



Stem Bark Extracts of *Ficus exasperata* protects the Liver against Paracetamol induced toxicity in Wistar Rats

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ABSTRACT: *Ficus exasperata* is an important medicinal plant with a wide geographical distribution in Africa particularly in Nigeria. In this study, aqueous stem bark extracts of *Ficus exasperata* were administered to investigate its hepatoprotective effects on Paracetamol induced liver toxicity in Wistar rats. A total of Twenty Five Wistar rats were randomly divided into five groups labeled A-E and kept in a well ventilated room. Group A served as control and were treated with distilled water. Rats in groups B-E were all treated with Paracetamol (800mg/kg body weight) but rats in group C, D and E were however pretreated with Silymarin (50 mg/kg bw), 100mg/kg bw aqueous stem bark extracts of *Ficus exasperata* and 200mg/kg bw aqueous stem bark extracts of *Ficus exasperata* respectively one hour before Paracetamol administration for fourteen days. Animals were sacrificed twenty four hours after the last treatment. Blood samples were collected into heparinized bottles for biochemical estimation of liver enzymes and the liver was harvested for routine histological examination. Paracetamol produced significant changes in biochemical parameters (increases in serum Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), with a reduction in Total protein) and Liver histology (damage to hepatocytes). Pretreatment with Silymarin and aqueous stem bark extracts of *Ficus exasperata* significantly prevented the biochemical and histological changes induced by Paracetamol in the liver. In conclusion, our histological and biochemical findings indicate that aqueous stem bark extracts of *Ficus exasperata* possesses hepatoprotective properties. © JASEM

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Introduction: The Liver is a key organ in the human body and is a frequent target for a number of toxicants (Meyer *et al.*, 2001). Liver damage due to hepatotoxic agents is of dire consequences, prompting an ever increasing need of an agent which could protect it from such damage. There is a worldwide trend to go back to traditional medicinal plants in the treatment of liver diseases as synthetic drugs used in the treatment of liver diseases have serious adverse side effects and are often times inadequate (Mitra *et al.* 2000).

Ficus exasperata Vahl (Moraceae) is an important medicinal plant with a wide geographical distribution in Africa particularly in Nigeria. (Hallan, 1999; Abbiw, 1990). In Nigeria there are over 45 different species of *Ficus* (Keay, 1964), such as *Ficus glomosa*, *Ficus lecardi*, *Ficus goliath*, *Ficus capensis*, *Ficus ingens* and *Ficus elastica*, which can be found besides rivers and streams and also in the Savannah, rainforest. The leaves are oval, it has elliptic leaves with a very rough surface and are alternately arranged making them look like sand paper. *Ficus exasperata* (Vahl) is commonly known as sand paper tree (“*Ewe Ipin*”) in

Yoruba. Other various local names include; *Anwerenwa* (Igbo), *Erepin* (Yoruba), *Kawusa* (Nupe), *Ameme* (Edo), (Bafor *et al.* 2009).

The leaf extract from *Ficus exasperata* is reported to have diverse uses such as treating hypertensive patients (Buniyamin *et al.* 2007), coughs and haemorrhoid (Odunbaku *et al.* 2008). Numerous pharmacological actions such as lipid lowering, anti-diabetic and antifungal activities have been reported for *Ficus exasperata* (Sonibare *et al.* 2006), and more recently the use of *Ficus exasperata* for treating several problems like difficult child birth, bleeding and diarrhoea in traditional medicine was reported (Ijeh and Agbor 2006). It is also used for treating various infections and as sand paper for polishing woods (Cousins and Micheal 2002).

MATERIALS AND METHOD

Plant Material: The Stem bark of *Ficus exasperata* were collected from the University of Benin environment, in Benin City, Nigeria and was identified and authenticated by Mr. Sunny Nweke of the

Department of Pharmacognosy, Faculty of Pharmacy, University of Benin.

Preparation Of Extract: The stem bark of *Ficus exasperata* were washed with normal saline, air dried at room temperature and protected from direct sunlight for two weeks. The air-dried specimens were then pulverized using the laboratory hammer-mill and the powdered samples were stored in air and water-proof containers until required for extraction. 2.0 kg of the powdered stem bark of *Ficus exasperata* was extracted in distilled water by percolation for 24 hours. The mixture was filtered using Whatmann filtered paper and the filtrate evaporated at 60°C using a vacuum Rotary evaporator. An aliquot portion of the extract residue was stored in a refrigerator and a measured portion was dissolved in distilled water for use on each day of the experiment.

Animal Care: The School of Basic Medical Sciences, University of Benin granted approval before the work commenced. The adult male Wistar rats weighing between 230-260g were obtained and maintained in the Animal Holdings of the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin city, Edo State, Nigeria. The animals were fed with grower's mash obtained from Edo Feeds and Flour Mill Limited, Ewu, Edo State, Nigeria and given feeds liberally.

Drugs And Dosing Schedule: The Wistar rats were divided into five Groups; Group A (Control), Group B (Paracetamol treated), Group C (Paracetamol + Silymarin treated), Group D and E (Paracetamol + Extract). Rats in group A served as the control group and were given distilled water. Rats in groups B were administered Paracetamol only at 800mg/kg body weight per day for fourteen days consecutively via the oral route. Simultaneously but at different hours of the day, rats in groups C, D and E were administered with Silymarin (50mg/kg), aqueous stem bark extracts of *Ficus exasperata* in doses of 100 mg/kg and 200 mg/kg body weight respectively an hour before administration of Paracetamol (800mg/kg body weight, orally) via the orogastric tube for fourteen days consecutively.

Sacrifice of animals: At the end of the experimental period, the rats were grossly observed for general physical characteristics. Rats were sacrificed by anaesthesia with ketamine and a midline incision was made through the anterior abdominal wall. The liver was isolated from the surrounding organs and fixed in

Bouin's fluid for 48 hours for routine histological examination.

Biochemical Estimations: Blood samples were collected from the rats, transferred to anticoagulant sample bottles smeared with lithium-heparin. The blood samples were centrifuged in a Denley BS400 centrifuge (England) for 5 mins at 5000 rpm to obtain serum. Serum obtained was used for estimation of Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Alkaline Phosphatase (ALP) and Total protein (TP) using Random diagnostic kits (Reitman *et al.*, 1957).

Histological Procedure: The liver tissue was processed via the paraffin wax embedding method of Drury and Wallington (Drury *et al.* 1976). Serial sections of 5µm thick were obtained using a rotatory microtome. The deparaffinized sections were stained routinely with Haematoxylin and Eosin (H&E) for light microscopic examination of the liver tissue architecture. Photomicrographs of the results were obtained using research photographic microscope in the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin city, Edo State, Nigeria.

Statistical Analysis: Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS, version 16, Chicago, IL). The data were analysed using descriptive and inferential statistics. All values were presented as mean \pm standard error of mean (SEM). The significance of difference in the means of all parameters was determined using one way analysis of variance (ANOVA; 95% confidence interval). When Analysis of Variance was statistically significant, Least Square difference *post hoc* test was carried out for all groups compared with control group and comparison of all pairs of groups respectively.

RESULTS AND DISCUSSION

Table 1 shows the effects of Paracetamol, Silymarin, and different doses of *Ficus exasperata* extracts on the activity of AST, ALT, ALP and TP respectively in the serum of experimental groups A-E. The activity of AST, ALT and ALP significantly increased ($p < 0.05$) in Paracetamol treated rats (Group B) when compared to the control. The activity of these enzymes is significantly lowered in the Silymarin + Paracetamol treated group C, *Ficus exasperata* + Paracetamol treated groups D and E. Furthermore, the activity of TP is significantly ($p < 0.05$) reduced in Paracetamol treated rats (Group B) when compared to the control group A and treated groups D-E.

Table 1: Effects of *Ficus exasperata* on liver enzymes and serum total protein of rats in experimental groups.

Groups	Group A (Control)	Group B (PCM only)	Group C (PCM + Silymarin)	Group D (PCM + 100mg/kg <i>Ficus exasperata</i>)	Group E (PCM + 200mg/kg <i>Ficus exasperata</i>)
AST (IU/L)	16.75 ± 2.66	67.25 ± 6.66 ¹	19.25 ± 2.14 ²	40.50 ± 3.10 ^{1,2}	30.00 ± 10.98 ^{1,2}
ALT (IU/L)	20.25 ± 1.38	82.50 ± 4.41 ¹	23.00 ± 1.47 ²	31.50 ± 3.23 ²	32.50 ± 8.54 ²
ALP (IU/L)	35.75 ± 2.10	127.75 ± 4.50 ¹	37.75 ± 2.30 ²	53.25 ± 5.76 ^{1,2}	37.00 ± 9.48 ²
TP (Mg/dl)	6.75 ± 0.16	3.90 ± 0.13 ¹	6.78 ± 0.18 ²	5.55 ± 0.06 ²	5.83 ± 0.28 ²

Values are expressed as means (\pm SEM) of 5 rats in each group. ¹significantly different ($p < 0.05$) from control group; ²significantly different ($p < 0.05$) from Paracetamol (PCM) group B

Histology Results

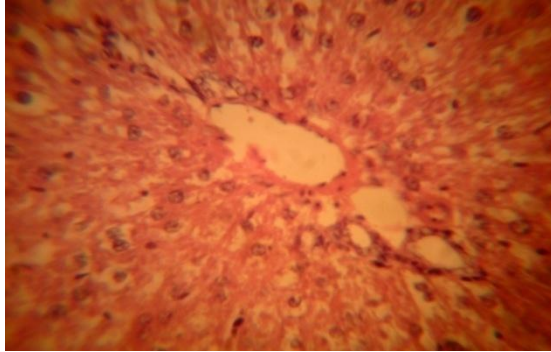


Plate 1: Liver tissue (Group A) showing normal architecture of portal tract and Normal Hepatocytes. (H&E x 400)

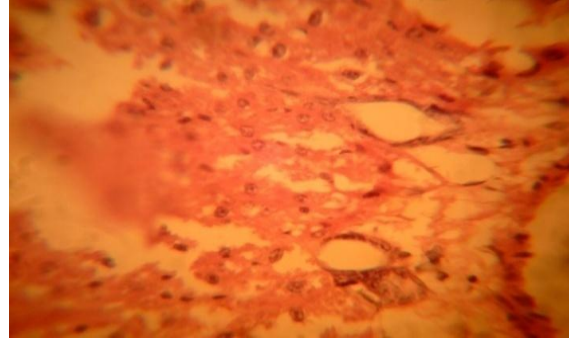


Plate 2: Liver tissue (Group B treated with Paracetamol 800mg/kg) showing necrosis of hepatocyte, severe distortion of the liver parenchymal and severe tissue separation. (H&E x 400)

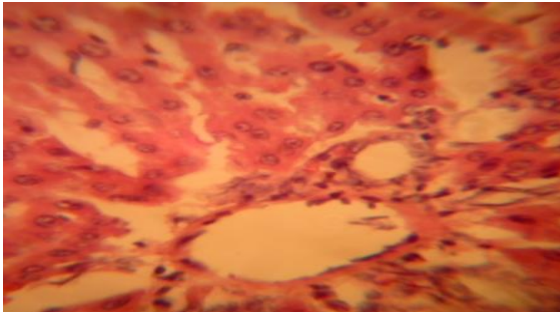


Plate 3: Liver Tissue (Group C treated with 50mg/kg Silymarin and 800mg/kg paracetamol) with better architecture showing mild portal area, mild tissue separation (H&E x 400)

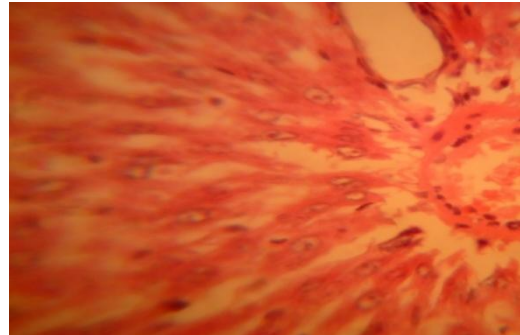


Plate 4: Liver Tissue (Group D treated with 100mg/kg *Ficus exasperata* stem bark extract and 800 mg/kg Paracetamol showing portal vein congestion and moderate tissue separation (H & E X 400)

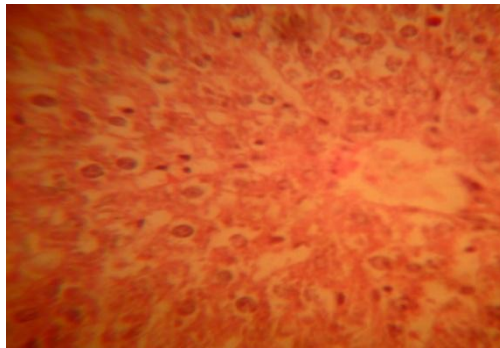


Plate 5: Liver tissue (Group E treated with 200mg/kg *Ficus exasperata* stem bark extract and 800 mg/kg Paracetamol) showing mild portal congestion and better liver architecture (H&E x 400)

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Drug induced liver injury has become a serious health problem in contemporary society, regrettably there are only a few drugs with serious side effects available for the treatment of liver ailments (Guntupalli *et al.* 2006). This therefore makes various researches on the mechanism and prevention of drug-induced liver injury very useful. Our histology results shows enormous liver tissue damage in the Paracetamol treated group which was also found to be reduced in groups that were administered with stem bark extracts of *Ficus exasperata*. The histology of the normal control group (Plate 1) showed normal portal tract, central vein and normal hepatocytes. The histological findings showed that there was visible distortion in the architecture of the liver tissue due to the administration of Paracetamol (Plate 2). These distortions were less visible on tissue sections of animals pretreated with silymarin. On treatment with both doses of *Ficus exasperata*, sections (Plates 4-5) began to show better architecture as compared to Paracetamol treated groups. Serum Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Total Protein (TP) activities were also assessed to corroborate and evaluate the hepatic functions (Degirmenchi *et al.* 2002). The increase in aminotransferases levels may be due to the cellular damage in the liver caused by Paracetamol induced hepatotoxicity. Various experimental studies have shown that subtle membrane changes are sufficient to allow passage of intracellular enzymes to the extracellular space (Garella 1997). Cell damage increases permeability causing cytosolic isoenzymes to spill into the sinusoids and from there into the peripheral blood (Garella 1997). Ezeonwu (2013), had earlier reported an increase in AST, ALT and ALP in Paracetamol hepato-nephrotoxicity in rats. The research work of Syed *et al.* (2012) also showed that administration of CCl₄ to normal rats increased serum levels of AST, ALT, ALP, and bilirubin. Salimuddin *et al.* (2008) reported that alterations in hepatic function may be because of increase activity and mRNA levels of araginase. These earlier reports therefore made evaluation of ALT, AST, ALP and TP activities necessary for our assessment of hepatocellular dysfunction. Consequently, the results of our study showed that Paracetamol induced toxicity in rats produced alterations in the hepatic functions as well as the histology of liver tissue. The increase in the levels of AST, ALT and ALP in diabetic rats after treatment for 14 days had been reported earlier by Arhoghro (2009). The reversal of increased serum enzymes in Paracetamol-induced liver damage by the extract may be an indication of its alleviation of plasma membrane

damage or its membrane stabilizing activity thus preventing the leakage of intracellular enzymes. The effective control of total protein (TP) can be attributed to an improvement in the hepatic cells secretory mechanisms. Preliminary phytochemical screening indicated the presence of tannins, saponins and flavonoids (Bafor *et al.* 2009; Adebayo *et al.* 2009). The antioxidant and hepatoprotective activities of flavanoids (Yoshikawa *et al.* 2003) and Saponins (Baek *et al.* 1996) are well known. This goes to strengthen our belief that the hepatoprotective activity of the extract may in also be due to the presence of Flavonoid and Saponin compounds. In conclusion, aqueous stem bark extracts of *Ficus exasperata* had protective effects on liver damage induced by high doses of Paracetamol. Isolation of the active principles majorly responsible for its hepatoprotective activity are recommended to corroborate our findings.

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