. On the whole, herbal medicines pose a risk of adverse effects and drug-drug and drug food interactions [3]. Therefore, there is the need to assess the safety or toxicity profile of herbal products in herbal and medicinal research.

Herbal remedies are commonly used by African traditional medicine practitioners and some individuals for the treatment of ailments such as infertility, high blood pressure, diabetes and sexually transmitted diseases. These herbs are either used as a single treatment or in the form of decoction [4]. Research has been focused on finding scientific evidence for the claims as to the therapeutic efficacy of some of these African herbs since eighty per cent of African populations use some form of traditional herbal medicine [5, 6]. China, India, Nigeria, the United States of America (USA) and WHO have all made substantial research investments in traditional herbal medicines [5, 7]. The rationale for utilizing medicinal plants for the treatment of diseases rested largely on the belief that they were easily available and affordable. Regardless of the widespread use of medicinal plants, few scientific studies have been undertaken to ascertain the safety and efficacy of traditional remedies. To determine the safety of drugs and plant products for human use, toxicological evaluation is carried out in various experimental animals to predict toxicity and to provide guidelines for selecting a 'safe' dose in humans. The highest overall concordance of toxicity in animals with humans is with haematological, gastrointestinal, and cardiovascular adverse effects [8, 9].

In herbal medicine, crude plant extracts in the form of infusion, decoction, and tincture are traditionally used by the population for the treatment of diseases. Although their efficacy and mechanisms of action have not been established scientifically in most cases, these simple medicinal preparations often mediate beneficial responses due

to their active chemical constituents [10]. Few plants and their phytochemical constituents have been shown through pharmacological research to have medicinal values. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these chemically active (bioactive) constituents of plants are: alkaloids, tannin, flavonoid and phenolic compounds [11, 12].

*M. aboensis* occurs in the forest zones of Nigeria, Cameroun and Equatorial Guinea. They are mostly tropical trees or shrubs, producing slowly streaked dark-reddish or chocolate-coloured wood. It belongs to the family Fabaceae which contains isoflavones, some of which show molluscicidic and schistomicidic activity. This investigation reports on the effect of the aqueous leaf extract of *M. aboensis* on the liver of Wistar rats.

## 2. Materials and Methods

### 2.1. Collection and Identification of Plant Materials

Fresh leaves of *M. aboensis* were collected from Omuihuechi, Aluu Community- a neighbouring village close to the University of Port Harcourt, Rivers State, Nigeria. The plant was identified and authenticated by a Biotechnologist Dr Edwin Nwosu and a specimen was deposited at the department of Plant and Science Biotechnology for reference purposes with accession number UPH 587 [13].

### 2.2. Extraction of Plant Materials

The leaves of *M. aboensis* were air-dried at room temperature ( $29 \pm 1^\circ\text{C}$ ) for 3 weeks, after which it was ground using Marlex Exceller grinder (Mumbai, India). Fine uniform powder was obtained after passing it through a sieve of 0.5mm pore size.

### 2.3. Chemicals/Reagents

The Reagents used for liver enzyme assays were products of Radox Commercial Kits (United Kingdom). Formalin was used for preservation of the dissected liver.

### 2.4. Methods for Phytochemical Screenings and Gravimetric Analysis

Phytochemical screenings and gravimetric analysis of the aqueous leaf extract and on the powdered samples were carried out using standard procedures to identify the constituents as described by Trease and Evans [14], Sofowara [15]; Rajpal [16]; Payal, *et al.* [17].

### 2.5. Extraction of Flavonoid

Fifteen grams of *M. aboensis* extract with 30ml of distilled water was placed in a water bath to heat, the mixture was filtered when hot and the filtrate was allowed to cool. The filtrate was mixed with chloroform, and then subjected to rapid extraction (twice its volume of chloroform using a separating funnel), chloroform precipitates the flavonoids. The solution was concentrated under reduced pressure to a small bulk, allowed to cool and stand for several days. The crude flavonoids crystallized out and was filtered off and then recrystallized for five times its volume of boiling methanol. This was allowed to stand at low temperature for crystallization to occur slowly.

### 2.6. Extraction of Tannins (African Pharmacopoeia Method)

Ten grams of plant material was extracted with 100ml of distilled water and then filtered. The filtrate was evaporated to dryness to obtain the residue. Fifty milligram of this residue was dissolved in distilled water followed by addition of 0.1gm of the protein and filtered. The filtrate was then dried and weighed and total bound tannins were then calculated by difference. A blank devoid of the test plant sample was determined and used as a correction factor.

### 2.7. Extraction of Saponins

Powdered leaves of *M. aboensis* were extracted by successive extraction in Soxhlet apparatus using petroleum ether and methanol as a solvent. The solvent was removed under vacuum by rotary evaporation, producing dry extracts. The methanol extract was further fractionated with butanol: water (1:1) proportion to get butanol extract which was precipitated in solvent ether to get crude saponins.

### 2.8. Extraction of Cardiac Glycoside

Fifteen grams of dried sample was subjected to grinding in a mill with 30mg of finely powdered ammonium sulphate. The product was passed through a roller mill to bring about intimate mixing; the fluid was then pressed in a hydraulic press. The fluid was separated and the solid material was collected for further processing. To the solid cake material 1litres of chloroform was added and the solution was subjected to stirring for several hours and filtered. The residue was again extracted with chloroform and filtered; the filtrates were combined and evaporated under reduced pressure at temperature of  $35^\circ\text{C}$ . The chloroform was removed by extracting with ether, leaving a green product which is an active compound of cardiac glycosides which was then weighed.

## 2.9. Experimental Animals

Wistar rats of both sexes weighing 154 – 186gm obtained from the Animal House of the Department of Biochemistry, Faculty of Sciences, University of Port Harcourt, Rivers State, Nigeria were used. The rats were allowed to acclimatize in the experimental animal house unit of the Department of Biochemistry, University of Port Harcourt, for 7days, during which they were fed with standard rodent diet. Water was given liberally.

## 2.10. Experimental Design

Thirty Wistar rats of both sexes weighing 154 – 186gm were housed in separate plastic cages and acclimatized for 7days on conventional rat feed. The rats were divided into six groups of five rats per group.

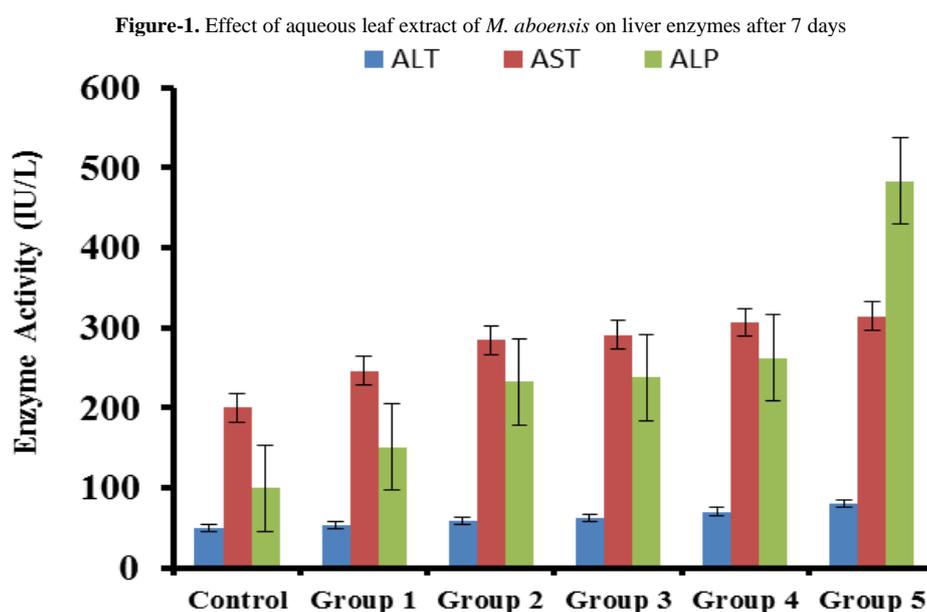
Rats in Groups 1-5 were given aqueous leaf extracts of *M. aboensis* at concentrations 1000, 2000, 3000, 4000 and 5000mg/kg body weight respectively for 7days. Treatment of the rats (Groups 1-5) with the *M. aboensis* aqueous leaf extracts was through oral intubation tube. Each rat group was also adapted to the commercial feed and water *ad libitum* for the 7-day study period. Rats in the control group were also given commercial rat feed and water *ad libitum*. Analysis of liver function parameters was carried out 24hours prior to the administration of the extract to determine the baseline parameter in all the groups. The administration of the extract lasted for 7days and analyses were done on the 8<sup>th</sup> day.

## 2.11. Collection and Analysis of Sample

The animals to be sacrificed were first anaesthetized with chloroform (inhalational anesthesia) followed by cervical dislocation. Each animal was then placed on a dissecting slab and then cut along the thorax down the abdominal region; blood was collected via cardiac puncture and dispensed into the Heparin bottle for biochemical assays (ALT, AST and ALP). ALT, AST and ALP were analyzed by kinetic methods kits from Randox (United Kingdom) using a double-beam spectrophotometer. Freshly dissected liver from each animal was rapidly fixed in buffered neutral formalin (10%). The tissues were subjected to standard routine histological procedures as described by Brown [18]. The slides were viewed using the light microscope and histopathological changes and observations were recorded at X40 magnification identifying both the normal and the degenerated hepatocytes. The results of the study were reported as mean  $\pm$  standard error of mean (SEM) of triplicate determinations. Data were analyzed using one way analysis of variance (ANOVA) and differences were considered significant at  $p < 0.05$ .

## 3. Results

Gravimetric analysis of the leaves shows that Saponin present in the leaves was relatively higher than the other metabolites in percentage which is 20.10%, followed by tannin with 13.58%, cardiac glycoside with 2.10% and flavonoids 1.83%. Results for the assay of liver enzymes (Figure 1) showed that AST activity increased in the different groups as compared to the control group whose AST activity was  $200.1 \pm 3.12$  IU/L. There was a corresponding increase in enzyme activity with increase in the concentration of the extract administered. AST activity increased from  $246.2 \pm 2.05$  IU/L in Group 1 to  $314.5 \pm 3.00$  IU/L in Group 5. For ALT, values ranged from  $53.13 \pm 2.12$  IU/L in Group 1 to  $80.67 \pm 0.68$  IU/L in Group 5 while for ALP values ranged from  $150.5 \pm 3.00$  IU/L in Group 1 to  $483.2 \pm 1.68$  IU/L in Group 5.

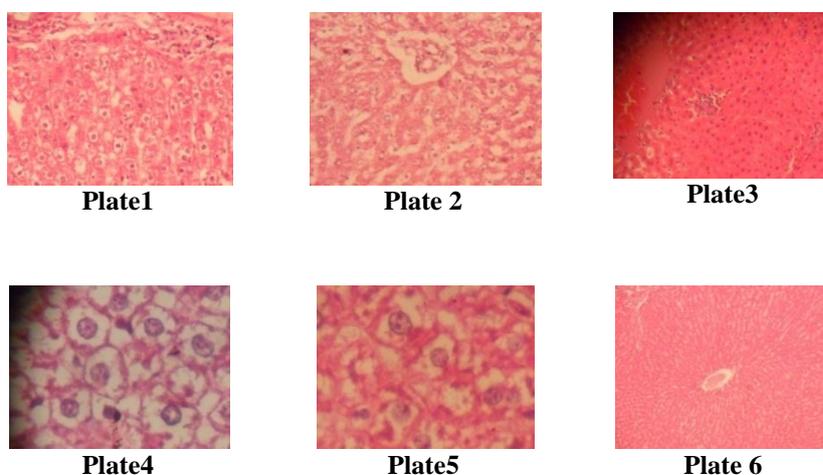


AST – Aspartate Transaminase ALT - Alanine Transaminase ALK - Alkaline Phosphatase

## 3.1. Histological Examination of the Liver

Photomicrographs of hepatocytes for rats in Groups 1 and 2 (Plates 1 & 2) showed normal liver with preserved central vein and normal cytoplasm. Group 3 (Plate 3) showed moderate distortion and mild degeneration of the

hepatocyte; there were inflammatory intraparenchymal liver cells. Group 4 (Plate 4) showed severe vacuolar degeneration of the hepatocytes while Group 5 (Plate 5) showed ballooning and vacuolar changes in the hepatocytes. The photomicrograph of the hepatocytes taken from the control group (Plate 6) showed a normal liver architecture with preserved central vein and normal cytoplasm that was not vacuolated



#### 4. Discussion and Conclusion

Liver enzymes are group of enzymes that give information about the state of a patient's liver [19]. AST, ALT and ALP are important in medical research as they help to determine the extent of liver health and damage. Results from this study showed progressive increase in AST, ALT and ALP activities with increased concentration of *M. aboensis* extract. An elevated level of AST, ALT and ALP helps identify liver diseases or damage and any damage to the liver causes the enzymes from the liver cells to leak into the blood stream and their activity therefore increases [20]. The reason for some of the changes observed had been explained by Singh, *et al.* [21] who reported that high levels of the aminotransferase (i.e. both AST and ALT) indicate the level of inflammation of liver cells due to hepatocyte injury. However, Ugwueze, *et al.* [22] reported that ethanolic and aqueous extracts of *M. aboensis* roots at 431 and 215 mg/kg body weight respectively, showed significant hepatoprotective activity at dose dependent level. Similarly, Attama, *et al.* [23] evaluated hepatoprotective effect of aqueous and ethanolic root extracts of *M. aboensis* on acetaminophen-induced hepatotoxicity in experimental rats and reported that ethanolic plus aqueous root extracts of *M. aboensis* roots showed potential significant hepatoprotective activity at 431 and 215 mg/kg body weight respectively. The effect was dose dependent and could be useful in the attenuation of paracetamol-induced lipid peroxidation.

Histological section of the liver obtained from the experimental rats treated with 1000 and 2000mg/kg body weight *M. aboensis* leaves showed normal liver architecture with preserved central vein and normal cytoplasm that was not vacuolated. However, Groups 3-5 treated with 3000-5000mg/kg body weight showed moderate distortion and mild degeneration of the hepatocyte with Group 5 showing ballooning/vacuolar change (distortion and degeneration) of hepatocyte. This finding suggests that high dose of the extract (above 3000mg/kg body weight) increases health risk. Moreover three of the experimental animals died after day 5 of administration of the extract, one from Group 4 and two from Group 5. This degeneration of the hepatocytes may be due to consumption of a higher dose of the extract and this is in agreement with the findings of Mosaid [24], that higher doses of Henna leaves increase health risk. According to Sofowara [15], medicinal plants have been found to be toxic to the liver at high doses. The degeneration of the liver hepatocyte may also be due to the high percentage of tannins (13.58%) earlier reported by Onyegeme-Okerenta, *et al.* [13]. Stewart and Kim [25] reported that in sensitive individuals, a large intake of tannins may cause bowel irritation, kidney irritation, liver damage and irritation of the stomach. These findings may be related to the fact that the liver plays a significant role in detoxification, and the accumulation of a wide variety of toxic by-products within the liver tends to cause harmful effects.

Aqueous leaf extracts of *M. aboensis* is consumed liberally by locals because of the claimed medicinal or therapeutic benefits. The increased activity levels of AST, ALT and ALP in the plasma and photomicrographs of liver architecture as a result of consumption of higher concentrations of the extract show that hepatotoxicity of aqueous leaf extract of *M. aboensis* was dose-dependent.

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