Hepatotoxicity of Aqueous Leaf Extract of Bridelia ferruginea on the Liver of Albino Rats

Odunlade A. K.1 Taiwo I. A2 Arojojoye O. A3 Lawal T.O.4
1. Department of Biological Science, Yaba College of Technology, Yaba, Lagos State, Nigeria
2. Department of Cell Biology and Genetics, University of Lagos Akoka, Lagos State, Nigeria
3. Department of Biochemistry, Leeds City University Ibadan, Oyo State, Nigeria
4. Department of Statistics, Yaba College of Technology, Yaba, Lagos State, Nigeria

Abstract
The hepatic effect of aqueous extract of Bridelia ferruginea leaves on the liver of albino rats (Rattus norvegicus) was investigated. The rats were fed with their feed (pellets) and clean water and were left for a period of four weeks to acclimatize to their new environment and thereafter the experiment commenced. The rats were grouped into four groups; the control group which did not receive the extract at all and three other groups according to dose of extracts administered orally. There was a steady increase in weight in both control and treated group in the treated group. The alanine aminotransferase (ALT) concentration was a mean value of 10.4 +1.0U/I for the control group while the treated groups were 38.1 + 3.8U/I, 57.7 + 19.3U/I, and 77.6 + 6.0U/I (at the doses of 50, 100, 150 and 200mg/kg weight/day) respectively. The aspartate aminotransferase (AST) concentration had a mean value of 11.5 + 0.5U/I for the control group and 45.6 + 1.3U/I, 44.6 + 4.1U, 41.5 + 2.4U/I and 50.5+3.3 UI (at the doses of 50, 100, 150 and 200mg/kg weight/day). The transaminases (AST and ALT) are well known enzymes used as biomarkers to predict possible toxicity to the liver. Possible damage to liver cells resulted in elevation of both these transaminases in the serum. Furthermore, measurement of enzymatic activities of AST and ALT is of clinical and toxicological importance as changes in their activities are indicative of liver damage by toxicants or in diseased condition. Histological section of the control group had a normal architecture where the central veins,portal traits hepatocytes and sinusoids appear normal. The lobula unit is also well define. However, group rats treated with 50mg/kg/bw and 100 mg/kg/bw showed disintegration of the hepatic cells represented by the separation and disruption of the cells in the tissue with karyolitic nuclei. Also, in rats group treated with 150mg/kg/bw showed extensive area of patchy and confluent hepatocyte necrosis and lobular inflammation

Keywords: Hepatotoxicity, Bridelia ferruginea, Albino rats

INTRODUCTION
The use of plant and their products form medicinal benefits has played a significant role in nearly every culture on earth. However, increasing in the use of some plant extracts has caused damaged to some vital organs in the body due to their toxicity (Richardson, 2001)

Hepato-toxicity (from hepatic toxicity) implies chemical-driven liver damage. The liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity of these agents. Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ. Other chemical agents such as those used in laboratories and industries, natural chemicals (e.g microcystins) and herbal remedies can also induce hepatotoxicity. Chemicals that cause liver injury are called hepatotoxins.

More than 900 drugs have been implicated in causing liver injury (Friedman et al, 2003) and it is the most common reason for a drug to be withdrawn from the market.

Chemicals often cause subclinical injury to liver which manifests only as abnormal liver enzyme tests. Drug induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures (McNally, 2006).

The human body identifies most drugs as foreign substances and subjects them to various chemical processes (i.e metabolism) to make them suitable for removal. This involves chemical transformations to reduce fat solubility and to change biological activity. Though most tissues in the body are capable to an extent to metabolize chemicals, smooth endoplasmic reticulum is the main metabolic clearing house for both endogenous chemicals (e.g drugs) (Donald et al, 2006). The central role played by liver in the clearance and transformation of chemicals also makes it susceptible to drug induced injury.

In the endoplasmic reticulum, is located a group of enzymes known as cytochrome P-450 which is the most important family of metabolizing enzyme in the liver. Cytochrome P-450 is the terminal oxidase component of an electron transport chain. It is not a single enzyme but rather comprises of a family of closely related 50 isofoms of which six of them metabolize 90% of drugs (Skett et al, 2001, Lynch and Price, 2007).

Liver function tests portray a wide range of normal functions carried out by the liver. The transaminases, ALT (alanine aminotransferase) and AST (aspartate aminotransferase), are two closely related
enzymes of great clinical significance especially in the assessment of liver function (Huncrantz et al, 1986). Significant amount of tissue levels of AST are found in skeletal muscles and kidney, lower levels are found in spleen, lungs, pancreas and erythrocytes (Ono et al, 2005) and the highest levels in the heart and levers (Calbeath, 1992).

ALT (Alanine aminotransferase) is present in different concentrations in the liver, heart, skeletal muscle, kidney, pancreas, spleen, lung and blood cells (Sherman, 1991). Both enzymes (ALT and AST) increase in many disorders associated with liver damage and thus have been proven to be sensitive indicators of liver cell injury (Pratt et al, 2000). ALT is higher or more elevated than AST in different neuro-inflammatory conditions of the liver thus pointing out its greater efficacy as a liver disease marker (Rosenthal et al, 1989). Due to their great use as serum markers of liver diseases, the ALT/AST ratio has been accepted to be good indicators of hepatic diseases in adults (Rosenthal & Haight, 1989).

*Bridelia ferruginea* is an indigenous medicinal species in Nigeria. It has different local names given to it by different tribes such as kirni (Hausa), iralodan (Yoruba), Ola (Igbo), Mareni (Fulani). (Kolawole and Olayemi, 2003). Decoction of its stem, bark and leaves are used in the treatment of different ailments and for other commercial purposes. The bark extract of *B. ferruginea* is being used for the coagulations of milk and also in lime juice for making traditional gargle “ogun efu” (Orafidiya et al, 1996). It is also used for purgative and worm-expeller (Cimanga et al., 1997). (Adeoye et al 1999) reported that the bark extract of the plant has antimicrobial activities against some microbes known to cause enteric and secondary upper respiratory tract infection. (Iwu 1984) asserted that the plant possesses molluscidal activities. In the Northern part of Nigeria, the bark is used in treating infections caused by arrow wounds (Irobi et al, 1994).

**MATERIALS AND METHODS**

**ANIMALS FOR THE EXPERIMENT**

Female albino rats obtained from NIMR (Nigerian Institute of Medical Research, Yaba) were used for this study. The experimental animals were sixteen female rats, weighing between 120-160g. They were kept in cages in the animal house of Yaba College of Technology. They were fed with their feed, which is in form of pelleted (obtained from NIMR), once daily and they were also provided with clean drinking water. They were allowed to acclimatize for a period of four weeks before the commencement of the experiment. The animal house was cleaned at least 4 times weekly and the cages were cleaned daily to provide a suitable and germ free environment for them to live in.

**PLANT MATERIALS**

*Bridelia ferruginea* leaves were gotten from the wild in Ibadan, Oyo State, Nigeria and were identified at the herbarium of the University of Lagos. They were air-dried to get rid of moisture and ground to powder afterwards with the aid of a grinding machine and stored in an air-tight container.

**PREPARATION OF PLANT EXTRACT**

This was achieved by aqueous extraction. 50gms of the powdered-leaf of *Bridelia ferruginea* was soaked in 500ml of boiled distilled water and left to stand for 24hrs and filtered thereafter to obtain a solution. The concentration of the crude plant extract was given.

**DETERMINATION OF THE CONCENTRATION OF THE PLANT EXTRACT**

Three evaporating dishes were obtained and labeled A, B and C. They were weighed individually with a weighing balance. 1ml pipette was used to pipette the extract into each of the evaporating dishes which were then placed on a hot – plate and the plant extract was heated to dryness (shown in plate). The evaporating dishes were weighed again and the initial weight (evaporating dish) was subtracted from the final weight (evaporating dish with dry extract). The average was gotten by dividing the final weights of the three evaporating dishes by three and the concentration of the plant extract was determined. The formula used was:

Concentration in g/ml= average weight of dry extract on evaporating dish
Concentration in mg/ml= concentration of extract in g/ml x 1000

**EXPERIMENTAL DESIGN**

The animals were grouped into four groups consisting of four rats each, group 1 was the control group that is the group that did not receive the extract at all. Group II was given the extract of *B. ferruginea* at the dose of 50mg/kg body weight. Group III animals receive the dosage of 100mg/kg body weight and Group IV received the dosage of 150mg/kg body weight. This was achieved through oral administration with the aid of a cannular and syringe for twenty eight days (as shown in plate 2).

The formula for calculating dosage is:

Volume=weight of organism (g) x dosage (mg/kg)

Concentration (mg/ml) x 1000

**BIOLOGICAL AND PHYSIOLOGICAL DATA OF THE EXPERIMENTAL RATS**

The rats were weighed with the aid of a weighing balance periodically at seven days interval for twenty eight days. This was made easy by marking each of the animals in each cage by a V-shaped cut on their ears i.e either up, middle or down part of the left or right ear. The animals were also observed for signs of mortality.
URINALYSIS
The urine of the rats was collected via a metabolism cage and tested with Combi 10, a urinalysis strip, which is used to detect some metabolites in urine. The strip was immersed into the urine and the readings were taken immediately. The essence of this test was to check if the plant extract had any toxic effect on the kidney. The urinalysis was done before and after dosing the rats with the plant extract.

COLLECTION OF BLOOD SAMPLES AND THE ORGANS
At the end of the experimental period, the final body weight of the animals were taken and the animals were sacrificed (Plate 3). This was achieved by putting them, one at a time, in an enclosed anaesthetic chamber with diethyl ether to make them unconscious so that they'll be oblivious to the pain and also for the temporary pumping of blood via their heart to facilitate easy and sufficient blood collection. The blood samples were collected by cutting the jugular vein with a sharp sterile vein. Their livers, kidneys and hearts were removed to determine the body/organ ratio.

LIVER ENZYME ANALYSIS
The biochemical analysis of the liver was carried out with the use of standard kits (Randox, UK) to check the activities of the liver marker enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The blood samples (sixteen) were centrifuged at 1500rpm for 5mins and the supernatant (plasma) was separated with the aid of Pasteur’s pipette. 50ul of plasma was dispensed into each of 32 test-tubes (16 for ALT & 16 for AST) labeled accordingly. The activities of ALT and AST were assayed according to the procedure of Schmidt & Schmidt (1963).

ALANINE AMINOTRANSFERASE (ALT) MEASUREMENT
Plasma (50ul) was deposited in each of the 16 test-tubes. 250ul of reagents 1(R1(buffer consisting of phosphate buffer, L-alanine and α-oxoglutarate)) was added to each of the test-tubes. 50ul of distilled water was added to a test-tube as blank and 250ul of R1 was also added to it. The solution in each test-tube was mixed and incubated for 30mins at 37C. After which 250ul of solution 2 (R2 (2,4-dinitrophenylhydrazine) was added, then the solution was mixed and allowed to stand for 20mins at 20 to 25C. Thereafter, 2.5ml of 0.4mol/l sodium hydroxide (NaOH) was added. The solution was mixed and the absorbance of the samples were read against the reagent blank after 5mins with the aid of a spectrophotometer, using water as blank at wavelength of 546nm.

ASPARTATE AMINOTRANSFERASE (AST) MEASUREMENT
Plasma (50ul) was deposited in each of the 16 test-tubes. 250ul of reagent 1(R1 (buffer consisting of phosphate buffer, L-aspartate and α-oxoglutarate)) was added to each of the tubes. 50ul of distilled water was added to a test-tube as blank and 250ul of R1 was also added to it. The solution in each test-tube was mixed and incubated for 30mins at 37C. After which 250ul of solution 2 (R2 2, 4-dinitrophenylhydrazine) was added, then the solution was mixed and allowed to stand for 20mins at 20 to 25C. Thereafter 2.5ml of 0.4mol/l sodium hydroxide (NaOH) was added. The solution was mixed and the absorbance of the samples were read against the reagent blank after 5mins with the aid of a spectrophotometer, using water as blank at wavelength of 546nm.

Histological Examinations
Small specimens of the organs of liver and kidney were taken from each experimental group, fixed in neutral buffered formalin, dehydrated in ascending concentration of ethanol (70, 80 and 90%), cleared in xylene and embedded in paraffin. Sections of 4-6 µm thickness were prepared and stained with hematoxylin and eosin according to Bancroft et al., (1996)

STATISTICAL ANALYSIS
Comparison of means between two groups was done by Student’s t-test. Analysis of variance (ANOVA) was employed when comparison involves more than two groups. Significant differences between the treatment means were detected at 5% confidence level using Duncan’s Multiple Range Test. Values are expressed as mean ± SEM.

RESULTS AND DISCUSSION
Table I: Effect of different concentrations of Bridelia ferruginea on bodyweight and liver weight.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weigh</th>
<th>Liver weight</th>
<th>Kidney weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>167.2±41.7</td>
<td>7.19±2.05</td>
<td>1.04±5.8</td>
</tr>
<tr>
<td>50mg/kg</td>
<td>157.2±3.8</td>
<td>6.36±1.3</td>
<td>1.13±6.1</td>
</tr>
<tr>
<td>100mg/kg</td>
<td>172.0±41.5</td>
<td>26.19±19.5</td>
<td>1.39±5.1</td>
</tr>
<tr>
<td>150mg/kg</td>
<td>182±2.30</td>
<td>26.6±23.0</td>
<td>2.30±3.0</td>
</tr>
<tr>
<td>200mg/kg</td>
<td>193±2.00</td>
<td>32.3±22.2</td>
<td>4.0±2.0</td>
</tr>
</tbody>
</table>
Table 2: The Effect of Different Concentration of Plant Extract on The Levels Of Alanine Aminotransferase (ALT) and The Levels Of Aspartate Aminotransferase (AST) (U/I)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration of ALT</th>
<th>Concentration of AST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.4 ±1.0</td>
<td>11.5 ± 0.5</td>
</tr>
<tr>
<td>50mg/kg</td>
<td>38.1 ± 3.8</td>
<td>45.6 ± 1.3</td>
</tr>
<tr>
<td>100mg/kg</td>
<td>57.7 ± 19.3</td>
<td>44.6 ± 4.1</td>
</tr>
<tr>
<td>150mg/kg</td>
<td>77.6 ± 6.0</td>
<td>41.5 ± 2.4</td>
</tr>
<tr>
<td>200mg/kg</td>
<td>88.6±5.0</td>
<td>50.5+3.3</td>
</tr>
</tbody>
</table>

Table III: Effect of different concentrations of Bridelia ferruginea on urinalysis

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pre-dosing urine</th>
<th>Post-dosing urine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Density</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>Ph</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Nitrite</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Blood</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Protein</td>
<td>500mg/dl</td>
<td>500mg/dl</td>
</tr>
<tr>
<td>Glucose</td>
<td>Norm</td>
<td>Norm</td>
</tr>
<tr>
<td>Ketone</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Uroglobulin</td>
<td>2 (35)</td>
<td>2 (35)</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Positive, - = Negative

Plate i. Light micrograph of liver treated with distilled water (control)
Plate ii. Light micrograph of liver treated with 50 mg/kg bw of *Bridelia ferruginea*

Plate iii. Light micrograph of kidney treated with 100mg/kg bw of *Bridelia ferruginea*
The mean body weight gain of the *B. ferruginea* extract administered groups (at the doses of 50, 100, 150 and 200mg/kg body weight/day) shows no appreciable difference when compared to the control after the twenty one days duration of study.

The plasma concentration of the liver function parameters are given in Table 2, showing ALT and AST levels in both the control and *B. ferruginea* extract treatment group. The liver function absorbance was gotten by the use of spectrophotometer. The AST and ALT values were derived by the use of a calibration curve.

The alanine aminotransferase (ALT) concentration was a mean value of $10.4 \pm 1.0U/I$ for the control group while the treated groups were $38.1 \pm 3.8U/I$, $57.7 \pm 19.3U/I$, and $77.6 \pm 6.0U/I$ (at the doses of 50, 100, 150 and 200mg/kg weight/day) respectively. The aspartate aminotransferase (AST) concentration had a mean value of $11.5 \pm 0.5U/I$ for the control group and $45.6 \pm 1.3U/I$, $44.6 \pm 4.1U$, $41.5 \pm 2.4U/I$ and $50.5 \pm 3.3$ U (at the doses of 50, 100, 150 and 200mg/kg weight/day).
Bridelia ferruginea is used in the treatment of various ailments and abnormalities. The findings of this study support the need for further investigation on the effect of the aqueous leaf extract of Bridelia ferruginea grown in Nigeria to assess and ascertain its hepatotoxic, anti-diabetic and anti-microbial properties and also the combination of this plant with other medicinal plant extracts on the treatment of various ailments. (Cimmanga et al., 2010)

Histopathological studies of the liver section of control and experimental animals were shown in plate I-V. It was carried out to test the hepatotoxicity effect of the aqueous leaf extracts of Bridelia ferruginea.

The observed increase in the activities of ALT and AST levels in the bridelia-treated rats may be an indicator of liver dysfunction. The increase in the activities of these enzymes may be as a result of their leakage into the blood stream from the cytosol of the liver which indicates the hepatotoxic effect of B. ferruginea. The result obtained from this study supports earlier studies by (Singh et al., 2001) and (Navarro et al., 1993), where administration of the extract of B. ferruginea resulted in increased activities of AST and ALT.

The histology results indicated that Bridelia ferruginea contain toxic phytochemical components and had a direct inhibitory effect on liver microsomal enzymes.

In Nigeria, high intake of medicinal plants can be observed especially in the south-west, B. ferruginea is used in the treatment of various ailments and abnormalities. The findings of this study supports the need for further investigation on the effect of the aqueous leaf extract of B. ferruginea grown in Nigeria to assess and ascertain its hepatotoxic, anti-diabetic and anti-microbial properties and also the combination of this plant with other medicinal plant extracts on the treatment of various ailments. (Cimmanga et al., 2010)

CONCLUSION

Bridelia ferruginea increases the AST and ALT levels in rats and likely in humans as both of their metabolism is similar. This is an indication of liver injury/damage (hepatotoxicity). It may therefore be stated that caution should be exercised in prolonged use of the plant in ethnomedicine. Further studies are therefore needed in human and other experimental animals to firmly establish possible hepatotoxicity activities of Bridelia ferruginea.

RECOMMENDATIONS

Bridelia ferruginea increases the AST and ALT levels in rats and likely in humans as both of their metabolism is similar. This is an indication of liver injury/damage (hepatotoxicity). Further studies are therefore needed in human and other experimental animals to firmly establish possible hepatotoxicity activities of the Bridelia ferruginea.
ACKNOWLEDGEMENT
We thank Mr. Oladele Samuel of Nigerian Institute of Medical Research and Mr. Adebayo Olotu for the typesetting work done and the entire staff and technician of the Animal house of Yaba College of Technology, Yaba, Lagos

REFERENCES
The IISTE is a pioneer in the Open-Access hosting service and academic event management. The aim of the firm is Accelerating Global Knowledge Sharing.

More information about the firm can be found on the homepage: http://www.iiste.org

CALL FOR JOURNAL PAPERS

There are more than 30 peer-reviewed academic journals hosted under the hosting platform. Prospective authors of journals can find the submission instruction on the following page: http://www.iiste.org/journals/ All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Paper version of the journals is also available upon request of readers and authors.

MORE RESOURCES

Book publication information: http://www.iiste.org/book/

Academic conference: http://www.iiste.orgconference/upcoming-conferences-call-for-paper/

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digital Library, NewJour, Google Scholar