

HISTOMORPHOLOGICAL IMPACT OF AQUEOUS EXTRACT OF *TALINUM TRIANGULARE* ON PARACETAMOL-INDUCED HEPATIC CELL INJURY IN WISTAR RATS

ABSTRACT

Background and Aim: Many vital bodily processes are carried out by the liver, and if it develops a disease, those processes may cease, which might seriously affect the function of the body. *Talinum triangulare* has been documented to possess hepato-protective, antioxidant and anti-inflammatory activities. This study investigated the potentials of *Talinum triangulare* extract against paracetamol-induced hepatic cell injury. **Method:** For the investigation, thirty-five (35) albino rats of both sexes were employed. They were assigned into 5 groups of seven rats. The dosage of the extract given to each group was determined by the average weight of the albino rats. The negative control group was given regular animal feed and water, whereas the positive control group was given just 200 mg/kg body weight of paracetamol. Animals in Groups 3, 4 and 5 were given 200 mg/kg body weight of paracetamol to induce toxicity and thereafter treated with 100, 200 and 300 mg/kg body weight of aqueous extract of *T. triangulare* respectively. The experiment lasted for 14 days. **Result:** Histomorphological examination after the experimental period revealed serious liver deterioration in the untreated group (Group 2), but the *T. triangulare*-treated groups (Groups 3, 4 and 5) demonstrated significant healing. **Conclusion:** The findings from this research reveals that *T. triangulare* is capable of reducing the hepatotoxic effect incurred by paracetamol. **Significance:** The result from this study will create more awareness on the need to avoid the abuse of paracetamol.

Keywords: Paracetamol, *Talinum triangulare*, Liver injury, histology

INTRODUCTION

The liver is a vital organ that regulates several important biochemical and biological activities like homeostasis, growth, energy, nutrient delivery, drug and other xenobiotics, detoxification, and healing infection. It is highly vulnerable to injury from hepatotoxic substances [1,2]. An alternated liver function resulting in illness is liver disease or hepatic disease. Many vital bodily processes are carried out by the liver, and if it develops a disease, those processes may cease, which might seriously affect the function of the body [3]. Liver diseases are quickly becoming acknowledged as a public health priority. In India, acute liver disease mortality is 5-6.3%, chronic liver disease, including cirrhosis (hepatitis B virus), mortality is 17.6-47.9%, and liver cancer (HBV) mortality is 40-60% [4].

Paracetamol, also known as acetaminophen, is frequently used to treat pain and fever in everyday conditions. It is one of the main causes of drug-induced liver injury especially when taken in excess of prescribed dosages [5,6]. Intentional or unintentional overdoses are the leading cause of drug-induced liver injury in the United States and a major global health concern [7,8]. The widespread use of combination drugs, such as painkillers and antihistamines, may conceal the presence of paracetamol, contributing to more than half of acute liver failure cases [8]. Furthermore, paracetamol levels might rise dangerously above the crucial 4-hour window for medical intervention when taken with other drugs [9,10].

Numerous population-based studies have revealed that large doses of paracetamol may also result in liver damage, which is consistent with the results of animal studies [11, 12, 6]. The link between paracetamol and the incidence of liver cancer has also been examined in earlier epidemiological studies [13,14,15]. Paracetamol poisoning is the leading cause of liver transplants and the second most prevalent cause globally. It causes 500 fatalities, 2600 hospitalizations, and 56,000 visits to the emergency department annually. Accidental overdoses account for half of these cases [16,17].

Herbal medicines are safe and devoid of major adverse reactions, which has led to a significant increase in the usage of herbal medicines to treat ailments worldwide [18,19]. They can also be quickly and readily derived from nature [20]. Pharmacological studies have demonstrated hepatoprotective, antioxidant and anti-inflammatory activities supporting the traditional uses of some leafy vegetables [21]. The current study focuses on the histomorphological impact of aqueous extract of *Talinum triangulare* on paracetamol-induced hepatic cell injury in Wistar rats.

MATERIALS AND METHODS

Drug purchase and preparation: Acetaminophen was purchased from a registered pharmaceutical store in Enugu. The drug was dissolved in water to appropriate concentration for administration. The drug was of analytical grade.

Plant Sample and Preparation: *Talinum triangulare* leaves were purchased at Ogbete market in Enugu, Enugu State. A sample was taken to the University of Nigeria Nsukka's Department of Botany for identification and authentication. After identification and authentication, it was given the voucher number; UHAE 2013/76. *T. triangulare* leaves were washed thoroughly with tap water and dried under normal atmospheric temperature. The dried leaves were pulverized into powder using a household blender and sieved to obtain a fine powder particle weighing 150 g. Thereafter, it was extracted in 500 ml distilled water for 48 hours. The mixture was filtered using Whatman no 1 filter paper and the yield was concentrated into paste using a Buchi Rotavapor.

Experiment Design: Thirty-five adult albino rats weighing between 150 to 200 g were purchased from the animal house of the Department of Human Anatomy, University of Nigeria Enugu Campus. They were housed in clean, well ventilated cages in the animal house of the department of Medical Laboratory Science, University of Nigeria, Enugu Campus. The animals were acclimatized for two weeks, and given unlimited access to water and pellets. The research procedure adhered to the International Ethical Norms on Animal Care and Use as outlined in NIH publication/85-23, which was amended in 1985. The rats were placed into five treatment groups, each containing seven rats. The following procedures were performed during the 14-day trial:
Group I: Negative control received feed and water only.

Group II: The positive control group (untreated) was administered 200 mg/kg body weight of acetaminophen for 7 days.

Group III: was administered 200 mg/kg body weight of acetaminophen for 7 days and treated with 100 mg/kg body weight of *T. triangulare* aqueous extract for 7 days.

Group IV: was administered 200 mg/kg body weight of acetaminophen for 7 days and treated with 200 mg/kg body weight of *T. triangulare* aqueous extract for 7 days.

Group V: was administered 200 mg/kg body weight of acetaminophen for 7 days and treated with 300 mg/kg body weight of *T. triangulare* aqueous extract for 7 days.

Tissue Homogenization Preparation: The liver was removed and washed with 0.25 M sucrose solution to eliminate any traces of sugar following the slaughter and dissection of the animals. Thereafter, it was weighed and homogenized in a cold 0.25 M sucrose solution (1:5 w/v) using a Teflon homogenizer before being utilized in the experiment. The supernatant was maintained frozen at -50°C in a freezer.

Histological Processing: The excised liver organ was sliced into 0.5 mm thick slabs and preserved in a 10% neutral buffered formalin solution. Utilizing an Automatic Tissue Processor, they were processed using the paraffin wax embedding procedure. The Rotary Microtome was used to obtain 5µm thick sections (2125 Leica Rotary Microtome, GmBh model). The technique of hematoxylin and eosin staining was used [22].

Microscopy and Photomicrography: An Olympus binocular microscope with an in-built illumination system was used to examine the sections. The sections were then photographed with a Samsung Model SS850 digital microscope camera coupled to an Olympus trinocular microscope.

RESULT

Photomicrographs showing histological changes in the liver after paracetamol treatment with and without *T. triangulare* aqueous extract (H&E 400x).

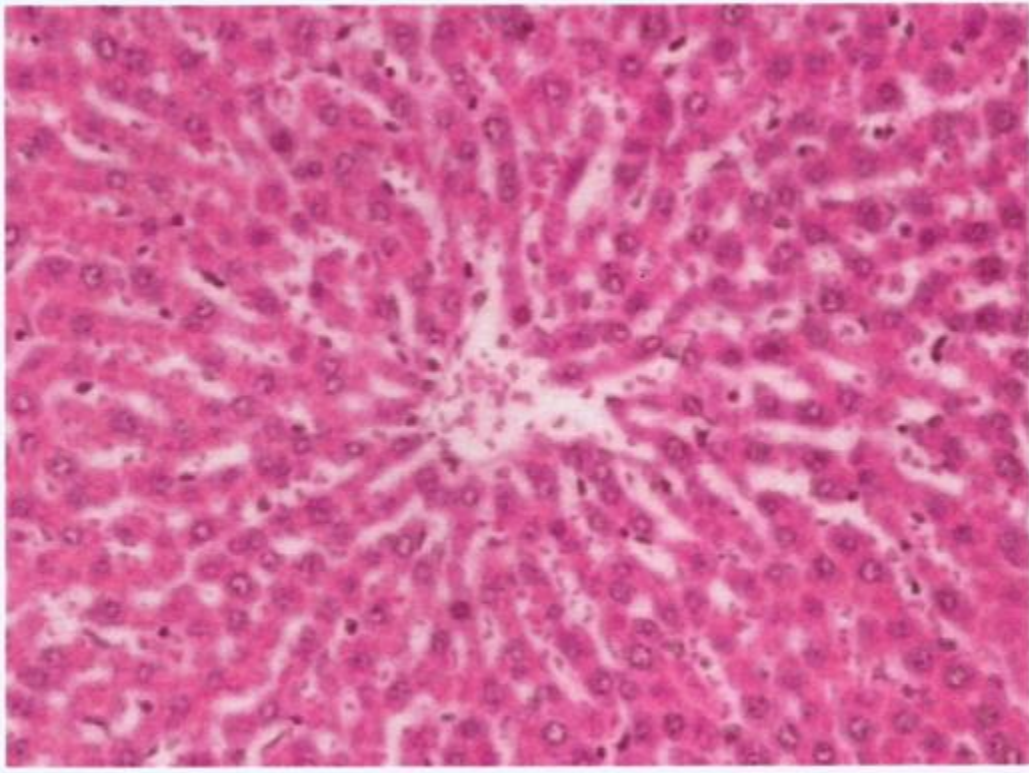


Figure 1: Group1 (Negative control): showing normal tissue morphology. (H&E X400). HC: Hepatocyte, CV: Central vein.

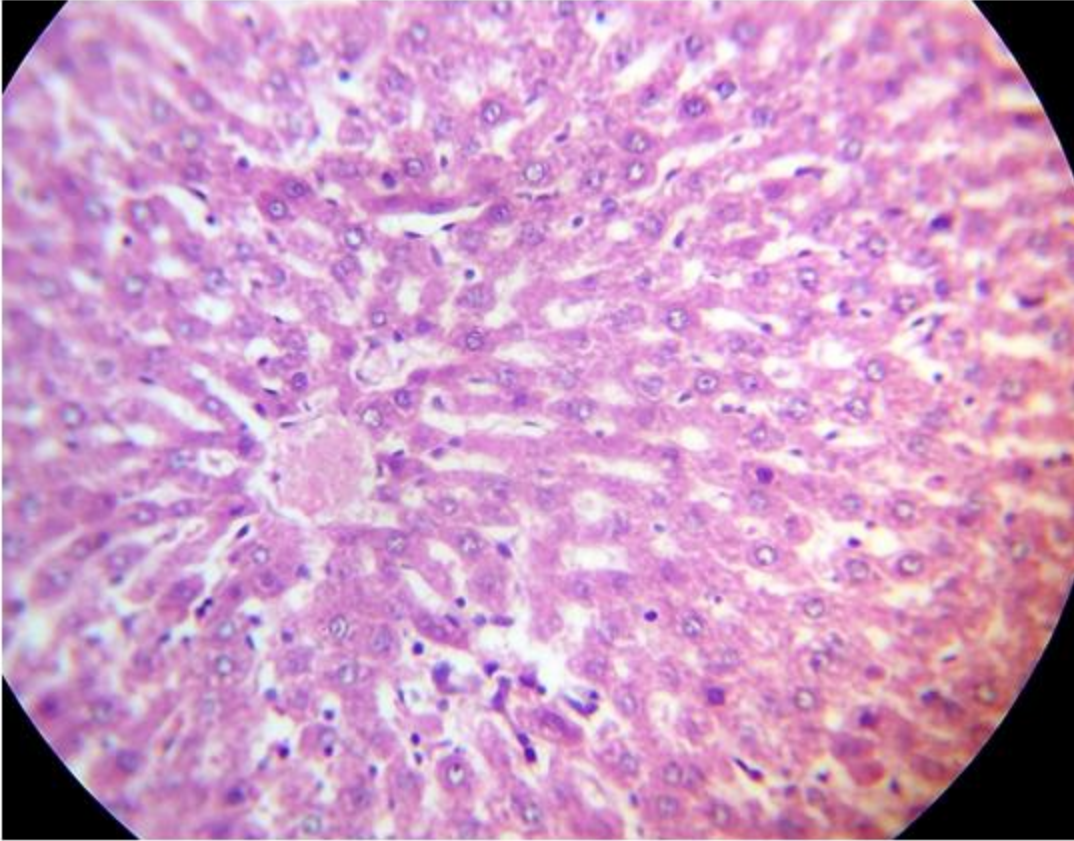


Figure 2: Group 2 (Positive control): Rat's liver tissue administered 200mg/kg body weight of paracetamol showed; hepatocyte deformation, a clogged and enlarged portal vein (CG), an expanded bile duct (EB), and an Area of Necrosis (NC).

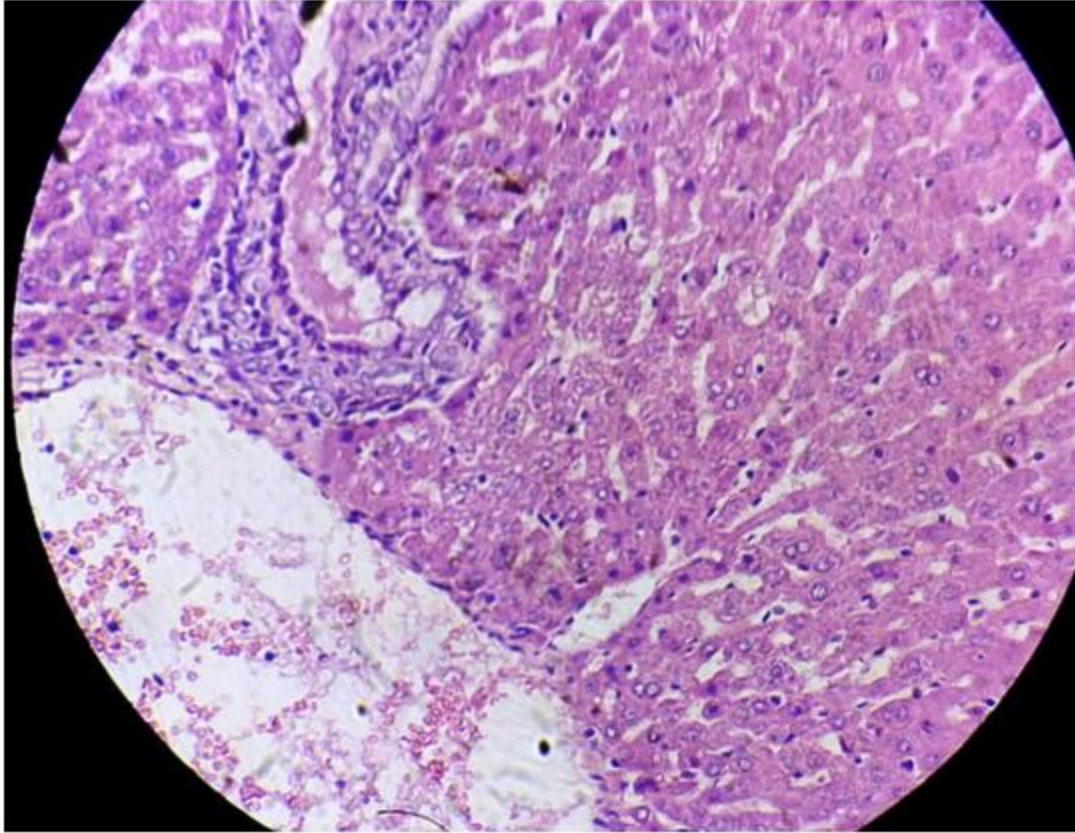


Figure 3: Group 3: Rat's liver tissue treated with paracetamol 200mg/kg followed by *T. triangulare* 100 mg/kg body weight extract showed; inflammatory cells infiltrated the periportal space in a moderate amount, and portal congestion was mild. (H&E X400)

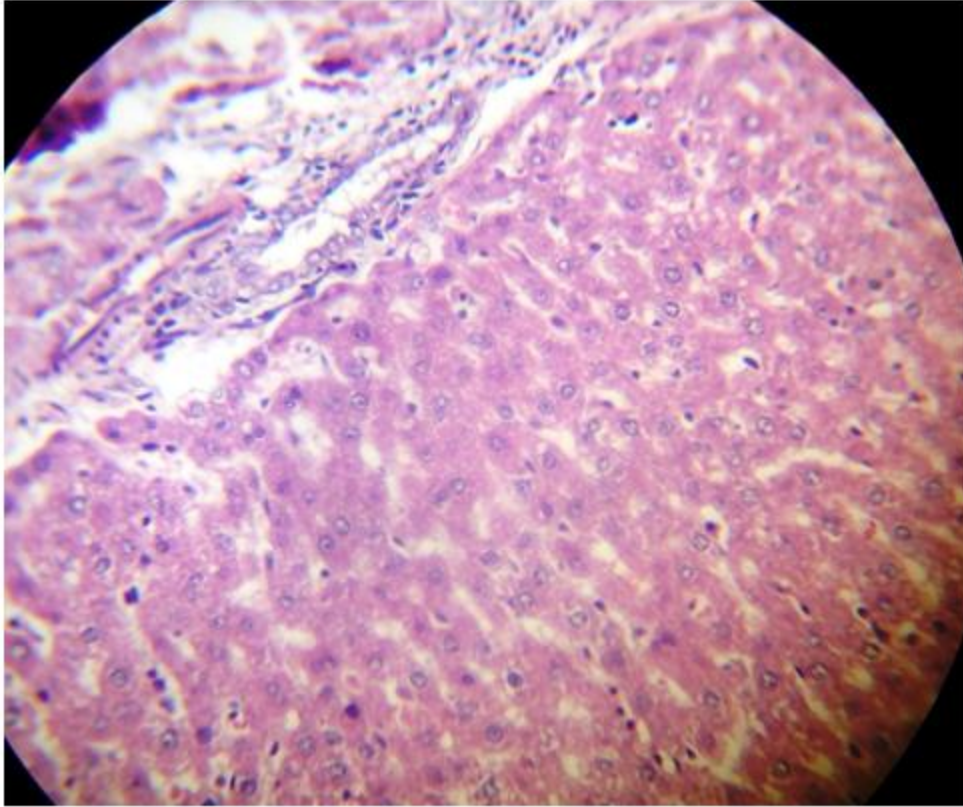


Figure 4: Group 4: Rat's liver tissue treated with paracetamol 200mg/kg followed by *T. triangulare* 200 mg/kg body weight extract showed; hepatocytes with significant diffuse vacuolar degeneration (VD) and minor portal congestion. Only a small percentage of hepatocytes appeared necrotic. (H&E X 400)

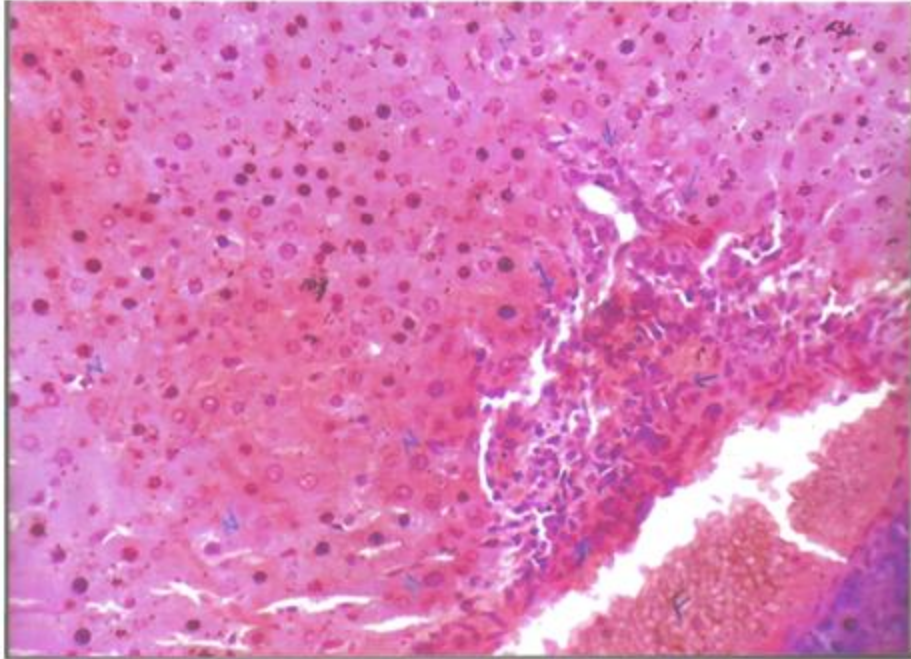


Figure 5: Group 5: Rat's liver tissue treated with paracetamol 200mg/kg followed by 300mg/kg body weight of *T. triangulare* extract showed; a moderate to high healing effect to periportal cellular infiltration by inflammatory cells and periportal fibrosis (PF) and a moderate regeneration of Hepatocytes. (H&E X400)

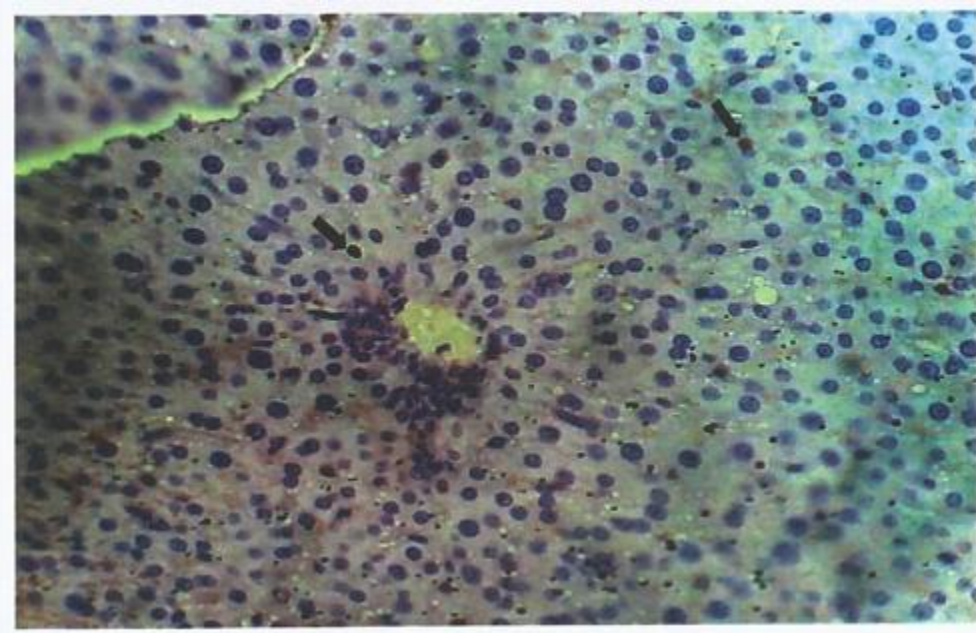


Plate 1: Immunohistochemistry of Liver section showing scanty expression of the marker Ki67

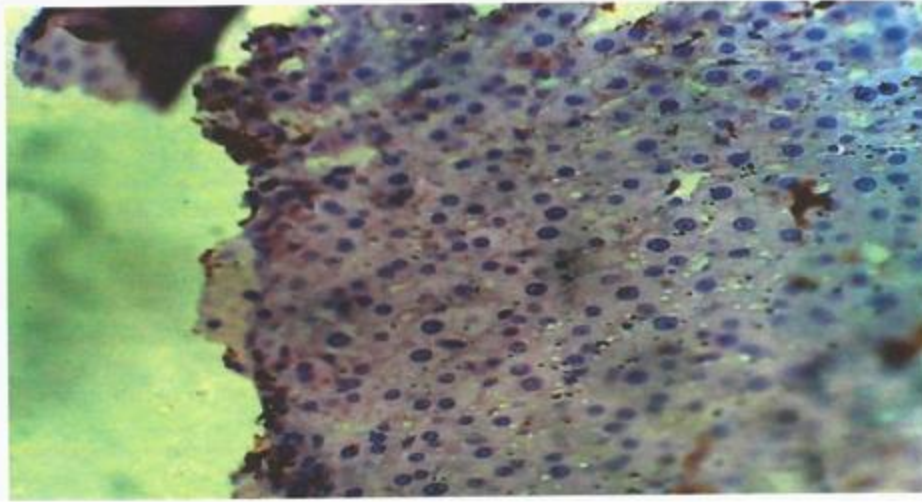


Plate 2: Immunohistochemistry of Liver section showing no expression of the marker Ki67

DISCUSSION

This research investigated the histomorphological impact of aqueous extract of *Talinum triangulare* on paracetamol-induced hepatic cell injury in Wistar rats. Studies have revealed that *Talinum triangulare* possess a variety of biological activities such as hepatoprotective, anti-inflammatory and antioxidative effects.

Figures 1-5 represents the histological effects aqueous extract of *Talinum triangulare* on histomorphological examination of liver tissue of paracetamol-induced hepatic cell injury in an animal model. Group 1 (negative control) had no lesion. Normal liver tissue morphology was observed. Group 2 (Negative control) treated with 200 mg/kg body weight of paracetamol had obvious lesions and necrosis. Group 3 (200 mg/kg b.w. of paracetamol+ 100 mg/kg b.w. of *T. triangulare* extract) revealed a weak to moderate hepatic cell repair and mild periportal cellular infiltration by inflammatory cells. Group 4 (200 mg/kg b.w. of paracetamol + 200 mg/kg b.w. of *T. triangulare* extract) showed considerable portal congestion with hepatocytes having moderate healing effects. It was also observed that just a small percentage of hepatocytes were necrotic. Finally, in Group 5 (200 mg/kg b.w. of paracetamol + 300 mg/kg of *T. triangulare* extract), it was observed that inflammatory cells infiltrated the periportal space, causing periportal fibrosis, and hepatocytes had a moderate to strong healing effect.

From the histological examination, necrosis, hepatocyte deformation, a clogged and enlarged portal vein (CG), an expanded bile duct (EB), and an area of necrosis was observed in the paracetamol-induced group. This is in line with documented studies by [23,24,12], where distorted hepatocyte, necrosis and inflammation progressed in the acetaminophen-induced hepatotoxic group [25]. Histology of the liver tissue showed significant activity of *Talinum triangulare* leave extract as revealed in the severity of damages observed only in paracetamol treated group (Group 2). The attenuating potentials of *Talinum triangulare* demonstrated that it possesses hepatocellular mitigating properties in the face of paracetamol intoxication, which agrees with the study by [26], which revealed that *Talinum triangulare* can protect the liver against hepatocellular toxicity which has been attributed to oxidative stress [14]. This research collaborates the pharmacognosis claims that *Talinum triangulare* is able to withstand intoxicants directed at the liver. This might be as a result of its abundant phytochemical properties which consists of primary and secondary metabolites collectively assisting in general protection of organs responsible for drug metabolism and elimination [27]. Necrosis appears to be the dominant cell death pathway in paracetamol intoxication, which is partially preventable with herbal treatment [2].

There was scanty expression of Ki67 marker in rats whose liver tissues were exposed to paracetamol when compared the control where no expression of the marker Ki67 was seen. This signifies a low proliferative activity of hepatocytes that may result to reduced regeneration of the liver thereby impacting negatively on liver function and overall health [28].

CONCLUSION

Administration of aqueous extract of *Talinum triangulare* was able to restore the hepatic damage caused by paracetamol treatment. It can therefore be concluded that *T. triangulare* has hepatoprotective effect and can form effective treatment for management of patients with liver injury.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

Consent: Not applicable

Ethical Approval: Ethical clearance for this study was obtained from Research and Ethics Committee, University of Nigeria, Enugu.

Disclaimer (Artificial intelligence): Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

REFERENCES

1. Garcia-Cortes M, Robles-Diaz M, Stephens C, Ortega-Alonso A, Lucena MI, Andrade RJ. Drug induced liver injury: an update. *Archives of toxicology*. 2020; 94(10), 3381–3407.
2. Oraebosi MI and Elendu MU. Hepatoprotective Effect of *Andropogongayanus* against Paracetamol-and Carbon Tetrachloride Induced- Liver Toxicity in Rats. *Trop. J. Nat. Prod. Res.* 2021; 5(1):188-193.
3. Osna NA, Donohue TM Jr, Kharbanda KK. Alcoholic Liver Disease: Pathogenesis and Current Management. *Alcohol Res.* 2017;38(2):147-161
4. Mondal D, Das K, Chowdhury A. Epidemiology of Liver Diseases in India. *Clin Liver Dis (Hoboken)*. 2022; 19(3):114-117.
5. Rotundo L, Pysopoulos N. Liver injury induced by paracetamol and challenges associated with intentional and unintentional use. *World J Hepatol.* 2020; 12(4):125-136
6. Xu L, Yang D, Chen D, Xu H, Zheng J, Cheng T, Ren H, Wang Y, Zhao X, Yang Z. Paracetamol-induced Liver Injury in An Experimental Rat Model: Noninvasive Assessment using DKI and the Effect of Gadoxetate on DKI Parameters. *Current medical imaging.* 2024; 20, e15734056292975.
7. Devarbhavi H, Aithal G, Treeprasertsuk S, Takikawa H, Mao Y, Shasthry SM, Hamid S, Tan SS, Philips CA, George J, Jafri W, Sarin SK, & Asia Pacific Association of Study of

- Liver. Drug-induced liver injury: Asia Pacific Association of Study of Liver consensus guidelines. *Hepatology international*. 2021; 15(2), 258–282.
8. Athersuch TJ, Antoine DJ, Boobis AR, Coen M, Daly AK, Possamai L, Nicholson JK, & Wilson ID. Paracetamol metabolism, hepatotoxicity, biomarkers and therapeutic interventions: a perspective. *Toxicology research*. 2018; 7(3), 347–357.
 9. Jasani B, Weisz DE, & McNamara PJ. Evidence-based use of acetaminophen for hemodynamically significant ductus arteriosus in preterm infants. *Seminars in perinatology*. 2018; 42(4), 243–252
 10. Rajaram P, Subramanian R. Management of Acute Liver Failure in the Intensive Care Unit Setting. *Clinics in liver disease*. 2018; 22(2), 403–408.
 11. Ramachandran A, Jaeschke G. Mechanisms of acetaminophen hepatotoxicity and their translation to the human pathophysiology. *J. Clin. Transl. Res*. 2017; 3(1):157-69.
 12. Chidiac AS, Buckley NA, Noghrehchi, Cairns R. Paracetamol (acetaminophen) overdose and hepatotoxicity: mechanism, treatment, prevention measures, and estimates of burden of disease. *Expert Opinion on Drug Metabolism & Toxicology*. 2023; 19(5), 297–317. <https://doi.org/10.1080/17425255.2023.2223959>
 13. Tian L, Mi N, Wang L, Huang C, Fu W, Bai M, Gao L, Ma H, Zhang C, Lu Y, Zhao J, Zhang X, Jiang N, Lin Y, Yue P, Xia B, He Q, Yuan J, Meng W. Regular use of paracetamol and risk of liver cancer: a prospective cohort study. *BMC cancer*. 2024; 24(1), 33.
 14. Wang L, Wei W, Xiao Q, Yang H, Ci X. Ferrerol ameliorates APAP-induced hepatotoxicity via activation of Nrf2 and autophagy. *Int. J. Biol. Sci*. 2019; 15(4):788-99.
 15. Bhushan B, Apte U. Liver regeneration after acetaminophen hepatotoxicity: mechanisms and therapeutic opportunities. *Am. J. Pathol*. 2019; 189(4):719-29.
 16. Kennon-McGill S, McGill M. Extrahepatic toxicity of acetaminophen: critical evaluation of the evidence and proposed mechanisms. *Journal of clinical and translational research*. 2017; 3(3), 297–310.
 17. Caparrotta TM, Antoine DJ, Dear JW. Are some people at increased risk of paracetamol-induced liver injury? A critical review of the literature. *European journal of clinical pharmacology*. 2018; 74(2), 147–160.
 18. Almasi F, Khazaei M, Chehre S, Ghanbari A. Hepatoprotective effects of *Tribulus terrestris* hydro-alcoholic extract on nonalcoholic fatty liver- induced rats. *Int. J. Morphol*. 2017; 35(1):345-50.
 19. Zhou HC, Wang H Shi K, Li J, Zong Y, Du R. Hepatoprotective effect of Baicalein against acetaminophen Induced acute liver injury in mice. *Molecules*. 2019; 24(1):131.
 20. Edavalath M. Ayurvedic dietary principles in prevention and management of Diabetes: A review. *Curr Opin Endocrinol Diabetes Obes*. 2018. 1(1), 2.
 21. Perez GRM. Review of Cucurbita pepo (Pumpkin), its Phytochemistry and Pharmacology. *Medicinal Chemistry* 2016; 6:012-021.
 22. Bhusan RP, Kishore GA, Manish G, Chakraborty G." Hepatoprotective activity of different extracts of Grains of Eleusine coracana" *pharmacology online* 2011; 2: 279-286.

23. Papackova Z, Heczko M, Dankova H, Sticova E, Lodererova A, Bartonova L, Poruba M and Cahova M. *Silymarin* prevents acetaminophen-induced hepatotoxicity in mice. *PLoS One*. 2018;13(1): e0191353.
24. Kabruk, CE, Amuzat AO, Ibrahim MI, Yakubu N. Biochemical and Histopathological Analysis of Aqueous Extract of an Aphrodisiac Formulation against Acetaminophen Induce Hepatocyte Injuries in Wistar Rats. *International Journal of Medical Science and Dental Health*. 2024; 10(10), 110-120.
25. Pettie JM, Caparotta TM, Hunter RW, Morrison EE, Wood DM, Dargan PI, Thanacoody RH, Thomas SHL, Elamin MEMO, Francis B, et al. Safety and efficacy of the SNAP 12-hour acetylcysteine regimen for the treatment of paracetamol overdose. *E Clin. Med*. 2019; 11:11-7.
26. Theophilus OE, Ifueko MMO, Anthony CN, Efosa BO, Blessing EA, Ramatu Y. *Talinum triangulare* (Waterleaf) Stem Extract Has Reno-Hepato Protective Potentials than Ameliorative Effects on Acetaminophen Toxicity in Sprague-Dawley Rats. *Sokoto Journal of Medical Laboratory Science* 2022; 7(1): 68 – 80
27. Swarna J, Ravindhran R. Pharmacognostical and phytochemical evaluation of *Talinum triangulare* (Jacq.) Wild. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2013; 5(2): 249-256.
28. Wang X, Li L, Wang L, Chen M. The expression of Ki-67 and Glypican – 3 in hepatocellular carcinoma was evaluated by comparing DWI and 18F-FDG PET/CT. *Frontiers of Oncology*. 2023; 13.1026245. doi: 10.3389/fonc. 2023.1026245