ISSN: 2141 – 3290
www.wojast.com

PHYTOCHEMISTRY AND EFFECT OF *Alchornea cordifolia* ON PARACETAMOL-INDUCED NEPHROTOXICITY IN MALE AND FEMALE WISTAR RATS

ETTE[1], B. B., EYONG[1], E.U.,
EDEM[2], D. O. and AKPAN[2], H. D.
[1]*Department of Biochemistry, University of Calabar, Nigeria*
[2]*Department of Biochemistry, University of Uyo, Nigeria*
benedictbette@yahoo.com

ABSTRACT: The nephroprotective effects of ethanolic leaf extract of *Alchornea cordifolia* against Paracetamol-induced toxicity in male and female Wistar rats was investigated. Phytochemical screening of the leave extract revealed the presence of alkaloids, saponins, tannins, flavonoids and reducing compounds. Histological examination of the kidney tissue slices on the experimental animals treated with 400 mg/kg bwt of Paracetamol alone recorded hepatocellular and renal tissue derangement, which ameliorated following co-administration of *Alchornea cordifolia* with Paracetamol. The administration of paracetamol for 21 days recorded comparable levels of creatinine, electrolytes; sodium, Potassium, chlorides and bicarbonates. The study has revealed the nephrotoxic ameliorating potential of *Alchornea cordifolia* leaf extract that can be harnessed for medical purposes.

INTRODUCTION

The kidneys serves to convert more than 1700 litres of blood per day into 1 litre of a highly specialized concentrated fluid called urine. As the primary organs responsible for the excretion of waste product of metabolism, it regulates the bodies' concentration of waste as well as electrolyte, acid-base balance and serves as endocrine organs for the secretion of hormones, and excretion of drugs and toxins (Cotran *et al.*, 1999).

Like in liver failure, damage to the kidneys are as complex as its structure base on the affected areas of the kidneys; (Glomeruli, tubules, interstitial and blood vessels) Tisher and Brenner, 1999 due to toxic agents resulting to nephrotoxicity (Boutis and Shannon, 2001) implicating acute renal failure, acute glomerulonephritis, obstruction of the tubules, and renal dysfunction as a result of paracetamol, carbon tetrachloride or heavy metal intoxication.

Consequently, kidney function test signaling kidney dysfunctions or failure could be assessed by determining the levels of urea, creatinine, electrolytes [sodium (Na^+, K^+ Cl^- CO_3^{2-}] in the serum of the experimental animals. Higher levels of urea, above the recommended average excreted for adult males and female of 30g daily and creatinine levels of male adults of 0.8 – 1.2 mg/dl or (60 – 90) µmol/L and female adults of 0.6 – 0.9 mg/dl or 45 – 70 µmol/L indicate kidney failure (Monica and Cheers Brough, 2002).

The markers of renal functions, urea and creatinine were assessed based on reported kidney toxicity studies associated with the use of medicinal plants. *Alchornea cordifolia* has been shown to contain flavonoids which have been demonstrated to inhibit nephrotoxicity because of its strong anti-oxidant activity (Satyaranayana, 2001). The leaf extract of the medicinal plant, *Alchornea cordifolia* is also used to treat wounds, sores, and cuts, (Abbiw, 1990). Phytochemical analysis of the leaves has identified the presence of several compounds including flavonoids, saponins, tannins and reducing compounds (Ognungbamila and Samuelsson, 1990).

Co-administration of Paracetamol with *Alchornea cordifolia* is becoming popular to reduced the histopathological changes on the liver and kidney tissues; consequent upon Paracetamol intoxication. The present study was designed to evaluate the phytochemistry and nephro-

protective effects of ethanolic leaf extract of *Alchornea cordifolia* on paracetamol-induced nephrotoxicity in male and female Wister rats.

MATERIALS AND METHODS

Collection, preparation and extraction of plant materials for analysis

The fresh leaves of *Alchornea cordifolia* were collected from the University of Calabar, Nigeria Botanical garden and was identified and authenticated. 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% alcohol (5 liters) for 40 minutes in a Soxhlet extraction apparatus at room temperature (28 ± 2^{o}C). The residue was freeze-dried, filtered, and concentrated under pressure to obtain crude extract used for the phytochemical screening.

Phytochemical Screening of *Alchornea cordifolia* Leaf

Phytochemical analysis of the crude extract of *Alchornea cordifolia* was carried out according to Harbone (1994) and Evans (1998) methods. Screening for alkaloids was carried out using Mayer's, Aragendroff's test and Picric acid reagents. The tannins were detected by the Ferric chloride test, phlobatannins by hydrochloric acid test, saponins by the Frothing and Fehling tests, flavonoids by Shinoda's test, anthroquinones by the Borntraggers test and reducing compounds by titrmetric method using Fehling's solution. The presence of these Phytochemical in the plant extract were graded as being slightly present (+), strongly (++), very strongly present (+++) and not present (-).

Source and Treatment of Test Animals

The Wistar rats were obtained from the animal's house of the Department of Biochemistry, University of Calabar. The rats which were kept in plastic cages in 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 incubation, the rats were fed with commercial rats chew palletized and water for 21 days.

12 male and 12 female mature rats with average weight of 150-220 mg 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	No. of male rats treated	No. 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
4	400mg /kg Paracetamol	3	3

The treated rats were kept under constant environmental and nutritional condition through out the experiment. The Wistar rats were allowed free feeding 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 the blood and tissues of the kidney were collected for analysis.

Collection and Handling of Blood and Kidney Tissue for Biochemical 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 was removed using a Pasteur pipette into another set of tubes after spinning in a MSE clinical centrifuge at 1000 rpm for 5 minutes and used for biochemical assays. The kidney tissues were surgically removed and

washed immediately with ice cold saline. A sliced section of the kidney tissues were fixed in a suitably treated formalin reagent for histo-pathological examination. The later was carried out within 48 hours of tissue collection.

Biochemical Analysis of the Kidney 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. These biochemical markers were done by spectrophotometric determination of their absorbance using analytical grades of laboratory reagents from Bio-systems Laboratories.

Estimation of total protein was done using Biuret method (Thomas, 1995 & Tietz, 1990). Serum albumin was determined by BCG method using Dualab kit (Webster, 1974 & Tietz, 1994), ALT activity measurement was carried out by modified IFCC method using Dialab kits, while the ALP estimation was by the colorimetric endpoint method, based on Randox kits. All absorbance reading were taken with DREL, 3000 AACH model spectrophotometer.

Histopathological Assay of the Kidney Tissues

Histological examination of the kidney tissue slices was carried out via the analysis of creatinine, electrolytes; sodium, potassium, chlorides and bicarbonates. Prior to analysis, the tissues 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

The assessment of the phytochemical constituents and protective effects of ethanolic leaf extract of *Alchornea cordifolia* on Paracetamol-induced kidney damage and its implications on biochemical indices in Wistar rats of both sexes were measured and compared with the control. The result of the phytochemical screening of the leaf extract of *Alchornea cordifolia* (Table 1) revealed very strong presence of saponins, tannins, flavonoids and reducing compounds in the plant leaves. Also detected in the medicinal plant extract was alkaloid. Their presence in the plant has previously been reported by (Yokozawa, 1991). However, phlobabitanin and anthraquinones were absent in the plant extract.

Table 1: Phytochemistry *Alchornea cordifolia* of leaves

Chemical Constituents	Ethanolic Extract
Alkaloids	++
Saponins	+++
Tannins	+++
Flavonoids	+++
Reducing compounds	+++
Phlobatanins	-
Anthraquinones	-

		KEY
+	=	Slightly present
++	=	Strongly present
+++	=	very strongly present
-	=	Not Present

Nephrotoxicity was assessed from the activities of the electrolytes concentration and other parameters considered in body fluids; concentration of urea; creatinine and the histological architectural of the kidney of the experimental animals. The results presented in Table 2 revealed significant increase in serum urea in Group 4 male and female Wistar rats treated with 400mg/kg body weight of Paracetamol, while the creatinine level is suggestive of progressive impairment caused by the toxicity of Paracetamol. Urea is a waste product of protein catabolism and is excreted by the kidneys. The serum concentration of urea and creatinine may therefore be use as indices for assessment of renal function (Bolarin, 2002).

Table 2: Serum electrolyte levels of the experimental animals

Group	Treatment	Sodium (meq/L)		Potassium (meq/L)		Bicarbonate (meq/L)		Chloride (meq/L)	
		Male	Female	Male	Female	Male	Female	Male	Female
1.	Control(distilled water)	90.00±0.13	105.00±2121	7.34±0.19	7.34±0.19	26.17±2.29	22.75±1.38	86.75±0.99	87.07±1.22
2.	Extract(300mg/kg b.wt+ paracetamol (250mg/kg)b.wt.	100.00±17.32	99.00±10.13	7.96±0.27	7.40±0.11	26.67±2.29	23.00±1.30	87.36±0.99	87.01±1.30
3.	Extract (300mg/kg b.wt + paracetamol (500mg/kg)b.wt	90.00±1.21	98.00±1.13	7.89±0.19	7.40±0.11	25±1.35	22.50±1.50	87.07±1.22	87.93±1.25
4.	Paracetamol 400mg/kg b.wt	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

Result are presented as Mean + SD: n = 3

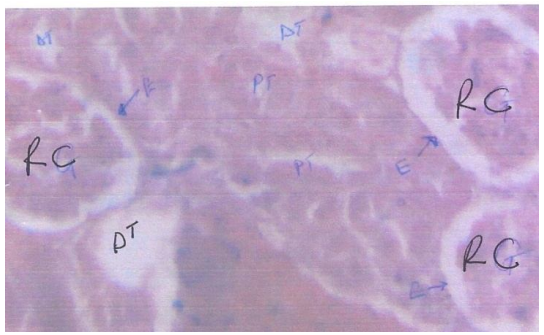

Plate 1 (a) Group 1 – K
Photo Micrograph of Kidney tissue shows normal central vein renal Cortex and dilated bowmans space.

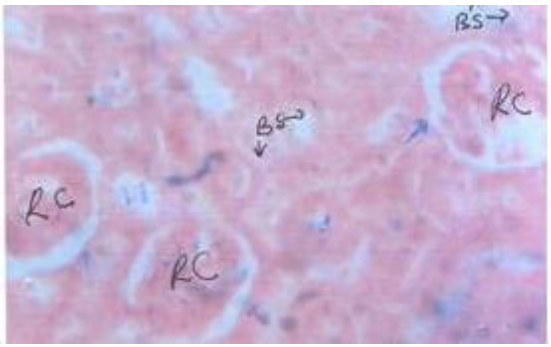

Plate 2 (b) Group 2 – K
Partial normal architecture, glomerulus appears normal in renal cortex

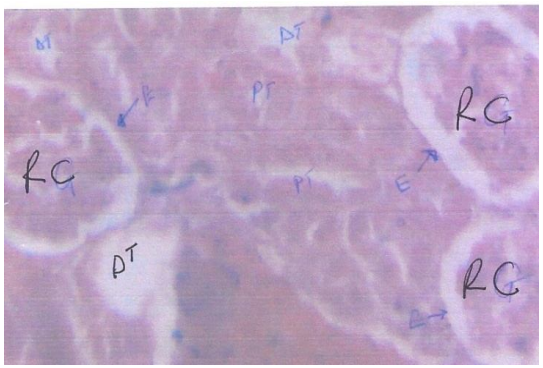

Plate 3 (b) Group 4
2 Kidney sections 400 mg/kg paracetamol alone. Kidney dilation of bowmans capsule fibrosis and hypochromatic nucei severe renal alteration and damage due to overdose paracetamol.

The fact that other experimental groups treated with Paracetamol and *Alchornea cordifolia* did not record significant increases in serum creatinine and urea, concentrations also indicates that *Alchornea cordifolia* may protect against Paracetamol-induced kidney dysfunction in male and female rats. This protective ability of the extract may be ascribed to the ant-oxidative properties of the plant (Olaleye *et al.,* 2007 and Okuda *et al*., 1983). Tannins are known to offer protection against Nephrotoxicity and it is possible that this component of phytochemical constitutions of *Alchornea cordifolia* offered protection to the treated animal in ameliorating the effect of nephrotoxicity of Paracetamol. Similar observation has previously been reported by Okuda *et al.* (1983) and Satyaranayana (2001).

Histological evidence from the micrographs of Wistar rats kidney tissue treated with the extract when compared to the animals that were treated with both extract and Paracetamol (Plates 1-2) and that treated with 400mg/kg body weight of Paracetamol alone (Group 4) (Plate 3) revealed distinct variation in the histological status of the kidney tissue analyzed. The pictures shows that animals treated both with extract and Paracetamol did not show severe renal alteration when compared to animals treated with Paracetamol alone (400mg/kg bwt of Paracetamol). This implies that *Alchornea cordifolia* leaf extract dose of 300mg/kg body weight was effective to protect against Paracetamol induced renal damage. Similar result has earlier been reported by Blakely and Mc. Donald (1995).

Examination of kidney tissue slices of normal, control, animals (Plates 1- 3) and *Alchornea cordifolia* treated animals did not reveal any significant histological alteration, however Wistar rats treated with overdoes of Paracetamol, showed dilation of Bowman's space, fibrotic capsule, and hypochromatic nuclei (Plate 3). These observations were remarkable in animals treated with 400mg/kg body weight of Paracetamol (Group 4) although the alterations were less severe in animals co-administered with Paracetamol and the leaf extract (Plate 3).

The serum electrolyte levels results of the treated rats are presented in Table 2. The levels were not significantly altered and this indicates that the impairments noticed in the histological section were not severe enough to have induced a disturbance in the maintenance of the normal concentrations of the electrolytes. Additionally, the proximal distal tubules of the glomeruli viewed in all groups appeared normal and the electrolytes measured are usually reabsorbed from the region of the nephron. This may be the reason why the levels were relatively comparable despite the histological differences observed on the kidney tissues.

CONCLUSION AND RECOMMENDATION

The study has shown that Wistar rats treated with *Alchornea cordifolia* and paracetamol did not show severe renal histological alterations as compared to animals treated with Paracetamol alone. This suggests that the extract may protect against paracetamol induced renal damaged. It is evident that ethanolic extract of *A. cordifolia* possesses the ability to ameliorate nephrotoxicity induced in rats by an overdose of Paracetamol. However, the ameliorating effect was more severe in males than in female rats. This protective ability may be as a result of a flavonoids and tannins content of the extract since these components have been reported to scavenged free radicals. It is recommended that the nephrotoxicity-ameliorating potential of *Alchornea cordifolia* should be harnessed for medical purposes.

REFERENCES

Abbiw, D. (1990). *Useful Plants of Ghana*, London, UK: Intermediate Technology Publication and the Royal Batanic garden kew, 147p.

Ansah, C., Oppong, E., Woode, E. and Duwiejua, M. (2009). Toxicity studies on *Alchornea cordinea* leaf tract in mice, *Journal and Science and Technology*, 29: 8 – 16.

Blakey, P. and McDonald, B. R. (1995). Acute renal failure due top acetaminophen injection; A case report and review of the literature, *J. Am Soc Nephrol* 6(1): 1853 - 1857

Bolarin, D. M. (2002). *A Handbook of Clinical Chemistry*, Nelson Publishers limited, ILepeja, Lagos, Nigeria, pp 412 -415.

Boutis, K. and Sharmon, M. (2001). Neprotoxicity after acute severe acetaminophen poisoning in adolescents. *Journal of Clinical Toxicity* 39 (5); 441-445.

Clark, P. M., Clark, J. D. and Wheatley, T. (1986) Urine discoloration after acetaminophen overdose. *Clinical Chemistry* 32(9): 1777-1778

Cotran, R. S., Kumar, V. and Collins T. (1999), *Robbins Pathologic Basic Diseases*, 6th Ed; New York, W. B. Saunders, London.

Evans, W. C. (1998) *Trease and Evans Pharmacognosy*, Balliere Tindall, London

Harbone, J. B. (1984) Phytochemical Methods, 2nd Edition, Chapman and Hall, London, pp 20 – 22.

Joy, K. L. and Kuttan, R. (1999). Anti dissetic activity of prorrhizza kurroa effect, *Journal of Ethnopharmacology*, 67(2): 143 -148.

Lami Kanra, A., Ogundani, A. O. and Ogungbamila, F. O. (1990), Anti-bacterial constituents of *Alchornea cordifolia* leaves, *Phylother Research*, 4:198-200.

Lollta, M. A; Bonnie, L. A. Baker W. L. Ball, G. D. Carol, B. K; Berkowitz D. M; Broadwell, D. C; Brown, F. L. and Clark , R. J. (2005). *Clinical Laboratory Test Values and Implications.* 1st ed). Library of congress, spring House cooperative , Punny Sylvania. 205-222.

Lollta, M. A., Bonnie, L. A., Baker, W. L., Ball, G. D., Carol, B. K., Berkowitz, D. M., Broadwell, D. C., Brown, F. L. and Clar , R. J. (2005). *Clinical Laboratory Test Values and Implications* 1st ed). Library of Congress, Spring House Cooperative , Punny Sylvania, pp. 205-222.

Mavar-Manga, H., Call-Haded, A. D. M., Pieter. L., Nacelli, C., Penge A. and Quetin – Leclkeroq, J. (2005). Anti - inflammatory compounds from leaves and root bank of *Alchornea cordifolia*. (Schumach, and Torn). *Ethnopharmacol*ogy, 115: 25-29.

Mayne, P. (2005). *Clinical Chemistry in Diagnosis and Treatment*, 6th Edition, Gillingham, India, pp. 63 – 74.

Monica, F. I. And Cheersbrough, G. R. (2002). District Laboratory Practice in Tropical Countries, part 2, 2nd Ed. Cambridge University, Pren London, pp 342-345.

Olaleye, M. T, and Rocha, J. B. (2008) Acetaminophen-induced liver damage in mice: Affects of some medicinal plants on the oxidative defense system. *Experimental Toxicology and Pathology*, 59: 319 -327.

Olaleye, M. T, Ayodele, O. K. and Joshua, O. A. (2007). Antioxidant properties and glutathione s- transferases. Inhibitory activities of Alchonea cordifolia leaf extract in acetaminophen induced. *Liver Injury,* 7(6): 63-66 .

Olaleye, M. T., Adegboue, O. O., Akindahnsi, A. A. (2006). *Alchornea cordifolia* extract protects Wistar albino rats against acetaminophen – induced liver damage. African Biotechnology, 5: 2439-2445.

Ogungbamila, F. O. and Samuelsson, G. (1990). Smooth muscle relaxing flavonoids. from *Alchornea cordifolia*, *Acta Pharm. Nord*, 1: 421 – 422.

Okuda, T., Kimur a, Y., Yoslida, T., Hating, T., Okuda, H. and Archie, S. (1983). Studies on the activities of tannins and related compounds from medicinal plants and drugs inhibiting effect on lipid peroxidation in mitochndria and macrodomes of liver *Chem. Bulletin*., 31: 1625 – 1631.

Satyanrayana, P. S., Singh, D., Chpra, K. and Quarcetin, A. (2001). Bio-flavonoid, protects. Against oxidative stem related renal dysfunction by cyclosporine in rats, methods stress. *Exp. Clin. Pharmacol* , 34: 175-181.

Tisher, C. and Brenner, B. M. (1999). Renal Pathology, with Clinical and Pathological Correlations, 3rd Edition, J B Lippin COH., Philadelphia

Wu, S. J. Wang, J. S. Lin; G. C, Chang C. H (2004). Education on hepato protective activity of legumes. *Phytomedicine*, 8(3): 223 – 239.

Yokozawa, T., Fujioka, K., Oura, H., Nonaka, G. and Nishioka, I. (1991). Effect of rhubarb Tannis on uremic Totins, Nephron 1991; 58; 155-160.