

paracetamol induced toxicity in Wistar rats

ABSTRACT

This study investigated the effects of ethanolic extract of *Anacardium occidentale* on paracetamol induced liver toxicity in Wistar rats. Twenty (20) Wistar rats were randomly divided into four (4) groups of five (5) rats each. The groups were designated as follows; A, B, C and D. Group A served as the normal control and received 2ml/kg of distilled water, group B served as the paracetamol treated control and received 1000 mg/kg body weight of paracetamol only, group C received 1000 mg/kg of paracetamol then treated with 250 mg/kg bw of *A. occidentale* extract, group D received 1000 mg/kg bw of paracetamol then treated with 500 mg/kg of *A. occidentale* extract daily for 4 weeks respectively. After the last day of administration, the rats were anaesthetized by cervical dislocation and their blood samples collected for analysis. The result revealed significant decrease ($P=0.05$) in Alanine aminotransaminase (ALT), Aspartate aminotransaminase (AST) and Alkaline phosphatase (ALP) in the groups treated with *A. occidentale* compared to the paracetamol treated groups. It can be deduced that *A. occidentale* has the potential of protecting the liver against paracetamol induced hepatotoxicity and thus, should be recommended in the management of liver disorders.

Keywords: *Anacardium occidentale*, Liver enzymes, Paracetamol, Blood, Wistar rats

1.0 INTRODUCTION

The liver is the key organ in the regulation of homeostasis. It fights against the hazards of harmful drugs and chemicals by metabolism and during metabolism free radicals are generated which could cause liver damage [1]. Liver injury can be induced by so many factors such as acetaminophen, carbon-tetrachloride (CCl₄), ethanol etc.

Paracetamol, sometimes called acetaminophen is one of the most common analgesics and antipyretic drugs often used around the world to treat pains and mild feverish conditions [2]. In 1989, it was one

of the most common causes of death in the United Kingdom (UK)[3]. Toxic doses of paracetamol could cause serious abnormalities in the liver function and sometimes could lead to death [4]. These toxic effects occur when there is saturation of the liver enzymes catalysing the normal conjugation reactions, thereby causing the drug to be metabolized by the mixed function oxidases [5]. The resulting toxic metabolized, N-acetyl-p-benzoquinone imine (NAPQI) is inactivated by conjugation with glutathione, but when there is depletion of glutathione, the toxic intermediate accumulates and reacts with nucleophilic constituents in the cell causing damage.

The use of medicinal plants in the management and treatment of ailments in both developed and developing countries is on the increase. Their medicinal values are indisputable; however, their toxicities could sometimes limit their clinical effect. Medicinal plants have been known to have antioxidant compounds which are valuable in the treatment of liver disorder and protection against chemical poisoning and environmental toxins[6]. An example of **amedicinal** plant is *Anacardium occidentale*

A. occidentale commonly known as cashew is a member of the *Anacardiaceae* family. The bark and leaf of the tree are used for medicinal purposes while the fruit has market value as food. The root and stem have been reported to have anti-inflammatory[7] and antidiarrhoea activity[8]. The extract of the leaf of *A. occidentale* has been reported to be having antidiabetic[9,10], antimicrobial [11] and antibacterial activity [12]. The leaf of *A. occidentale* contains phytochemical constituents like tannin, saponin and flavonoids which are known to have antioxidant properties [13]. The leaves and barks are also used in Brazil in the treatment of psoriasis, eczema, cough, genital problems, bronchitis and intestinal colic [14]. The fruits are rich in vitamins, minerals and other essential nutrients which have a high amount of vitamins and mineral salts [15].

The aim of this study was **to evaluate** biochemically the hepatoprotective effect of ethanolic extract of the leaf of *A. occidentale* on paracetamol-induced liver toxicity in Wistar rats.

2.0 MATERIALS AND METHODS

2.1 Experimental Animals

A total of twenty (20) albino rats weighing between 200-220 g were used in this study and were procured from the animals house of the Faculty of Basic Medical Sciences Gregory **University**, Uturu,

Nigeria. Ethical clearance was obtained before commencement from the research and ethical committee of the college for animal care and use, Gregory University, Uturu which is in compliance with the National regulation for animal research. The rats were kept in standard cages under normal temperature and fed with guinea feed and water *ad libitum*. They were allowed to acclimatize for a period of two weeks before administration of treatment.

2.2 Preparation of the plants extract

Fresh leaves of *A. occidentaled* were collected from Uturu and washed in a bowl of water to remove dirt and debris. They were dried under room temperature and later grounded using manual blender into a coarse form. 30 g of the coarse powder was macerated in 1000 ml of ethanol for 48 hours and then filtered using a filter paper. The filtrate was concentrated using a rotary evaporator which was further dried using a thermostat oven into a gel-like form..

2.3 Experimental procedure

2.3.1 Acute Toxicity Test (LD₅₀)

Acute toxicity test described by Lorke [16] was carried out on paracetamol. A total of thirteen rats were used. Three groups of three rats each were used in the first phase and were administered 10 mg/kg, 100 mg/kg and 1000 mg/kg of paracetamol orally. The rats were observed for mortality for 24 hours. After 24 hours no mortality was recorded and the second phase commenced. Four groups of one rat each were administered with 1200 mg/kg, 1600 mg/kg, 2900 mg/kg and 5000 mg/kg of paracetamol respectively. The animals were observed for 24 hours for mortality. LD₅₀ was calculated using the formula:

$$LD_{50} = \sqrt{(a \times b)}$$

Where, a = Highest dose that gave no mortality

b = Lowest dose that produced mortality

2.3.2 Induction of toxicity

Paracetamol manufactured by Emzor was procured from a reputable pharmacy in Uturu. Fifteen (15) out of the twenty rats were induced with toxicity. 1000 mg of paracetamol was dissolved in 100 ml of distilled water giving a concentration 10 mg/ml.

2.4 Study design

The rats were randomly divided into four (4) groups of five rats each designated as groups A, B, C and D. The administration was given as follows;

GROUP A: Normal control and received 2 ml/kg of distilled water

GROUP B: Paracetamol treated control and was administered 1000 mg/kg body weight

GROUP C: 1000 mg/kg of paracetamol + 250 mg/kg body weight of the extract.

GROUP D: 1000 mg/kg of paracetamol + 500 mg/kg body weight of the extract.

The administration was given orally, once daily between the hours of 10 am and 12 pm for a period of 28 days. At the end of the experiment, the rats were sacrificed using cervical dislocation method and dissected. The thoracic cavity was opened and blood samples were collected via cardiac puncture using **sterile syringes**. The blood samples were then centrifuged at 3,000rpm for 10 minutes using bench top centrifuge (MSE, Minor, England) **and analyzed for hepatic** marker enzymes: Alanine aminotransaminase (ALT), Aspartate aminotransaminase (AST) and Alkaline phosphatase (ALP). The liver function analysis for serum levels of ALT, **ALP**, and AST were determined using standard methods [17,18].

2.5 Statistical Analysis

Data were presented as Mean \pm SEM (Standard Error of Mean) and was analyzed using one way Analysis of Variance (ANOVA). This was achieved with the use of **the** Statistical Package for Social Sciences (SPSS) software (V20, USA). The results were considered statistically significant at $P = .05$ level of significance.

3.0 RESULT

3.1 LD₅₀ of paracetamol

The LD₅₀ of paracetamol was calculated to be 2154.07 mg/kg as follows

A = maximum dosed with 0% mortality is 1600 mg/kg

B = minimum dosed with 100% mortality is 2900 mg/kg

$$LD_{50} = \sqrt{axb}$$

$$\sqrt{1600 \times 2900} = \sqrt{4640000}$$

$$LD_{50} = 2154.06 \text{ mg/kg}$$

Table 1: Phases of the acute toxicity level test of paracetamol

	Dosage mg/kg body	
	weight	Mortality
Phase I		
Group 1	10	0/3
Group 2	100	0/3
Group 3	1000	0/3
Phase II		
Group 1	1200	0/1
Group 2	1600	0/1
Group 3	2900	1/1
Group 4	5000	1/1

3.2 Biochemical Analysis - Level of Serum liver enzymes

The result in Table 2 shows a significant increase ($P < 0.05$) in the serum level of ALT, AST, and ALP in paracetamol treated rats. However, there was a significant decrease ($P < 0.05$) in the serum level of ALT, AST, and ALP when treated with different doses of *A. occidentale*.

Table 2: Effect of *A. occidentale* (250 mg/kg and 500 mg/kg) and paracetamol (1000 mg/kg) on liver enzymes

Enzyme	Group A	Group B	Group C	Group D
ALT (U/L)	15.33 ± 3.52 ^a	65.66 ± 5.81 ^{ab}	39.66 ± 0.88 ^{abc}	32.00 ± 1.73 ^b
AST (U/L)	13.00 ± 1.15 ^a	58.66 ± 0.88 ^{ab}	39.00 ± 1.52 ^{ab}	29.33 ± 0.88 ^b
ALP (U/L)	27.33 ± 1.45 ^a	96.00 ± 8.71 ^{ab}	55.33 ± 1.85 ^{abc}	40.66 ± 2.96 ^b

All values are expressed as Mean ± S.E.M for each group. Subscripts of the same alphabets show significance at the level of $P = .05$;

Group A – normal control; Group B – Paracetamol – treated; Group C – Paracetamol + 250 mg/kg of extract;
Group D– Paracetamol + 500 mg/kg of extract

4.0 DISCUSSION

Disorders of the liver have raised a great concern in the health care system. Though Liver protective drugs may not be readily available, medicinal plants could play an important role in the management of liver disorders. Hepatotoxicity is being associated with liver damage and serves as a diagnostic tool. The mechanism of hepatotoxicity of paracetamol was reported by Hazai *et al.* [5], in which they explained that toxicity occurs due to its active metabolite N-acetyl-pbenzoquinoneimine (NAPQI). NAPQI is known to exert its toxicity through its oxidative effect on cellular proteins, which induces lipid peroxidation. The toxic peroxidative products cause widespread damage of macromolecules.

The activities of enzymes ALT, AST, and ALP in serum are used routinely to assess the functional status of the liver both in clinical and experimental settings. The hallmark of paracetamol hepatotoxicity is the presence of elevated serum enzymes [19]. An elevated level of these serum enzymes in the paracetamol treated group indicates liver dysfunction when compared to that of group A and other treated groups. Groups treated with *A. occidentale* significantly reduced the level of the enzymes when compared with the paracetamol treated group; with the higher dose having a more protective effect. This reduction could be due to the presence of polyphenols and flavonoids in the extract which acts as an antioxidant [20]. This shows that the ethanolic extract of *A. occidentale* could maintain the functional capacity of the liver. This is in agreement with reports of [21,22]. However, it contradicts the work of (Famurewa *et al.* [23] who reported a significant increase in ALT when treated with aqueous extract of *A. occidentale* stem-bark at a dose of 400 mg/kg. The lowering of enzymes level is a definite indication of hepatoprotective action of the drug [24]. Hepatoprotective action may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity [22].

5.0 CONCLUSION

Findings from this research indicate that high doses of paracetamol could result in hepatic damage and ethanolic extract of *A. occidentale* leaf has the potential of protecting the liver against paracetamol induced hepatotoxicity. This hepatoprotective activity could be of great therapeutic potentials to clinicians and is attributed to the antioxidant activities. It is recommended that the ethanolic leaf extract of *A. occidentale* should be used in the management of liver disorders

ETHICAL APPROVAL

All authors hereby declare that principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

REFERENCES

1. Biswas K, Kumar A, Babaria BA, Prabhu K, Ramachandra SS. Hepatoprotective effect of leaves of *Peltophorumpterocarpum* against paracetamol induced acute liver damage in rats. J Basic Clin Pharm. 2010;1:10-15.
2. Blieden M, Paramore LC, Shah D, et al. A perspective on the epidemiology of acetaminophen exposure and toxicity in the United States. Expert Rev ClinPharmacol2014;3:341–8.
3. Rang HP, Dale MM, Ritter JM, Moore PH. Pharmacology, 5th ed, Philadelphia., 2003. p245-252.
4. Chun LJ, Tong MJ, Busuttill RW et al. Acetaminophen hepatotoxicity and acute liver failure. J ClinGastroenterol. 2009;43:342-349.
5. Hazai E, Vereczkey L, Monostory K. Reduction of toxic metabolite formation of acetaminophen. BiochemBiophys Res Commun. 2002;291(4): 1089-1094.
6. Barar RS. Essentials of Pharmacotherapeutics. 3rded. New Delhi: Chand and Company Ltd.; 2000. P.340-341.
7. Mota ML. anti-inflammatory actions of tannins isolated from the bark of *Anacardiumoccidentale*. J. Ethnopharmacol. 1985;13:289-300.
8. Goncalves JL, Lopes RC, Oliveira DB, Costa SS, Miranda MM, Romanos MT, et al. In vitro anti-rotavirus activity of some medicinal plants used in Brazil against diarrhea. J Ethnopharmacol. 2005;14:403-407.
9. Esimone CO, Okonta JM, Ezugwu CO. Blood sugar lowering effect of *Anacardiumoccidentale* leaf extract in experimental rabbit model. J Nat Remed. 2001;1:60-63.
10. Kamtchoury P. protective role of *Anacardiumoccidentale* extract against streptozotocin-induced diabetes in rats. J Ethnopharmacol. 1998;62:95-99.

11. Akinpelu DA. Antimicrobial activity of *Anacardium occidentale* bark. *Fitoterapia*. 2001;72:286-287.
12. Kubo J. *Anti-Helicobacter pylori* agents from the cashew apple. *J Agric Food Chem*. 1999;47:533-537.
13. Jaiswal YS, Tatke PA, Gabhe SY, Vaidya A. *Antioxidant activity of various extracts of leaves of Anacardium occidentale* (Cashew). *Res J of PharmaBiolCheml Sci*. 2010;4:112-119.
14. Franca F, Cuba CA, Moreira EA, Miguel O, Almeida M, das VirgensMde L, et al. an evaluation of the effect of a bark extract from cashew (*Anacardium occidentale* L.) on infection by *Leishmania(Vlannia)braziliensis*. *Rev Soc Bras Med Trop*. 1993;26:151-155.
15. Dare SS, Hamman WO, Musa S, GojiAD, Oyewale AA, Abba S, et al. *Effects* of aqueous extract of *Anacardium occidentale* (cashew) leaf on pregnancy outcome of *Wistarrats*. *Int J Anim Vet Adv*. 2011;3:77-82.
16. Lorke, U.C. Determination of lethal dose of xenobiotics in experimental animals. *Nature*. 1983;45: 264-266.
17. The committee on enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology Recommended methods for determination of four enzymes in blood. *Invest Scand J Clin Lab*. 1974;33:291-306.
18. Ahmed M, Khater MR. Evaluation of the protective effect of *Amrosia maritime* extract on acetaminophen-induced liver damage. *J Ethnopharm*. 2001;75:169-174.
19. Robert JF. Acute Liver Failure including Acetaminophen Overdose *Med ClinNorth America*.2008;92(4): 761–794.
20. Ojezele MO andAgunbiade S. Phytochemical Constituents and Medicinal Properties of Different Extracts of *AnacardiumOccidentale* and *PsidiumGuajavSa*. *Asian J BiomedPharma Sci*.2013;3(16), 20-23.
21. Daniel I, Coston P, Abel NA. *Hepatoprotectiveeffect of methanolic leaf extract of Anacardium occidentale* (Cashew) on carbon-tetrachloride-induced liver toxicity in *Wistar rats*. *Sub-Saharan African Journal of Medicine*. 2014;1(3):124-131.
22. Sahreen S, Khan MR, Khan RA. Evaluation of antioxidant activities of various solvent extracts of *Carissa opaca* fruits. *Food Chem*. 2010;122:1205-11.

23. Famurewa AC, Sowunmi FA, Folawiyo AM, Ogbu PN, Epete MA, Igwe EC. Hepatotoxic and nephrotoxic potentials of aqueous extract of stem-bark of cashew tree (*Anacardium occidentale*) in rats. Toxicology International. 2015;22(3):108-114.
24. Thapa BR, Anuj W. Liver function tests and their interpretation. Indian J paediatr. 2007;74:663-671.

UNDER PEER REVIEW