Quest Journals
Journal of Research in Humanities and Social Science
Volume 13 ~ Issue 1 (2025) pp: 193-200
ISSN(Online):2321-9467
www.questjournals.org

Research Paper

Hepatoprotective Activity ofAqueous Extract of *Borreria stachydea*Leaves inAcetaminophen-InducedAcute Liver Injury

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Abstract
Acetaminophen (Paracetamol) overdose is a common cause of liver injury. To evaluate the possible hepatoprotective effects of Aqueous extract of Borreria stachydeaLeaves(BSAE), albino rats were pre-treated with graded doses of the extract(100, 200 and 400 mg/kg b.wt. p.o.) once daily for 7 days. On the 8th day of the experiment after an overnight fast, paracetamol was administered to the rats at a dose of 2g/kg b. wt. this produced a significant effect on the liver, which was evidenced by an increased level of serum activities of liver enzymes (AST, ALT, and ALP), total cholesterol, triglycerides, bilirubin, total protein and albumin. Histopathological examination also revealed hepatic necrosis. Results revealed that BSAE at all doses administered produced varying degrees of protection by significantly (P<0.05) decreasing the serum activities of ALT, AST, ALP, serum levels of total cholesterol; triglycerides and total bilirubin as well as significantly (P<0.05) increasing the serum concentration of total protein and albumin compared to the negative control that received only paracetamol. It was concluded that extract ofBorreria stachydeashowed promising hepatoprotection potentials in rats with acetaminophen-induced liver damage. Thus the plant could be useful in the management of liver diseases.

Key words: *Borreria stachydea,Acetaminophen, Paracetamol, Hepatic injury*

***R**eceived 14 Jan., 2025; **R**evised 26 Jan., 2025; **A**ccepted 28 Jan., 2025 © The author(s) 2025.*
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I. INTRODUCTION

The incidence of paracetamol (acetaminophen) poisoning is usually due to the abuse or overuse of paracetamol (Woolley & Woolley, 2017). When paracetamol is taken in therapeutic doses, it is usually harmless. However, it becomes extremely toxic when taken in excess of the requirement either as a single or repeated dose. Acute liver failure (ALF) which is the major manifestation of paracetamol poisoning, can result fromregular overuse, unintentional abuse, and purposeful intake for purposes of suicide(Tittarelli*et al.,* 2017). Paracetamol poisoning has become a major challenge globally because people tend to abuse it because of its accessibilityand affordability (Tittarelli*et al.,* 2017).

Despite the availability of varieties ofconventional drugs for a large number of health conditions, it is extremely difficult to see any drug that directly offers protection to the liver from damage or boost its function either by rejuvenation or regeneration of hepatocytes (Mc Govern *et al.,* 2015). This has necessitated the search for alternative agents that can offer protection to the liver by minimizing the damaging effects of hepatotoxic compounds or stimulate the regeneration of the hepatocytes after damage(Daly *et al.,* 1999). This has led researchers to medicinal plants which according to WHO are responsible for the health care needs of 80% of the population of the developing world. A number of studies have established the hepatoprotective potentials of medicinal plants. It has been documentedthat about 170 phytochemicals isolated from 110 different plants possess hepatoprotective activity (Handa, 1991).These phytochemicals include phenols, coumarins,lignans, terpenoids, flavonoids and essential oils. It has also been proven through research that medicinal plantsare efficacious in the treatment and management of liver diseases (Nikolaos & Laura, 2020). Despite the hepatoprotective potentials associated with these medicinal plants, only a small percentage of themhave been scientifically investigated. Also, some of these plants that are used traditionally for this purpose are also yet to

be pharmacologically investigated for its efficacy (Handa, 1991).One of such plants in use as gathered from our personal communication with a number of herbalists is*Borreria stachydea.*

Borreria is a very large genus of herbs. This genus is known to have about 100 species that are spread throughout the tropics (Neoh*et al*., 2010). *Borreria stachydea* is a specie that is very common in Africa and Nigeria in particular. In the Northern part of Nigeria, *Borreria stachydea*is commonly referred to as " Alkamartururuwa" while Fulanis called it "fairare". Ethnobotanical information made available from herbalists in northern Nigeria revealed that *Borreria stachydea*is used for the treatment of inflammation-related diseases. This have been confirmed and scientifically reported by Anas *et al.,* (2023). The antibacterial effectsof *Borreria stachydea* have also been reported. To the best of our understanding, there is little or no information confirming the hepatoprotective potential of this plant.Hence, this research evaluated the hepatoprotective potentials of *Borreria stachydea*with a view ofproviding a scientific backup for its claim as a potential in the management of liver diseases.

II. MATERIALS AND METHODS

Chemicals and Drugs

Paracetamol (PCM; Emzor Pharmaceuticals) was purchased from HealthSealed Pharmacy, Abuja. N-acetylcysteine (NAC), DMSOand all other chemicals/reagents used were of analytical grade and products of Sigma Chemical Co. Ltd (USA) purchased through a vendor in Jos, Nigeria.

Experimental Animals

Healthy adult male Wistar rats with weight range of 120–200 g were purchased from the Animal House Facility of the Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Research and Development (NIPRD), Abuja-FCT, Nigeria. They were accommodated in well-ventilated stainless steel cages and allowed to acclimatize under standard laboratory conditions for 7 days before the commencement of the experiment. The rats were fed with standard rodent feed and allowed access to clean water.

Plant Collection and Identification

The leaves of *Borreria stachydea*were collected from a natural habitat in Mararaba, NassarawaState, Nigeria. The plants wereauthenticated at theDepartment of Pharmacognosy,National Institute of Pharmaceutical Research and Development (NIPRD), Abuja-FCT, Nigeriawere **a** voucher specimen was deposited for future reference.

Preparation of Extract

After collection of the leaves of*Borreria stachydea*from its natural habitat, they were rinsed properly with distilled water to remove debris and subsequently driedunder shade for 5 days. The dried leaves were then pulverizedusing an electric blender to yield a coarse sample. A known quantity(2000g) ofthe pulverized leaves was cold-macerated in distilled water for 72 h then filtered usingmuslin sieve followed by Whatmann filter paper (Size No1). The resulting filtrate which is the extractwasthen concentratedusing rotary evaporator at a temperature of 40ºC and pressure of 204 mbar. The extractwas code-named BSAE and stored in the refrigerator till the commencement of the experiment.

Acute Toxicity Study

This study was carried out according to the method of Lorke*et al.* (1983). The study was carried out in two phases. In phase 1, nine adult rats were divided into 3 groups comprising of 3 animals each. The 3 groups were administered a single dose of 10, 100 and 1000 mg/kg BSAE and monitored for signs of toxicity within the first few hours of administration. Based on the observations made in Phase 1, 1600, 2900 and 5000 mg/kg BSAE were administered to 3 groups of animals (comprising on 1 animal each) respectively in Phase 2 of the study. The observations made in the second phase was used to estimate the LD_{50}.

Experimental Design for Hepatoprotective Assay

Thirty(30) healthyadult male Wistar rats were randomlyassigned to 6 groups made up of 5 animals and treated as follows;

Group A served as normal control and was administered5ml/kg 10% DMSO
Group B served as negative control and was administered 5 ml/kg 10% DMSO
Group C served as positive control and was administered 50 mg/kg 10% NAC
Group D served as a test group and was pre-treated with 100 mg/kg BSAE
Group E served as a test group and was pre-treated with 200 mg/kg BSAE
Group F served as a test group and was pre-treated with 400 mg/kg BSAE

The rats were subjected to a 48 h fast before the commencement of the experiment. However, they were allowed access to water *ad libitum*. After the fast, each group of animals was treated as stated above orally and once daily for 7 days.Acute PCM intoxication (2 g/kg) was carried out on the 8th day except for Group A which served as normal control. Forty-eight hours after the hepatic injury induction, all rats were fasted overnight and sacrificedunder slight anaesthesia and blood samples were collected viacardiac puncture into plain bottles for biochemical analysis.The following parameters were determined.

Assays/testsof liver function parameters

Both enzyme and non-enzyme liver function biomarkers were investigated. The activities of Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayedemploying the method of Reitman and Frankel (1957) as described in Randox kits while the activity of alkaline phosphatase (ALP) was assayed according to the method of Kind and King (1972). The concentrations of Total cholesterol (TC) and triacylglycerol (TAG) were estimated using the method of Trinder (1969). The concentrations of Total bilirubin (Tbil) and direct bilirubin were measured according to the method of Jendrassik and Grof (1938). The concentration of Total protein (TP) was determined by Biuret method (Doumas, 1975) while that of albumin was determined according to the method of Spencer and Price (1971).

Histopathological examination

The organ of concern which is the liver were collected from rats in all thegroups after sacrifice and fixed in 10% formalin for Histopathological examination.

Statistical Analysis

The Data generated in this study were presented as Mean ± Standard Deviation (SD). Statistical comparisons were performed by one-way ANOVA, followed by Duncan's multiple comparisons test and the values were considered statistically significant when p-value is less than 0.05.

III. RESULTS

Acute Toxicity

The resultsof the acute toxicity studies are presented in **Table 1**. BSAE up to a dose of 5000 mg/kg did not produced physical signs of toxicity nor mortality. The oral LD_{50} of the extract was then taken to be > 5000 mg/kg (Lorke's method).

Table 1: Observations from Acute Toxicity Study of the Aqueous Extract of *Borreria stachydea*Leaves (BSAE)in Rats

Phase	Group	Treatment (mg/kg)	D/T	Observed Sign of Toxicity
I	1	BSAE(10)	0/3	-
	2	BSAE (100)	0/3	-
	3	BSAE(1000)	0/3	-
II	1	BSAE(1600)	0/1	-
	2	BSAE(2900)	0/1	-
	3	BSAE(5000)	0/1	-

D=death, T= No of animals treated

Liver Function Parametersof Rats with Paracetamol-Induced Liver Damage

Table 2 shows theresultof the pre-treatment of rats with graded doses of BSAE on theserum activities of AST, ALT and ALP. Following paracetamol administrationa significant ($p< 0.05$) increase in the activities of these enzymeswere observed inthe negative control compared to the normal control. However, in the groups pre-treated with the positive standard and 200 and 400 mg/kgBSAE,no significant ($p> 0.05$) difference in the activities of AST, ALP and ALP was observed when compared to the normal control. As seen in **Tables 3 and 4**, similar trends were observed withthe serum concentrations of total cholesterol,triglycerides and bilirubin. Paracetamol poisoning produced a significant ($p< 0.05$) increase in the serum concentrations of these parameters as seen in the negative control when compared to the normal control. Treatment with graded doses of BSAEparticularly 200 and 400 mg/kg produced significant ($p< 0.05$) protection as there wasno significant ($p> 0.05$) difference in the cholesterol, triglycerides and bilirubin concentrations of these groups compared to the normal control. **Table 5** shows the result of the pre-treatment of rats with graded doses of BSAE on the serum concentrations total protein and albumin. Paracetamol administration produced a significant ($p< 0.05$) decrease in the concentrations of total protein and albumin as observed in the negative control compared to the normal control. Pre-treatment with 200 and 400 mg/kg BSAE produced a significant ($p< 0.05$) protection as no

significant (p> 0.05) difference was observed in the concentrations of total protein and albumin of the groups that received these pre-treatment when compared to the normal control.

Table 2:Effect of Aqueous Extract of *Borreria stachydea*Leaves(BSAE)on Enzymatic Liver Function Parameters of Rats with Paracetamol-Induced Liver Damage

Treatment	AST IU/L	ALT IU/L	ALP IU/L
Normal Control (5 ml/kg 10% DMSO)	40.11±1.74[a]	42.34±2.43[a]	28.26±1.25[a]
Negative Control (5 ml/kg 10% DMSO + PCM)	73.14±5.49[c]	60.72±5.24[c]	41.73±2.71[b]
Positive Control (50 mg/kg 10% NAC + PCM)	43.26±2.27[a]	45.25±3.22[a]	28.41±1.26[a]
Test (100 mg/kg BSAE + PCM)	55.85±4.34[b]	58.01±2.15[b]	38.32±1.08[b]
Test (200 mg/kg BSAE + PCM)	43.11±1.94[a]	43.22±1.26[a]	28.26±1.91[a]
Test (400 mg/kg BSAE + PCM)	40.26±1.06[a]	42.28±1.17[a]	29.16±1.45[a]

Values shown are mean ± S.D. (n = 5). Mean values with different alphabets as superscripts down the column are significantly different at $P<0.05$

Table 3:Effect of Aqueous Extract of *Borreria stachydea*Leaves(BSAE)on Lipid Profile Parameters of Rats with Paracetamol-Induced Liver Damage

Treatment	TC (mg/dl)	TAG (mg/dl)
Normal Control (5 ml/kg 10% DMSO)	65.25±2.73[a]	46.03±1.62[a]
Negative Control (5 ml/kg 10% DMSO + PCM)	100.31±9.01[b]	71.23±5.23[b]
Positive Control (50 mg/kg 10% NAC + PCM)	67.23±3.11[a]	45.63±3.17[a]
Test (100 mg/kg BSAE + PCM)	65.41±5.25[a]	46.38±3.15[a]
Test (200 mg/kg BSAE + PCM)	64.18±2.15[a]	48.41±2.23[a]
Test (400 mg/kg BSAE + PCM)	65.40±4.33[a]	46.16±3.08[a]

Values shown are mean ± S.D. (n = 5). Mean values with different alphabets as superscripts down the column are significantly different at $P<0.05$

Table 4:Effect of Aqueous Extract of *Borreria stachydea*Leaves(BSAE)on Bilirubin Concentration of Rats with Paracetamol-Induced Liver Damage

Treatment	Tbil (µmol/L)	Dbil (µmol/L)
Normal Control (5 ml/kg 10% DMSO)	4.34±0.28[a]	2.16±0.12[a]
Negative Control (5 ml/kg 10% DMSO + PCM)	8.73±0.15[b]	3.21±0.13[b]
Positive Control (50 mg/kg 10% NAC + PCM)	4.11±0.26[a]	2.13±0.15[a]
Test (100 mg/kg BSAE + PCM)	4.26±0.13[a]	2.26±0.11[a]
Test (200 mg/kg BSAE + PCM)	4.45±0.21[a]	2.28±0.12[a]
Test (400 mg/kg BSAE + PCM)	4.22±0.35[a]	2.30±0.19[a]

Values shown are mean ± S.D. (n = 5). Mean values with different alphabets as superscripts down the column are significantly different at $P<0.05$

Table 5:Effect of Aqueous Extract of *Borreria stachydea*Leaves(BSAE)on Serum Protein Concentration of Rats with Paracetamol-Induced Liver Damage

Treatment	TP (mg/l)	ALB (mg/l)
Normal Control (5 ml/kg 10% DMSO)	67.32±3.38[b]	40.22±2.53[b]
Negative Control (5 ml/kg 10% DMSO + PCM)	41.11±2.44[a]	20.11±1.37[a]
Positive Control (50 mg/kg 10% NAC + PCM)	57.41±4.22[ab]	38.15±2.29[b]
Test (100 mg/kg BSAE + PCM)	60.23±3.18[ab]	36.21±3.45[b]
Test (200 mg/kg BSAE + PCM)	65.32±3.46[b]	35.45±3.23[b]
Test (400 mg/kg BSAE + PCM)	65.04±3.21[b]	38.26±4.34[b]

Values shown are mean ± S.D. (n = 5). Mean values with different alphabets as superscripts down the column are significantly different at $P<0.05$

Histological Findings

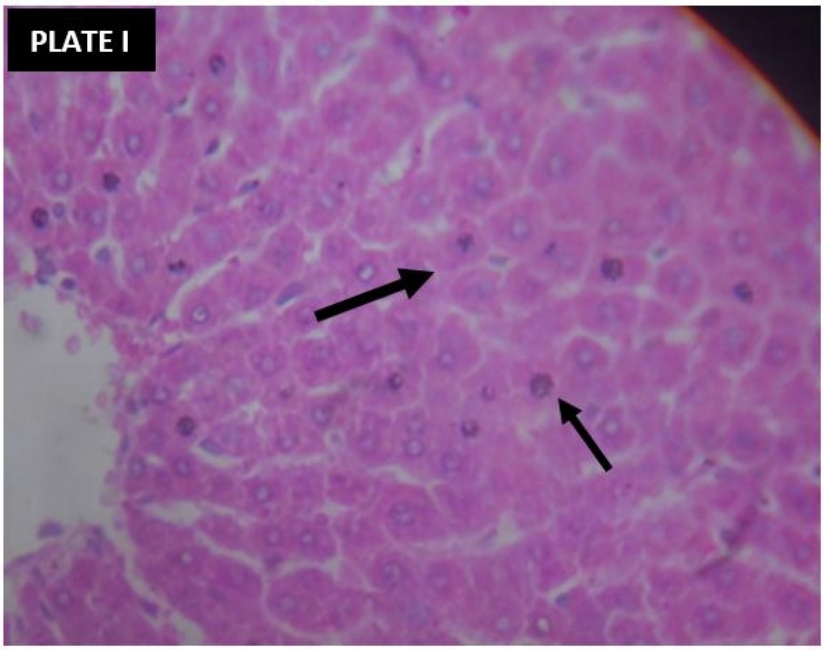

Plate I: Histologic section of a liver tissue from a normalcontrolrat. Normal structure of hepatocellular alignmentand Granular cytoplasm. H & E X400 mag.

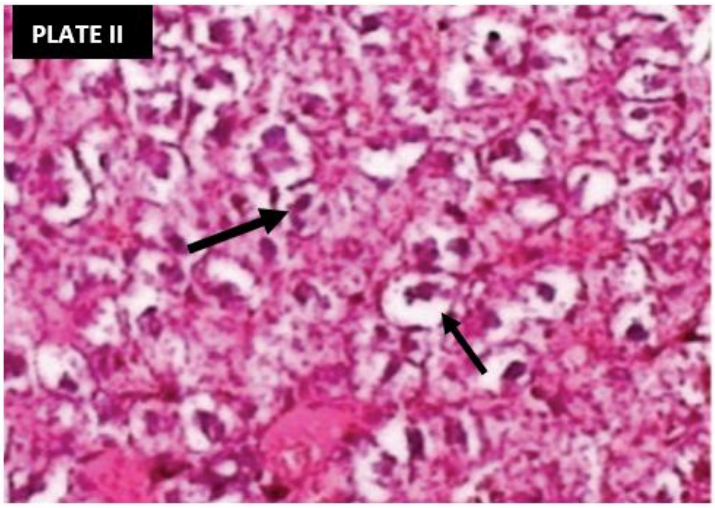

Plate II: Histologic section of a liver tissue from a negative control rat. Areas of necrosis and oedema present following injury caused by paracetamol. H & E X400 mag.

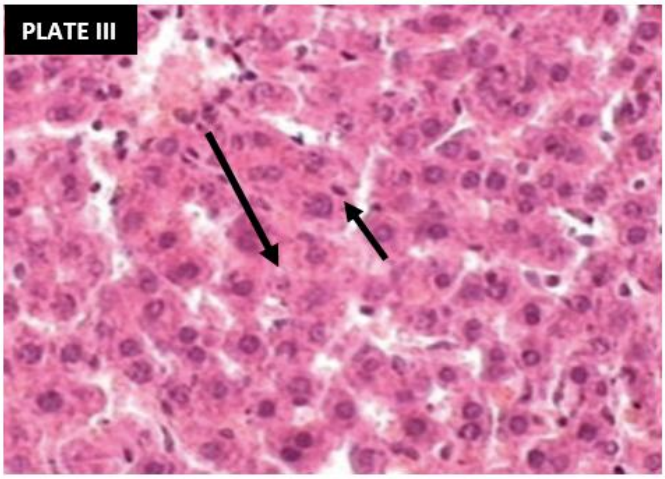

Plate III: Histologic section of a positive control rat.Newly proliferating and few necrotized hepatocytes seen following hepatic necrosis caused by paracetamol. H & E X400 mag.

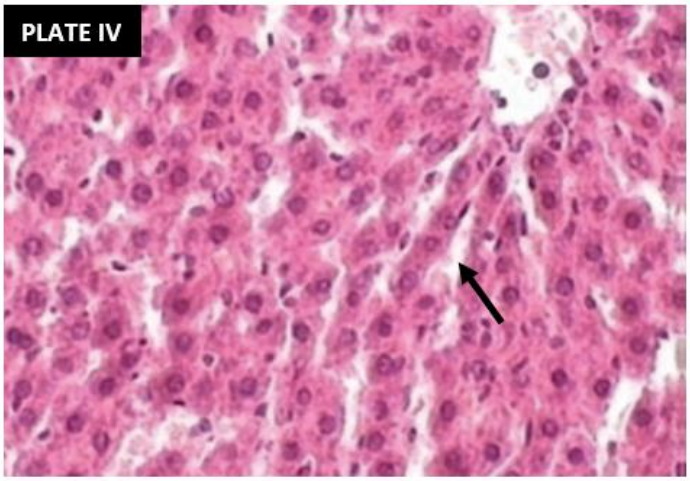

Plate IV: Histologic section of a 100 mg/kg BSAE treated-rat. Few necrotized hepatocytes seen. H & E X400 mag.

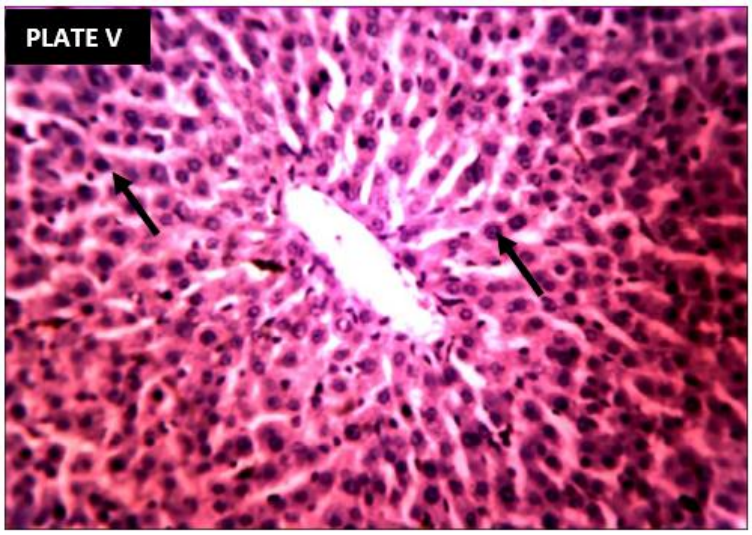

Plate V: Histologic section of a 200 mg/kg BSAE treated-rat. Normal structure of hepatocellular alignment and Granular cytoplasm. H & E X400 mag.

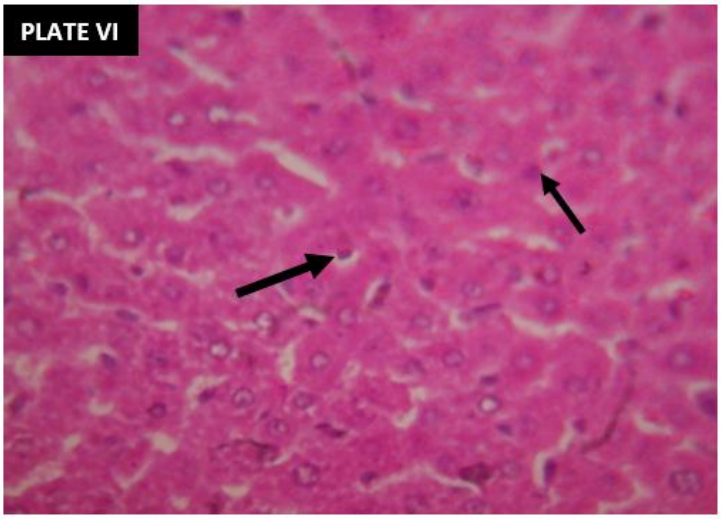

Plate VI: Histologic section of a 400 mg/kg BSAE treated-rat. Normal structure of hepatocellular alignment and Granular cytoplasm. H & E X400 mag.

IV. DISCUSSION

The liver is a vital organ to the existence of living things asit perform diverse rolesranging from biosynthesis of biologically important molecules to clearance of foreign substances such as drugs from the living system (Dancygier and Strassburg, 2010). Liver is the major organ responsible for metabolism and excretion of drugs as hepatocytes contain a class of enzymes known as Cytochrome p450 enzymes that are responsible for drugmetabolism (Lukong*et al.,* 2018). There are situations where these enzymes are overwhelmed due to large intake of drugs (overdose). When this happens, the maximum metabolic threshold of the liver is said to have been exceeded and this usually give rise to some deleterious effectson the liver and hence, liver diseases (Imo *et al.,* 2021).

Paracetamol when administered in large quantities as a single dose or repeatedly can induceacute liver injury and this has become a standard model for hepatotoxicity in pharmacological research. (Chakrabarti *et al*., 1978). In the liver, there are many pathways through which paracetamol can be metabolized. These include cytochrome P450 enzyme system,glucuronidation (conjugation with glucuronic acid) or sulfation (addition of a sulfate group) (Ghaffar *et al*., 2014). Glucuronidation and sulfation convert paracetamol into water-soluble and nontoxic compounds which are subsequently excreted inthe urine. Asmall percentage of paracetamol is however, excreted unchanged in the urine (Ullah *et al*., 2022). In a study by Larson *et al*., (2005), avery little amount of paracetamol was metabolizedmainly by CYP 2E1 which belongs to the family of the cytochrome P450 enzymes. The product of this metabolism was a highly reactive and toxic metabolite, N-acetyl-benzoquinone imine (NAPQI), which was subsequentlyinactivated by glutathione (Lancaster *et al*., 2015). At doses that exceeds the metabolic capability of the liver, the cytochrome P450 enzymes and glutathione can become saturated, resulting in the accumulation of the intermediate- NAPQI and its subsequent interaction with important biomolecules in the cell such asproteins. This leads to cellular damage, jaundice, liver necrosis,reduced synthetic capability and bilirubin accumulation, and fulminant hepatic dysfunction (Ibrahim *etal*., 2013, Dokugan*et al.,* 2016).

In this study, pretreatment of the rats with BSAEat doses of 100, 200 and 400 mg/kg before intoxication with paracetamol produced significant protection to the liver as evident in the various biomarkers examined. The elevation of theactivities of AST, ALTthat occurred following paracetamol intoxication as seen in the negative control was not seen in the treated groups. This observation is indicative of the ability of the extract tooffer stabilization to the plasma membrane as well as rejuvenateany hepatic tissue damage caused by paracetamol. The ability of the extract to suppress the elevation in serum total cholesterol, triglycerides, total bilirubin and direct bilirubin is another pointer to the ability of the extract to stabilize the plasma membrane. The liver is usually the major site of synthesis for majority of the proteins found in the serum. Proteins such as albumin and fibrinogen are synthesized in the parenchymal cells of liver. Decreased concentration of albumin as produced by paracetamol in this study usually occurs as a result of increased vascular permeability due to inflammation and thisis indicative of liver disease. (Thapa and Walia, 2007, Orji *et al.,* 2020). The observed decrease in total protein might be as a result of reduced number of cells responsible for protein synthesis in the liver due to necrosis (Goldwasser and Feldman, 1997).In this study, an increase in the total plasma protein content suggests the ability of the extract to stabilize the plasma membrane and also regenerate the worn out cells of the liver (Mukherjee *et al.,* 2002).Theobservations were further confirmed by histopathological

examination. The study therefore shows the ability of the extract topreserve the structural integrity of the hepatocellular membrane and liver cell architecture.

Certain phytochemicals have been reported to be responsible for hepatoprotective activity. These include triterpenes, flavonoids and tannins (Babatunji*et al.,* 2017). The presence of such phytochemicals in *Borreria stachydea*have been reported. These phytochemicals might have probably contributed to its pharmacological activity as observed in this study. Resultsof this study also revealed the safety of the extract when consumed acutely. No sign of toxicity nor mortalitywas observed up toa dose of 5000 mg/kg of BSAE. The oral LD_{50} of BSAE was therefore taken to be > 5000 mg/kg according to Lorke et al., (1983).

V. CONCLUSIONS

In this study, the aqueous extract of *Borreria stachydea*leavesproduced a promising hepatoprotection activity in rats withparacetamol-induced liver damage. Thus the extractcould be useful in the management of liver diseases.

Conflict of interest: The authors declares no conflict of interest.

REFERENCES

[1]. Anas, A., AmbiA. A., Zainab, M. M. Saleh I. A. and Saifullahi, U.(2023). Evaluation of Phytochemical and Anti-Inflammatory Effects of Hexane Extracts of Borreria stachydea [(DC) Hutch. and Dalziel] (Rubiaceae) DUJOPAS; 9 (2a): 124-132.

[2]. Babatunji E.,Osunsanmi, O.,Ajiboye, B.,Ojo, A., Kappo, P. (2017) .Modulatory Effectof Methanol Extract of Piper guineense in CCl4-Induced Hepatotoxicity in Male Rats. Int. J. Environ. Res. Public Health, 14(9), 955; https://doi.org/10.3390/ijerph14090955

[3]. Chakrabarti, S, Choudhuri S. K, Sikdar S. (1978). Studies on the protective effect of livergen on hepatotoxicity produced by drugs. Ind. J Pharmac.; 10(1):85.

[4]. Daly, M. J: (1999). Hepatic Disease, Clinical Pharmacy and Therapeutics, Churchill Livingstone, New York, 195-212.

[5]. Dancygier, H. and C. P. Strassburg (2010).Hepatic Drug Metabolism and Drug Toxicity, Springer, Berlin, Germany.2 (1) 12.

[6]. Dogukan, C., Yasin, B., Nurcan, B.,Esen, S., Hasan, T., Fatma, B., … Zekai H., (2016) Paracetamol-induced nephrotoxicity and oxidative stress in rats: the protective role of Nigella sativa. Pharmaceutical Biology, 2016 Vol. 54, NO. 10, 2082–2091 http://dx.doi.org/10.3109/13880209.2016.1145701

[7]. Doumas, B. T. (1975). Standards for total serum protein assays- a collaboration study: Clinical Chemistry, 21: 1159- 1166..

[8]. Ghaffar U. B., Tadvi N. A. (2014). Paracetamol toxicity: A review. J Contemp Med A Dent. 2014; 2:12-15.

[9]. Handa S. S, (1990). Hepatoprotective activity of andrographolide from Andrographis paniculata against carbon tetrachloride. The Indian journal of medical research; 92:276-283.

[10]. Ibrahim T, Agnihotri S, Agnihotri A. K. (2013). Paracetamol toxicity-an overview. Emergency Medicine; 3(158):1-3

[11]. Imo, C.,Adebisi K., Ezeonu C.,Ikwebe, J.,Ejeh, O., Imo, N.,&Danlami, G. (2021). Biochemical and histological effects of ethanolic extracts of fruits of Xylopia aethiopica and seeds and leaves of Piper guineenseon liver and kidney function in male albino rats. Future Journal of Pharmaceutical Sciences 7:35.

[12]. Jendrassik, L. and Grof, P. (1938). Vere infachte photometric che method enzurbestm- mung des blutbiluribins. BiochemischeZeitschrift. 297: 82– 89.

[13]. Kind, P. R. N. and King, F. J. (1972). Alkaline phosphatase determination. Clinical Pathology, 7: 322- 360.

[14]. Lancaster E. M, Hiatt J. R, Zarrinpar A. (2015). Acetaminophen hepatotoxicity: an updated review. Archives of toxicology; 89:193-199.

[15]. Larson A. M., Polson J, Fontana R. J, Davern T. J, Lalani E,Hynan LS, (2005). Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study. Hepatology;42(6):1364-1372.

[16]. Lorke, D. (1983). "A new Approach to Practical Acute Toxicity Testing." Archives of Toxicology 54: 275-287.

[17]. Lukong, B., Ifemeje, C., Chukwuka, T., &Onah, C., (2018) Effects of aqueous extracts of Gongronemalatifolium leaves and Piper guineenseseeds on liver and kidney

[18]. McGovern,A.,Vitkovitsky, I., ones, D.,Mullins M., (2015) Can AST/ALT ratio indicate recovery after acute paracetamol poisoning. Clin Toxicol (Phila). 2015 Mar; 53(3):164-7. doi: 10.3109/15563650.2015.1006399. PMID: 25652258.

[19]. Mukherjee , P. K. (2002) Quality control of herbal drugs, 1st ed, Business Horizons Pharmaceutical Publication, New Delhi, pp. 531.

[20]. Neoh, B.K., Rahayu, U.U. Nordin, H.L, Tai, Y.C, Tu YL, Mawardi, R, and Mohd, A.S. (2010). The Malaysian Journal of Analytical Sciences. 14 (1); 6-11.

[21]. Nikolaos, P., and Laura R., (2020) Liver injury by paracetamol and challenges associated with international and unintentional use. World journal of hepatology. 125-136. Doi 10.4254/wjh.v12.i4.125.

[22]. Orji, B., Obi F., & Ibrahim M. (2020). Effects Of Chronic Exposure Paracetamol And Hibiscus Sabdariffalinn Calyx Extract On The Kidney Of Mice. FUDMA Journal of Sciences (FJS). ol. 4 No. 2, pp 60-66 DOI: https://doi.org/10.33003/fjs-2020-0402 116

[23]. Reitman, S. and Frankel, S. (1957). Method of alanine and aspartate aminotransferase determination. American Journal of Clinical Pathology, 28: 56– 58.

[24]. Spencer, K. and Price, C. P. (1971). Determination of serum albumin using bromoscresol techniques. Annals of Clinical Biochemistry, 14: 105-115.

[25]. Thapa, B. R, Walia A. Liver function tests and their interpretation. The Indian Journal of Pediatrics. 2007; 74:663-671.

[26]. TittarelliR.,Pellegrini, M., Scarpellini, M.,Marinelli, E.,Bruti, V.,di Luca, N.,Busardò, F., &Zaami S. (2017). Hepatotoxicity of paracetamol and related fatalities biomarkers of albino rats. Trop. J. Appl. Nat. Sci., 2(2): 26-33 (2018) ISSN: 2449-2043

[27]. Trinder, P. (1969). Enzymatic determination of triglycerides by GOP-PAP method. Annals of Clinical Biochemistry, 6: 24-27

[28]. Ullah H, Khan A, Bibi T, Ahmad S, Shehzad O, Ali H (2022). Comprehensive in vivo and in silico approaches toexplore the hepatoprotective activity of poncirin againstparacetamol toxicity. Naunyn-Schmiedeberg's Archivesof Pharmacology; 21(1):1-34.

[29]. Woolley, D., & Woolley, A., (2017). Practical Toxicology: Evaluation, Prediction, and Risk, Third Edition. CRC Press. p. 330. ISBN 9781498709309. Archived from the original on September 10, 2017. Retrieved July 5, 2017.