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

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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 were 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 100mg/kg/bw showed disintegration of the hepartic cells represented by the separation and disruption of these 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 forn 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 remediea can also induce hepatotoxicity. Chemiicals 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 isoforms 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 anti-microbial 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 pellet (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:

$$\text{Volume} = \frac{\text{weight of organism (g)} \times \text{dosage (mg/kg)}}{\text{Concentration (mg/ml)} \times 1000}$$

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 a-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 a-oxoglutarate)) was added to each of the tubes. 50ul of distilled water was added to a test-tube as blank and 250ul of R1was 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, fi xed in neutral buffered formalin, dehydrated in ascending concentration of ethanol (70, 80 and 90%), cleared in zylene 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.

Treatment	Body weigh	Liver weight	Kidney weight
Control	167.2±41.7	7.19±2.05	1.04±5.8
50mg/kg	157.2±3.8	6.36±1.3	1.13±6.1
100mg/kg	172.0±41.5	26.19±19.5	1.39±5.1
150mg/kg	182±2.30	26.6±23.0	2.30±3.0
200mg/kg	193±2.00	32.3±22.	4.0±2.0

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)

Treatment	Concentration of ALT	Concentration of AST
Control	10.4 ±1.0	11.5 ± 0.5
50mg/kg	38.1 ± 3.8	45.6 ± 1.3
100mg/kg	57.7 ± 19.3	44.6 ± 4.1
150mg/kg	77.6 ± 6.0	41.5 ± 2.4
200mg/kg	88.6±5.0	50.5±3.3

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

Parameters	**Pre-dosing urine**				**Post-dosing urine**			
	Rats				**Rats**			
	1	2	3	4	1	2	3	4
Leucocytes	Ca.25	Ca.25	Ca.25	Ca.25	Ca.25	Ca.25	Ca.25	Ca.25
Density	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ph	9	9	9	9	9	9	9	9
Nitrite	+	+	+	+	+	+	+	+
Blood	_	_	_	_	_	_	_	_
Protein	500mg/dl	500mg/dl	500mg/dl	500mg/dl	500mg/dl	500mg/dl	500mg/dl	500mg/dl
Glucose	Norm	Norm	Norm	Norm	Norm	Norm	Norm	Norm
Ketone	_	_	_	_	_	_	_	_
Uroglobulin	2 (35)	2 (35)	2 (35)	2 (35)	2 (35)	2 (35)	2 (35)	2 (35)
Bilirubin	+	+	+	+	+	+	+	+

+ = Positive, - = Negative

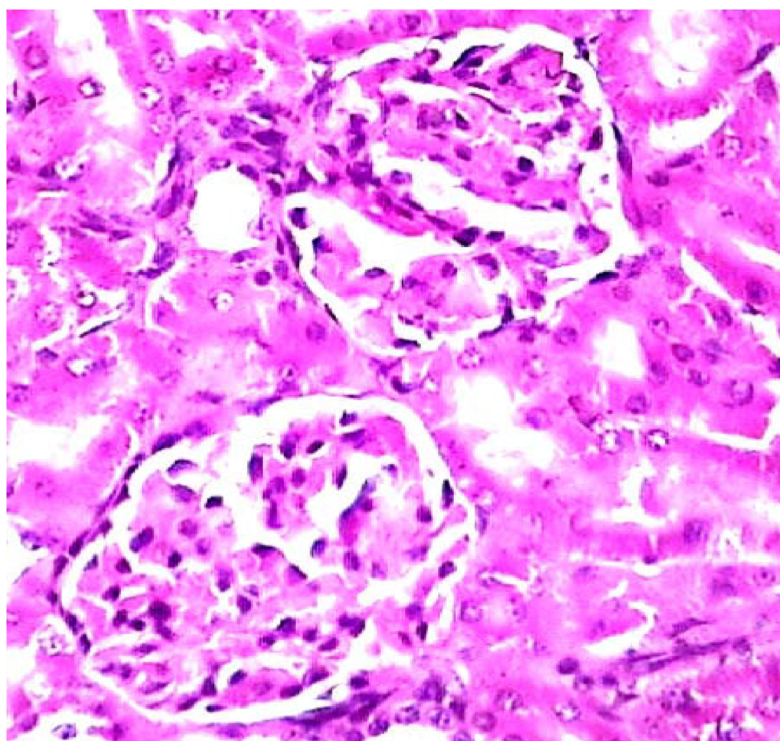

Plate i. Light micrograph of liver treated with distilled water (control)

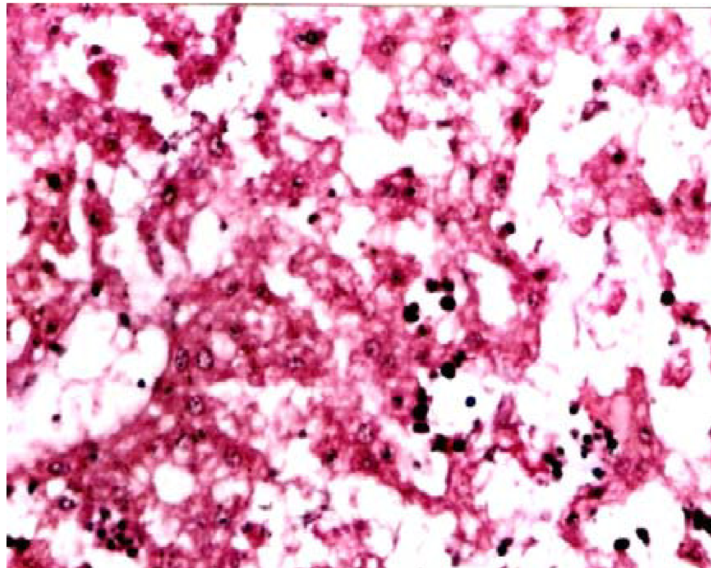

Plate ii. Light micrograph of liver treated with 50 mg/kg bw of ***Bridelia ferruginea***

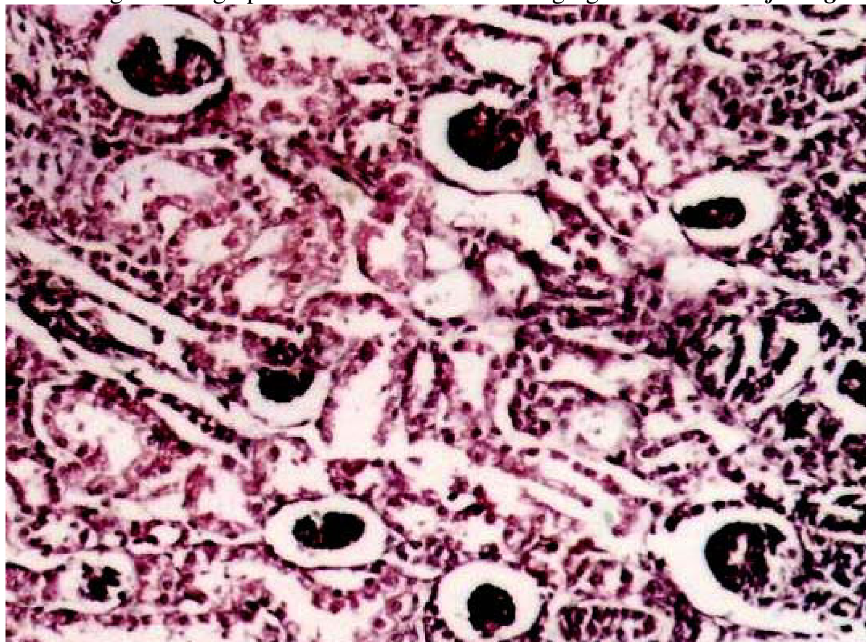

Plate iii. Light micrograph of kidney treated with 100mg/kg bw of ***Bridelia ferruginea***

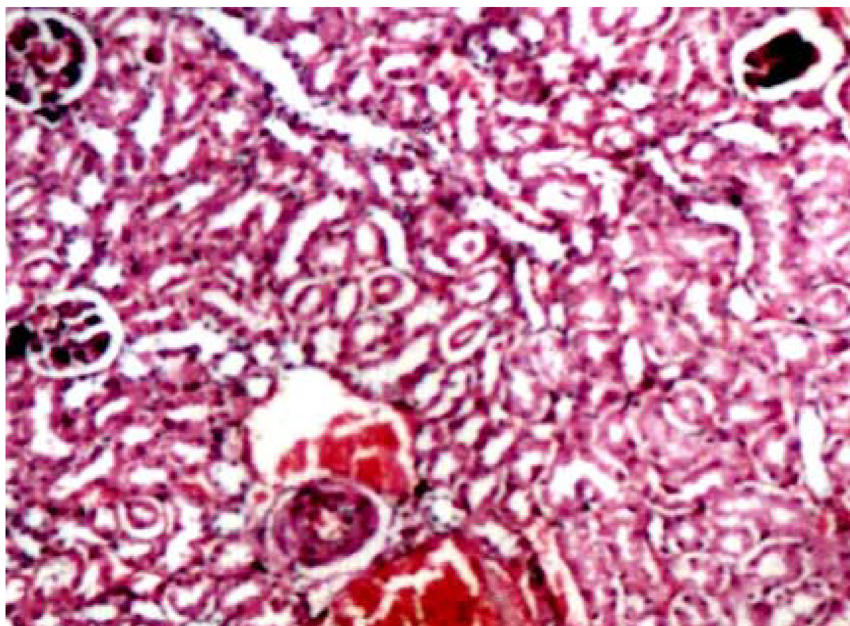

Plate iv. Light micrograph of kidney treated with 150mg/kg bw of ***Bridelia ferruginea***

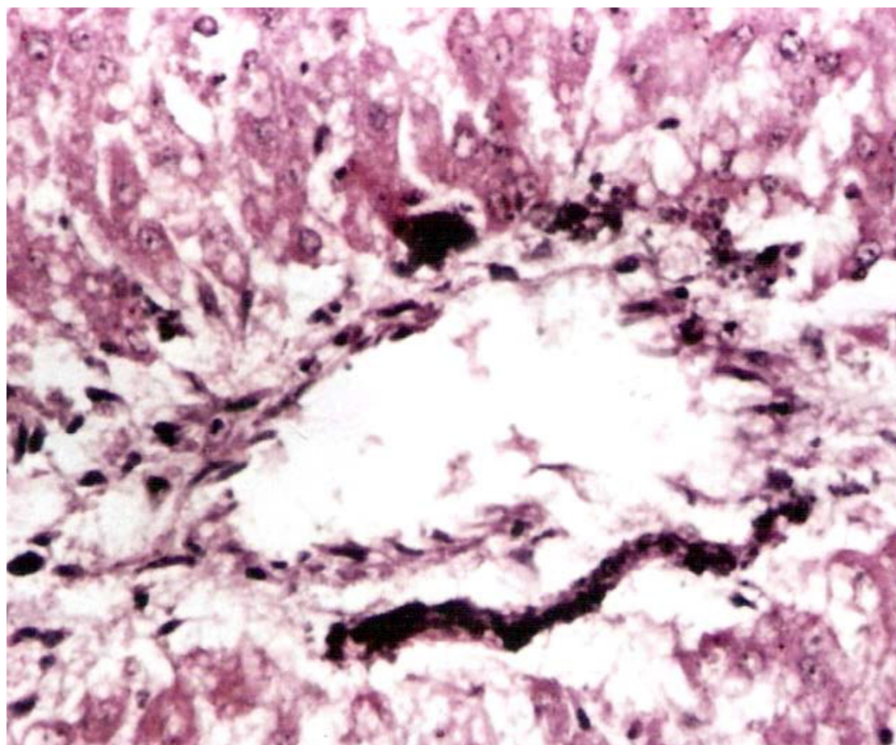

Plate v. Light micrograph of liver treated with 200mg/kg bw of *Bridelia ferruginea*

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 ±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).

There was a significant difference in the ALT and AST level of the treated rats and the control rats ($P<0.05$).

The urine of the rats before administration of the extract had a Ph of 9. There was absence of blood and glucose in the urine. All other factors were normal. This remained the same after administration of extract, thus signifying that the plant extract had no effect on the renal functions of the animals (Table 3).

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

The plate I shows the section of the control group which has normal architecture where the central veins, portal traits hepatocytes and sinusoids appear normal. The lobular unit is also well defined.

However, group rats treated with 50mg/kg/bw and 100mg/kg/bw showed disintegration of the hepartic cells represented by the separation and disruption of these cells in the tissue with karyolitic nuclei.(plate II & III)

Also, in rats group treated with 150mg/kg/bw showed extensive area of patchy and confluent hepatocyte necrosis and lobular inflammation.(plate IV & V)

During the course of the experiment, there was an increase in the body weight, liver weight and kidney weight production of the Rats in the treatment group which indicates the plant probably had some purgative effects. This is in accordance with the work carried out by *Cimmanga et al., (1997)* who described the plant as a purgative.

The transaminases (ALT and AST) are well known enzymes used as biomarkers to predict possible toxicity to the liver *(Rahman et al., 2001)*. They are both associated with inflammation and or injury to the liver cells and this is a condition known as hepatocellular liver injury. Although these enzymes are common liver enzymes because of their higher concentrations in hepatocytes, only ALT is remarkably specific for liver functions since AST is mostly present in the myocardium, skeletal muscle, brain and kidneys *(Witthawasku et al., 2003)*. Generally, damage to the liver cells will result in an elevated level of both of these enzymes. Furthermore, measurement of enzymatic activities of ALT and AST is of clinical and toxicological importance as changes in their activities are indicative of liver damage by toxicants or in diseased conditions *(Navarro et al., 1993)*.

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.

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The histology results indicated that *Bridelia ferruginea* contain toxic phytochemical componenets 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 (hepatoxicity). It may therefore be stated that caution should be exercised in prolonged use of the plant in ethnomadicine. 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*0

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