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HEPATO-TOXICITY AMELIORATING EFFECT OF *Alchornea cordifolia* ON WISTER RAT

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ABSTRACT: The hepatoprotective effect of ethanolic leaf extract of *Alchornea cordifolia* against Paracetamol-induced toxicity was investigated in male and female Wistar rats. Hepatoxicity was assessed based on the activities of biochemical enzymes; serum transaminases; alanine amino-transferases (ALT) and aspartate amino-transferases (AST); alkaline phosphatase (ALP), protein, albumin, bilirubin and urea. The result showed evidence increase levels of ALT, AST and ALP which were significantly high $P \leq 0.05$ due to Paracetamol intoxication and decrease AST/ALT ratio ($P \leq 0.05$) being also significant when compared to the control. Serum albumin and total protein levels were higher in animals treated with higher dosage (400mg/kg body weight) of Paracetamol alone, and un-conjugated bilirubin, but with comparable decreased activities in other groups co-administered with the plant extract. The concentration of urea, creatinine and electrolytes were not significantly different ($p \leq 0.05$) in all the experimental groups. The results of this study clearly indicate that *Alchornea cordifolia* extract possess the ability to ameliorate hepatotoxicity.

INTRODUCTION

The liver is the major organ responsible for metabolism, detoxification and secretary functions in the body. Therefore test of its functions have been devised in the hope that it will serve as diagnostic aids when a metabolic progress has been disturbed as a result of toxic chemicals, certain drugs, and environmental pollutants which is on the increase in the last few decades (Mayne, 2005). Hepatic damage is associated with the distortion of these metabolic functions and the liver is known to be one of the organs with high regenerative response to stimuli such as drug and toxins inducing liver diseases (Carither, 1992).

Damage to the liver or hepatotoxicity results not from paracetamol itself, but from one of the metabolites, N–Acetyl–p – benzoquinone imine (NAPQI) also known as N - acetylimindoquinone (Borne, 1995 and Teneabein, 2004). NAPQI depletes the livers natural anti-oxidant, glutathione and directly damages cells in the liver, leading to liver failure (Olaleye *et al.,* 2007). Hepatotoxicity results in the release of the liver enzymes obtained from the damaged liver into blood stream as their functions are disrupted, (Zakim and Boyer, 1996, Uboh *et al.,* 2009). Liver function test signaling liver damage most often could be assessed using liver diagnostic enzymes, alanine amino-transferases (ALT) and aspartate amino-transferases (AST) and alkaline phosphatase (ALP), with other biochemical assays, protein, albumin , and bilirubin, concentrations in the blood above normal values, implicating the degree of liver damage (Asator and King; 1954, Reitman and Frankel, 1997).

Reports on the biological activity of *Alchornea cordifolia* suggest that it is antibacterial (Laminkanra *et al.,* 1990), spasmolytic (Ogungbamila and Samuelsson, 1990), anti-inflammatory (Mavar-Manga *et al*., 2008), hepato-protective (Olaleye *et al.,* 2006) and anti oxidant (Olaleye and Rocha, 2008). Studies have also revealed the propensity of *Alchornea cordifolia* extract for provoking hepatic damage in mice, (Ansah *et al*., 2009). The hepato-protective activity of ethanol leaf extract of *A. cordifolia* against Paracetamol-induced toxicity

has been reported (Olaleye *et al.*, 2006) and shows that the extract has been found to offer the highest protection among other per-oxidant by increased lipid per-oxidation against Paracetamol-induced toxicity (Olaleye and Rocha, 2008).

In this study, the hepatotoxicity ameliorating effects of the plant extract was assayed based on the activities of biochemical markers enzymes; serum transaminase; alanine and aspartate (ALT and AST); alkaline phosphatase (ALP), protein, albumin, and bilirubin.

MATERIALS AND METHODS

Collection, preparation and extraction of plant materials for analysis

The fresh leave of *Alchornea cordifolia* were collected from the University of Calabar, Nigeria botanical garden and was identified and authenticated by a plant taxonomist in the Department of crop science. A voucher specimen (AC-2008) were kept in department of biochemistry laboratory, University of Calabar, used for biochemical assay within two days with appropriate refrigeration. The leaves were separated, sundried and powdered for easy extraction. Precisely 400g of well grounded fresh product of *Alchornea cordifolia* was extracted with 100cm^3 of 98% alchohol (5 liters) for 40 minutes in *a* Soxhlet extraction apparatus at room temperature (28 ± 2^{o}C). The residue was freez-dried, filtered, and concentrated under pressure to obtained crude extract referred to as extract in this study which was used for the phytochemical screening and other biochemical estimation.

Test Animals

The Wister rats used in this study were obtained from the animal's house of the Department of Biochemistry, University of Calabar. The rats which were kept in plastic cages in a well-ventilated animal house and were allowed to acclimatize for two weeks under standard condition of 28 ± 2^{o}C and 46% relative humidity with 12 hour light/dark cycles. During the period, the rats were fed with commercial rats chew palletized and water for 21 days.

Treatment of Test Animals

12 male and 12 female mature rats with average weight of 150-220 g were grouped according to their weight into 4 experimental groups of 3 rats each of both sexes, and treated. During treatment graded dosages of *Alchornea cordifolia* leaf extract and Paracetamol (Acetaminophen) purchased from Amela, pharmacy, Uyo, Akwa Ibom State; Nigeria, were administered orally as follows:

Group	Treatment	Number of male rats treated	Number of Female rats treated
1	Distilled water (control)	3	3
2	300mg /kg bwt of Extract + 250 mg/kg bwt. of Paracetamol	3	3
3	300mg /kg bwt of Extract + 500 mg/kg bwt of Paracetamol	3	3
	400mg /kg bwt Paracetamol	3	3

The treated rats were kept under constant environmental and nutritional condition throughout the experiment. The Wistar rats were freely fed with standard diet and drinking water *ad-libitum*. Body weight changes of the treated animals were monitored after every 4 days using animal balance. At the end of 21 days, the animals were sacrificed after overnight fast and thereafter, blood and tissues of the liver were collected for analysis.

Collection and Handling of Blood and Liver Tissue for Hepatotoxicity Assay

The animals were sedated with chloroform vapour and dissected for collection of blood and tissue specimens. Whole blood from each animal was collected by cardiac puncture into a well labeled plane screw-cap sample tubes collection. The serum used for biochemical assay was

removed using a Pasteur pipette into another set of tubes after spinning in a MSE clinical centrifuge at 1000 rpm for 5 minutes. Histopathological examination were carried out within 48 hours of tissue collection. The liver tissues were surgically removed and washed immediately with ice cold saline. A sliced section of the tissues were fixed in a suitably treated formalin reagent for histopathological examination.

Biochemical Analysis of the Liver Tissues:
Biochemical analysis carried out included measurement of the activities of serum alanine amino-transferases (ALT), aspartate amino-transferases (AST) and alkaline phosphatase (ALP). Serum total protein and albumin concentration of these biochemical markers were done by spectrophotometric determination of their absorbance using analytical grades of laboratory reagents kits from Bio-systems laboratories.

Estimation of total protein was done using Biuret method (Thomas, 1995 and Tietz, 1999). Serum albumin was determined by BCG method using Djalab kit (Wehster, 1974 and Tietz, 1994); ALT activity measurement was carried out by modified. IFCC method using Dialab kits while the ALP activity assay was based on Randox kits. All absorbance reading were taken with DREL, 3000 AACH model spectrophotometer.

Histopathological findings of the Liver Tissues:
The histopathological examination of the liver tissue was also carried out after rinsing the dissected tissues in normal saline, tissue section were taken from the organs and were fixed in 10% from saline dehydrated with 100% ethanol solution and embedded in paraffin. They were processed into sections, 4 - 5 µm thick stained with haematoxylin and eosin and observed under a light microscope for any morphological changes.

Statistical Analysis
The data were analyzed statistically using the "ANOVA and students T-test score values of ∑0.05 were considered as significant.

RESULT AND DISCUSSION

In this study, hepatoprotective activity of the ethanolic leaf extract of *Alchornea cordifolia* on Paracetamol- induced toxicity was evaluated in male and female wistar rats. The results of the hepato-protective effects of ethanolic leaf extract of the plant on liver damage induced by Paracetamol and its implications on biochemical indices in Wistar rats of both sexes were measured and compared with the untreated samples (control). The marker of hepatic functions; liver enzymes, total protein, albumin and bilirubin were examined and hepatotoxicity assessed from the activities of the serum and liver enzymes AST, AP, ALP (Table 1). Total and direct bilirubin levels, as well as total protein and albumin levels (Table 2).

Table 1: Effect of *Alchornea cordifolia* on serum enzymes activities of experimental animals

Gro -up	AST(IU)		ALP(IU)		ALT(IU)		AST/ALT	
	Male	Female	Male	Female	Male	Female	Male	Female
1.	35.0±7.81	32.50±0.71	29.41±2.92	38.75±4.28	11.00±0.58	10.00±0.11	3.08	3.25
2.	37.67±2.89	34.50±0.71	36.47±2.92	34.34±8.07	16.67±0.58	14.50±0.71	2.26	2.86
3.	44.00±4.24	47.50±0.71	38.25±4.17	35.24±8.17	21.50±2.21	15.50±0.71	2.05	3.07
4.	54.00±4.24	52.30±4.60	51.03±2.09	51.65±2.05	35.15±1.41	34.51±0.71	1.54	1.52

Significantly different from control ($P<0.05$); n = 3, Mean ± S.D using t-test and ANOVA

The results presented in Table 1 have shown that animals treated with 400mg/kg b.wt of Paracetamol alone, Group 4 recorded AST levels that were significantly higher ($P < 0.05$) than those obtained from other experimental groups. This observation suggests that the extract may be responsible for the low AST levels recorded for other experimental groups. The result is an indication that the plant extract tended to protect the Wistar rats against Paracetamol-induced hepatotoxicity even though the dose of Paracetamol was higher. The increase as compared to the controls indicates pathology of the heart while the reverse implicates that of the liver.

Increase in AST means a rise in the ratio. This produces additional evidence in support of the hepatotoxicity of Paracetamol overdose which is liver tissue directed.

The serum levels of conjugated bilirubin of all experimental animals are presented on Table 2. Group 2 and 3 results were not significantly different in both sexes. However both male and female animals treated with 400mg/kg b/wt of Paracetamol (Group 4) recorded significantly higher ($p \leq 0.05$) level of conjugated bilirubin when compared to controls (Group 1) which suggests progressive loss of the conjugating ability of liver due to toxicity effect. Increase in levels of serum albumin and total protein concentration were observed in all the animals treated with Paracetamol except for animals in group 4 which received 400mg/kg body weight of Paracetamol (Table 2). These observations were consistent for both male and female and are supportive of hepatocellular degenerations induced by Paracetamol intoxication. The observed decrease in serum albumin concentration may be indicative of progressive liver disease (Bolarin, 2002), since albumin is synthesized by the liver.

The increase of total serum protein concentration observed in animals which received 400mg/kg body weight of Paracetamol (Group 4) may be as a result of the increase in the serum level of ALT, AST and ALP. Moreover, hepatocellular damage will usually be associated with a spillage of these enzymes into blood (Mayne, 2005; Bolarin, 2002; Cotran *et al.*, 1999). Animals in all other experimental groups recorded comparable levels of serum albumin and total protein concentration except for animal in Group 4 which received 400mg/kg body weight of Paracetamol. Results show that animals in Group 4, recorded higher levels of serum albumin when compared to all other experimental groups. This observation was consistent both for male and female animals and the effect of the extract is comparable.

However, our finding have shown that in all cases of animals treated with Paracetamol (normal and overdose), *Alchornea cordifolia* did not cause any significant change in the serum levels of sodium, potassium, bicarbonate and chloride ions in both male and female experimental animals. This result may suggest that the treatments did not sufficiently perturb on the biochemical mechanism responsible for the maintenance of these electrolyte concentrations in the animal blood.

Animal treated with 400mg/kg b.wt of Paracetamol recorded a significant increase in serum and liver levels of AST, ALT and ALP when compared to control and other test groups. Animals with 300mg/kg b.wt of paracetamol recorded a significant increase ($P \geq 0.05$) in serum AST and ALT in males. This observation is suggestive of the fact that the extract may protect against Paracetamol induced hepatotoxicity. Our observation is consistent with previous report by other researchers investigating the hepatoprotective activity of various plant extract (Wu *et al.*, 2004 and Olaleye *et al.*, 2006).

Hepatoxicity also affected the food intake of the Wistar rats and reflected in the weight changes and growth rate of the animals treated (Table 3). The result shows that all experimented groups of rats recorded weight increases except for Group 3 male and female animals treated with 500mg/kg b.wt of Paracetamol plus 300mg/kg b.wt of leaf extract. The decrease in body weight may be due reduced food intake by the affected rats. Previous studies by Joy and Kultan (1999) and Lollta *et al.* (2005) revealed variation in weight of rats treated with *Alchornea cordifolia* leaf extract.

Histological evidence from the micrographs of wistar rats tissue treated with the extract when compared to the animals that were treated with both extract and Paracetamol (Plates 1-4) and that treated with 400mg/kg body weight of Paracetamol alone (Group 4) (Plate 5) revealed distinct variation in the liver tissue analyzed. The changes notice are supportive of previous observations reported by McClain *et al.* (1990), that activated Kuppfer cells and their secreted cytokins may contribute to liver injury. The injury is characterized by hepatic necrosis, sinusoidal dilation, vacoulations, cell shrinkages and less distinct nuclei (Plate 5).

The hepaprotective potential of the plants may be due to its anti-oxidative properties. Related studies by Ette *et al.* (2013), Olaleye *et al.* (2007) and Okuda *et al.* (1983) have shown that *Alchornea cordifolia* leaf is endowed with remarkable concentrations of phytochemical constituent: saponins, tannins, flavonoids and reducing compounds. Some flavonoids, have been demonstrated to inhibit hepatotoxicity because of its strong antioxidant activity. On the other hand Olaleye *et al.* (2007), has reported that the tannins component of *Alchornea cordifolia* is known to offer protection against hepatotoxicity and it is possible that this component of phytochemical constituents offered protection to treated animal by ameliorating the hepatotoxic effect the Paracetamol,.

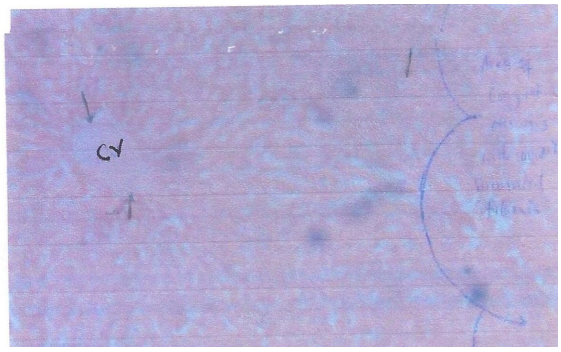

Plate 1 (a) Group 1 – L
(Liver Section – Control)
No evidence of hepato injury seen, normal architecture with distinct hepatic cell and sinusoidal space.

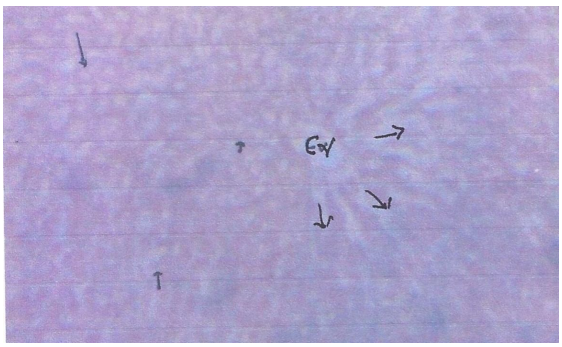

Plate 2 (b) Group 2 – L
(Liver Section – Control) Co-adminstered extract-paracetamol show normal cellular architecture with distinct and improved hepatocytes, distinct cell outline and nuclei

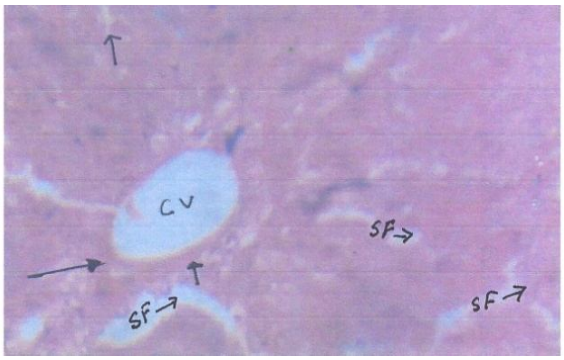

Plate 3 (c) Group 3 – L
(Liver Section of 300 mg/kg b.w.t extract + 500 mg/kg b.w.t paracetamol) shows improved architecture, hepatocytes, cell nuclear appears normal.

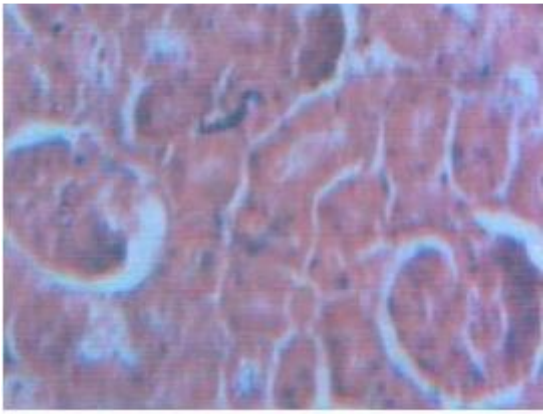

Plate 4 (d) Group 4 – L
(Liver Section) shows signs of hepatic necrosis Sinusoidal dilation vacuolations, cell shrinkages less distinct nuclei.

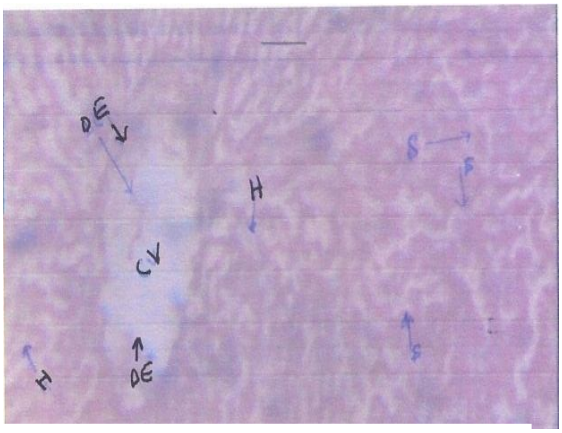

Plate 5 (e) Group 5 – L
(Liver Section) shows signs of hepatic necrosis sinusoidal Dilation vacuolations, cell shrinkages less distinct nuclei.

Table 2: Some serum biochemical indices of experimental animals

Group	Albumin g/dl		Total protein (g/dl)		Conjugated Bilirubin µmol/L		Unconjugated Bilirubin		Urea (mg/dl) µmol/L		Creatinine	
	M	F	M	F	M	F	M	F	M	F	M	F
1.	3.70 ±0.17	3.60± 0.33	11.17±0.129	11.75±0.30	3.33±0.14	3.36±0.16	5.09±0.15	5.34±2.35	6.33±0.44	6.40±0.28	4.70±0.14	3.70±0.78
2.	3.72 ±0.47	3.60±0.11	11.83±0.29	11.55±0.07	3.38±0.96	3.58±0.96	5.27±0.23	5.56±0.96	6.45±0.41	6.49±0.11	3.73±0.23	3.80±0.11
3.	3.65± 020	3.60±0.13	11.50±0.33	11.50±0.15	3.30±0.08	3.40±0.15	5.84±0.18	5.80±0.15	6.44±0.50	6.40±0.50	3.8-±0.12	3.78±0.23
4.	3.08± 0.26	3.08±0.28	13.76±0.53	12.96±0.11	3.83±1.08	3.17 ±1.08	7.67±0.10	8.67±0.10	8.67±0.20	8.35±0.38	5.80±0.28	5.90±0.20

Significantly different from control (P>0.05). Result are Presented as Mean **± SD: n = 3**

Table 3: Changes in weight and growth rate of the experimental animals

Group	Treatment	Initial body weight (g)		Final Body Weight (g)		Loss or gain in weight (g)		Percentage gain of loss in weight		Growth Rate (GR)	
		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1.	Control(distilled water)	169.83±8.22	165.67±1.15	178.17±29.11	176.63±15.02	8.34±7.87	10.96±6.87	4.9	62	23.38	29.90
2.	Extract(300mg/kg b.wt+ paracetamol (250mg/kg)b.wt.	218.17±12.39	176.50±2.78	226.00±19.09	174.83±7.18	7.83±2.12	1.67±0.76	3.46	0.96	16.48	4.57
3.	Extract (300mg/kg b.wt + paracetamol (500mg/kg)b.wt	228.50±1.32	180.00±15.00	187.67±6.93	5.33±9.61	7.67±4.54	2.28	4.09	10.86	10.86	19.48
4.	Paracetamol 400mg/kg b.wt	174.50±2.12	235.50	184.15±6.58	244.00±3.58	9.65±6.58	8.50±7.18	5.24	3.48	24.95	16.57

MEAN + SD, Result are presented as Mean ± SD; n = 3

CONCLUSION AND RECOMMENDATION

Liver diseases are considered as one of the serious health problems. It has been reported that steroids, vaccines and antiviral drugs that are employed as therapy for liver diseases have potential adverse effects especially when administered for long period (Schrawat *et al.*, 2006). Additionally, a number of drugs and pharmaceutical chemicals such as Paracetamol have been found to cause severe liver necrosis which sometimes becomes difficult to manage by medical therapies. This study has shown that chronic administration of Paracetamol may cause liver failure (cirrhosis) as indicated by histopathological alterations and marked elevated levels of serum total bilirubin, ALT, AST, and ALP in Wistar rats. However, the result of this investigation and treatment of the affected rats with *Alchornea cordifolia* leaf extract has revealed that ethanolic leaf extract possess the ability to ameliorate hepatotoxicity induced in rats by an overdose of paratamol.

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