Ikenazor et al. J Biomed & App Sci FUD (2025) 4:2
www.jobas.fud.edu.ng
ISSN: 2814-3221 (print), ISSN: 2955-0874 (online)

JOBASFUD

Journal of Biomedical and Applied Sciences

RESEACH ARTICLE

EFFECT OF ETHANOLIC EXTRACT OF TETRAPLEURA TETRAPTERA SEEDS ON THE HISTOLOGY OF RAT LIVER ADMINISTERED WITH PARACETAMOL

Herbert Okwudili Ikenazor, Adebayo Olugbenga Adegoke*, Kelvin Ebuka Udoh

Department of Medical Laboratory Science, Madonna University Nigeria

Abstract

Background: *Tetrapleura tetraptera* seed extract has been traditionally used in herbal medicine and possesses reported antioxidant and hepatoprotective properties, suggesting its potential as a therapeutic agent.. **Methods:** A total of 30 wistar rats weighing 64-97g were randomly grouped into 5 groups with 6 rats each. The rats in group A were fed rat diet and water *ad libitum* to serve as control. Group B were orally administered with 2g/kg of paracetamol only to induce hepatotoxicity. The rats in group C, D, E and F received intraperitoneal injection of *Tetrapleura tetraptera* seed extract at a dosage of 1000mg/kg, 200mg/kg and 400mg/kg respectively per day for 7 days prior to administration of 2g/kg of paracetamol and continued for 7 days post administration of 2g/kg of paracetamol. The rats were sacrificed and liver collected into labelled jars containing formalin. **Results:** The result in group A showed intact normal hepatocytes while that of group B showed pyknotic nuclei with Hepatocyte cytoplasmic vacuolation. The group C rat showed the presence of proliferated focal inflammatory cells, enlarged sinusoid and presence of binucleated hepatocytes while group D rat reported massive liver degeneration and severe (chronic) proliferation of predominace diffused inflammatory cells. Group E rat showed kupffer cells hyperplasia. **Conclusion:** These findings suggest that *Tetrapleura tetraptera* seed extract treatments can restore liver histological changes in paracetamol induced hepatotoxic conditions, emphasizing the importance of therapeutic interventions in managing liver-induced hepatotoxicity.

Keyword: paracetamol, hepatotoxicity, liver, *Tetrapleura tetraptera,* ethanol

Introduction

Tetrapleura tetraptera, (African pea) commonly known as Aidan fruit or "Aridan" in Nigeria, is a tropical plant native to West Africa. It belongs to the Fabaceae family and is widely recognized for its culinary and medicinal uses (Adjanohoun *et al.,* 1996). The pods, seeds, and leaves are traditionally used in various African cultures to treat conditions such as inflammation, diabetes, and hypertension (Adjanohoun *et al.,* 1996). The seeds are particularly notable for their rich phytochemical profile, containing alkaloids, flavonoids, tannins, saponins, and phenolic compounds (Akinmoladun *et al.,* 2015; Adeyemi *et al*., 2014). These compounds contribute to the plant's antioxidant, antimicrobial, and anti-inflammatory properties. In recent years, scientific studies have increasingly focused on the pharmacological potential of *Tetrapleura tetraptera*, including its hepatoprotective effects (Edeoga *et al*., 2005). Notably, research by Owolabi *et al.* (2016) identified various bioactive compounds in *Tetrapleura tetraptera* extracts, including flavonoids, alkaloids, and tannins, which contribute to its antioxidant, anti-inflammatory, and hepatoprotective effects.

*Corresponding: Adebayo Olugbenga Adegoke
Email: bayoadeghq@yahoo.com

The liver plays a key role in metabolism, storage, and secretion and in the detoxification of harmful chemicals (Hussain, *et al.,* 2019a). Carbohydrates, proteins, and fats are mainly metabolized in the liver (Nishikawa and Osaki 2015). It is highly susceptible to damage from various toxins, including medications like paracetamol (acetaminophen). Paracetamol is widely used for its analgesic and antipyretic properties, but in cases of overdose, it can cause severe liver injury, leading to acute liver failure (Larson *et al*., 2005; Olayinka *et al*., 2016). Hepatotoxicity is a significant concern, as it can lead to liver damage and dysfunction. Paracetamol-induced hepatotoxicity is primarily due to its metabolism in the liver, where it is converted into a toxic metabolite, N-acetyl-p-benzoquinone imine (NAPQI). Under normal circumstances, NAPQI is detoxified by conjugation with glutathione. However, in overdose situations, the glutathione stores are depleted, leading to the accumulation of NAPQI, which binds to cellular proteins and causes oxidative stress and hepatocyte damage (James *et al.,*2003).Current treatments for paracetamol-induced liver toxicity are limited and include the administration of N-acetylcysteine (NAC), which replenishes glutathione stores. However, NAC has a narrow therapeutic window and can cause adverse effects (Kaplowitz, 2005).

Therefore, there is a growing interest in finding alternative and complementary therapies from natural sources that can offer protective effects against liver damage with fewer side effects. Despite the widespread use of *Tetrapleura tetraptera* in traditional medicine, there is limited scientific evidence on its effects on liver histology, particularly in the context of paracetamol-induced hepatotoxicity. A study by Adeyemi *et al.* (2014) investigated the hepatoprotective effects of ethanol seed extract of *Tetrapleura tetraptera* against paracetamthe instol-induced hepatotoxicity in rats. Understanding the potential hepatoprotective effects of *Tetrapleura tetraptera* seeds against paracetamol-induced liver damage is essential for validating its traditional use and exploring its therapeutic potential. The aim of this study is to evaluate the effects of ethanol extracts of *Tetrapleura tetraptera* seeds on the histology of rat liver induced with hepatotoxicity using paracetamol.

Materials and Methods

Rats

In this study, male Wistar rats weighing between 150-200 grams were used. These rats were obtained from the animal house facility of Madonna university, where they were housed under controlled conditions (temperature: 22±2°C, humidity: 50-60%, 12-hour light-dark cycle) with access to standard rat chow and water. All experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee (IACUC) of Madonna University, ensuring compliance with ethical guidelines for animal research.

Chemical and reagents

Commercially prepared Haematoxylin and Eosin stains and other analytical grade reagents were purchased in Lagos.

Preparation of Plant Materials

The *Tetrapleura tetraptera* plant were bought from Elele Market Rivers state, identified by Pharmacognosy, Department of Madonna University. The *Tetrapleura tetraptera* seeds were thoroughly washed with distilled water to remove any dirt or contaminants. Ethanol extracts of *Tetrapleura tetraptera* seeds were prepared according to the procedure. Briefly, dried seeds of *Tetrapleura tetraptera* were ground into a fine powder and macerated in 95% ethanol for 72 h at room temperature. The extract was then filtered, and the solvent was evaporated under reduced pressure using a rotary evaporator. The resulting crude extract was stored in airtight containers at 4°C until further use.

Experimental Design

A total of 30 wistar rats weighing 64-97g were randomly distributed into 5 groups of 6 rats each. The rats in group A were fed rat diet and water *ad libitum* to serve as control. Group B were orally administered with 2g/kg of paracetamol only to induce hepatotoxicity. The rats in groups C, D and, E received intraperitoneal injection of *Tetrapleura tetraptera* seed extract at a dosage of 1000mg/kg, 200mg/kg and 400mg/kg respectively per day for 7 days prior to administration of 2g/kg of paracetamol which continued for 7 days

post administration of 2g/kg of paracetamol. The rats were sacrificed and the liver was collected into bottles containing formalin for histological analysis.

Haematoxylin and Eosin

Principle: This protocol is applied in the routine staining of cationic and anionic tissue components in tissue sections. This is the standard reference stain used in the study of histochemical tissue pathology.

Procedure: The sections were deparaffinized in xylene for20 minutes. The Hematoxylin was filtered. The sections were rehydrated by 100% alcohol for 2 minutes and then 95% alcohol for 2 minutes. It was rinsed in tap water and then rinsed in distilled water. The sections were stained with Hematoxylin for 5 minutes. It was washed in tap water. The sections were differentiated with 1% HCl in 70% alcohol 2 dips and check under microscope. The slides were washed in running tape water for 15 minutes. The slides were stained in Eosin for 4 minutes. The sections were dehydrated in ascending alcohol solutions (50%,70%,80%,95% x 2, 100% x 2)and Differentiated. The slides were cleared in xylene 2 times. The slides were mounted with DPX mountant observed with X40 and X100objectives and photomicrograph.

Results

Histopathology of rat's liver treated with paracetamol and administered with different concentrations of *T. tetraptera* seed extract is as shown below in Plates I -V.

Plate I showed Photomicrograph of liver of wistar rat dosed with normal saline + feed (control). This shows intact normal hepatocytes

Plate II showed Photomicrograph of liver of rat induced 2 g/kg paracetamolwith (1) Pyknotic nuclei (A) (white arrow) (2). Hepatocyte cytoplasmic vacuolation (B) (red arrow) which appear to be consistent with glycogen and liver cholestasis which has red dense patches(C) surrounding the liver lobules. Figure 3 showed Photomicrograph of liver of rat induced with 2 g/kg paracetamol and treated with 100 mg/kg of *T. tetrapteras*eed extract. There was presence of (1) proliferated focal inflammatory cells (A) (star (2) enlarged sinusoid (B) (black arrow) with distortion of hepatic cords, (3) Presence of binucleated hepatocytes (circles).

Plate IV: Photomicrograph of liver of wistar rat induced with 2 g/kg paracetamol and treated with 200 mg/kg*T. Tetraptera* seed extract. This shows (1) massive liver degeneration especially the hepatic cords which appeared destroyed. (2) severe (chronic) proliferation of predominace diffused inflammatory cells (A)(star). Plate V Photomicrograph shows liver of rat induced with paracetamol and treated with 400 mg/kg *T. tetrapteras*eed extract which indicated (1). Kupffer cells hyperplasia, (A) which is attached to the walls of enlarged sinusoid which participated in the removal of spent erythrocytes and other particulate debris from circulation.. (2). (H&E. mag.100X).

Discussion

The result showed that liver of wistar rat dosed with normal saline + feed (control) showed intact normal hepatocytes while the liver of rat treated with 2 g/kg paracetamol showed damaged liver cells with (1) Pyknotic nuclei (A) (2) Hepatocyte cytoplasmic vacuolation (B) which appear to be consistent with glycogen and liver cholestasis which has red dense patches(C) surrounding the liver lobules. This is suggestive that administration of 2g/kg of paracetamol caused hepatic damage. Al-Harbi *et al.,* (2014) and Hussain *et al.,* (2019b) reported that liver function can be negatively affected by oxidative stress resulting from exposure to various xenobiotics (naturally occurring harmful compounds such as free radicals and hydroperoxides). The liver can also be damaged by certain chemicals including paracetamol, CCl4, and polycyclic aromatic hydrocarbons. Hepatic injury caused by chronic exposure to these chemicals and via infectious agents may lead to progressive liver fibrosis with ultimate cirrhosis and liver failure (Al-Harbi *et al.,* 2014). Hence administration of 2 g/kg paracetamol which is a chemical caused changes to liver architecture suggestive of hepatic damage.

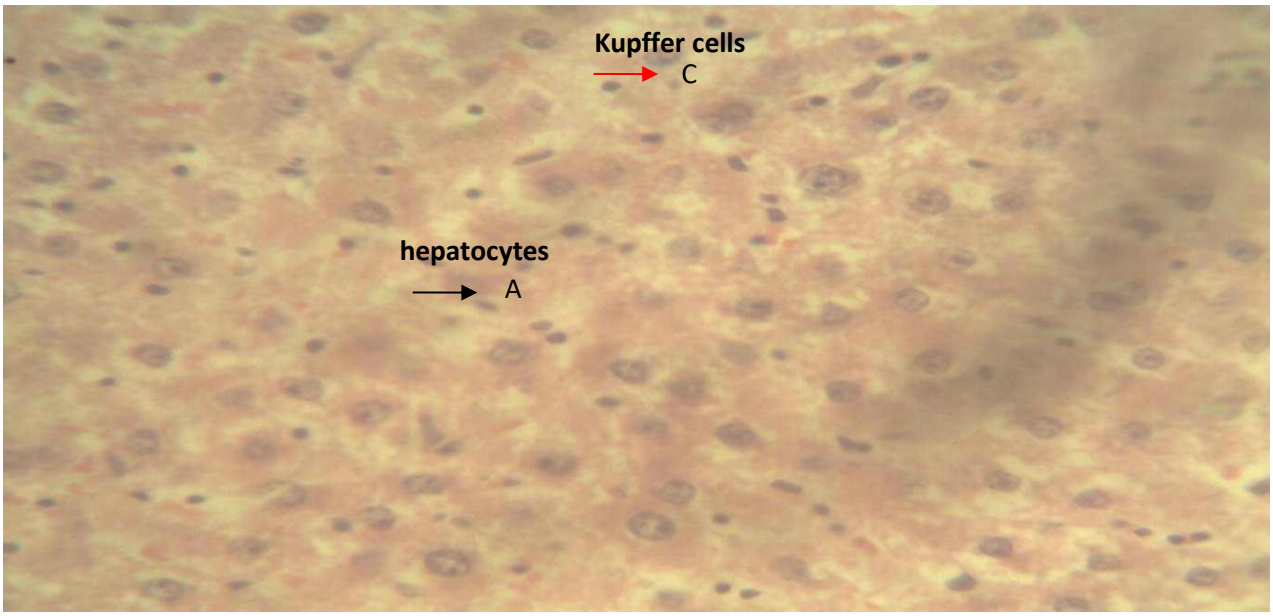

Plate I: Photomicrograph of liver of wistar rat dosed with water + feed (control). This shows intact hepatocytes (A) (black arrow) and kupffer cells (red arrow). (H&E. Mag.100X).

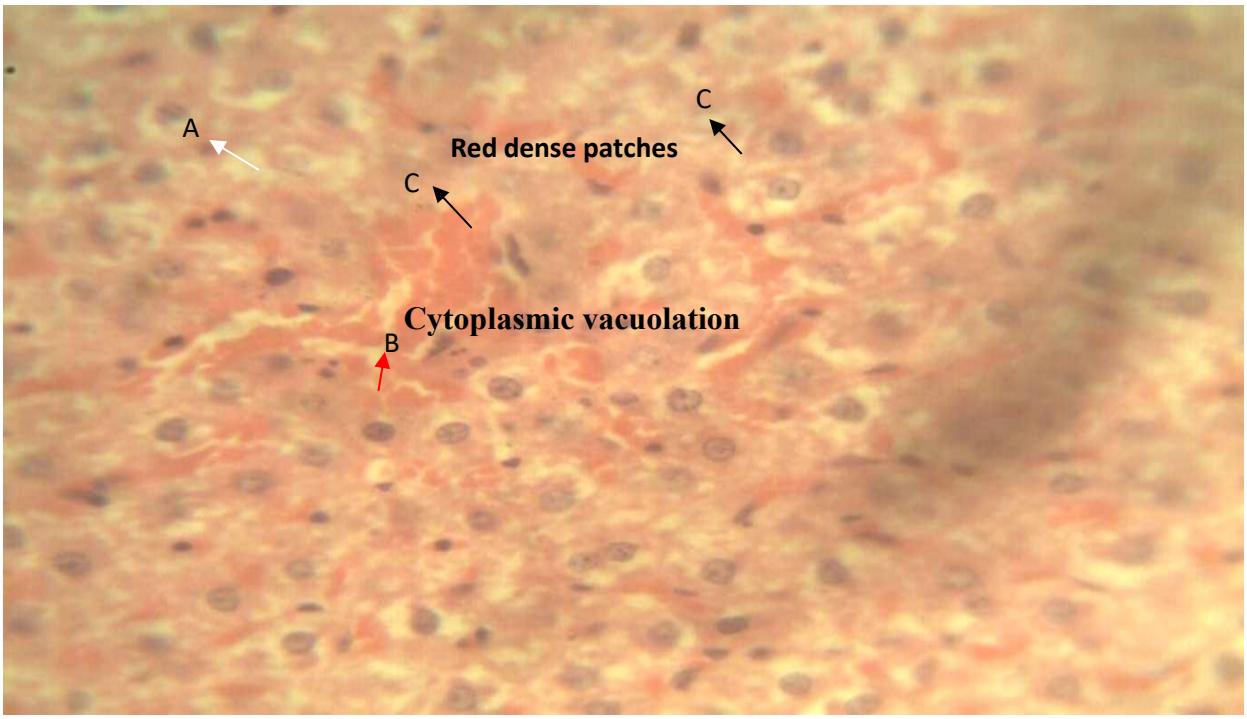

Plate II: Photomicrograph of liver of wistar rat induced 2 g/kg paracetamol (1) Pyknotic nuclei (A) (white arrow) (2). Hepatocyte cytoplasmic vacuolation (B) (red arrow) which appear to be consistent with glycogen and liver cholestasis which has red dense patches(C) surrounding the liver lobules (H&E. mag. 100X)

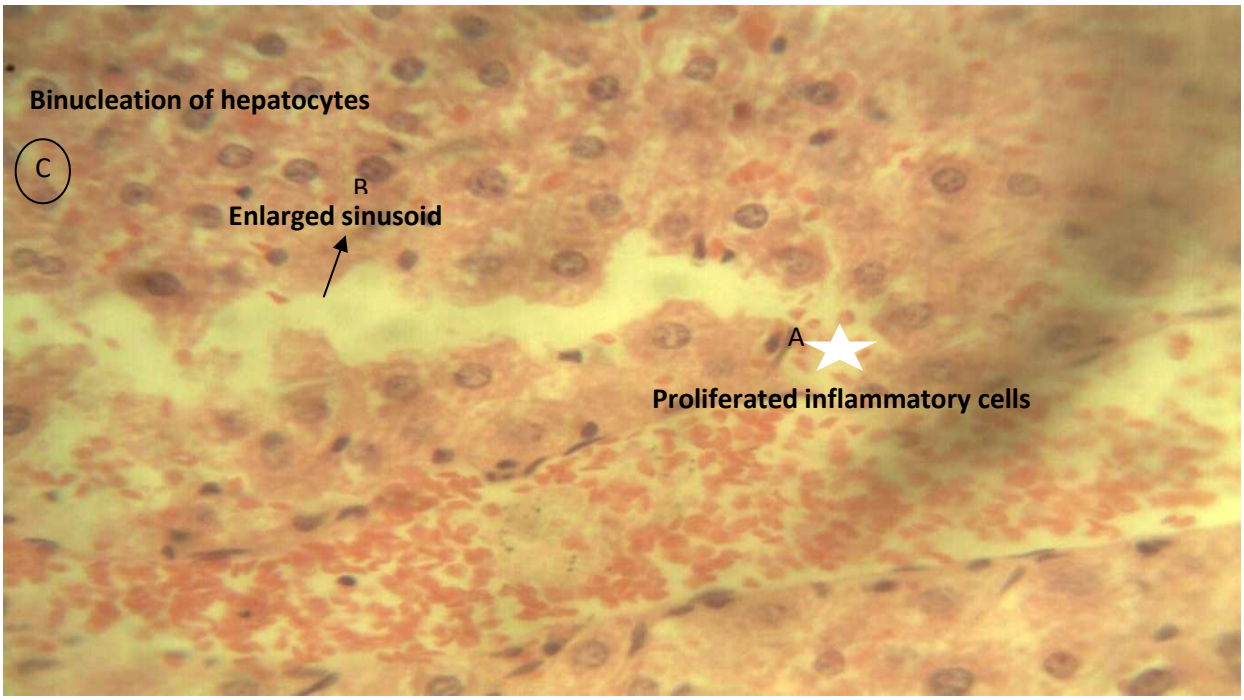

Plate III. Photomicrograph of liver of wistar rat induced with 2 g/kg paracetamol and treated with 100 mg/kg of *T. tetraptera* seed extract. This shows (1) proliferated focal inflammatory cells (A) (star) which is an indication of a response to parenchyma cell death with causes ranging from infectious agent, exposure to toxicants, tissue anoxia etc, (2) enlarged sinusoid (B) (black arrow) with distortion of hepatic cords, (3) Presence of binucleated hepatocytes (circles), (H&E. mag. 100X)

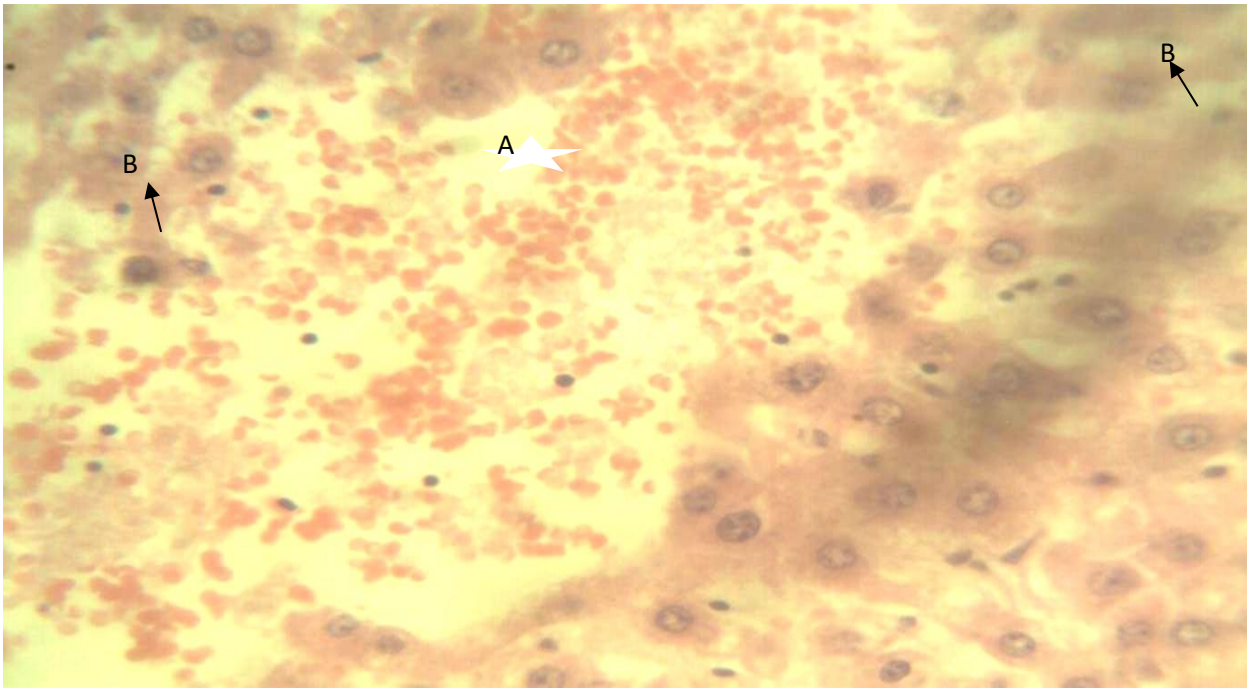

Plate IV: Photomicrograph of liver of wistar rat induced with 2 g/kg paracetamol and treated with 200 mg/kg *T. tetraptera* seed extract. This shows (1) massive liver degeneration especially the hepatic cords which appeared destroyed. (2) severe (chronic) proliferation of predominace diffused inflammatory cells (A)(star). the sequence of

liver damage appeares to progress as follows; swelling/vacuolation of hepatocytes, cytoplasmic swelling and eccentric displacement of the nucleus followed by necrosis of hepatocytes (B)(black arrow). (H&E.mag. 100x.).

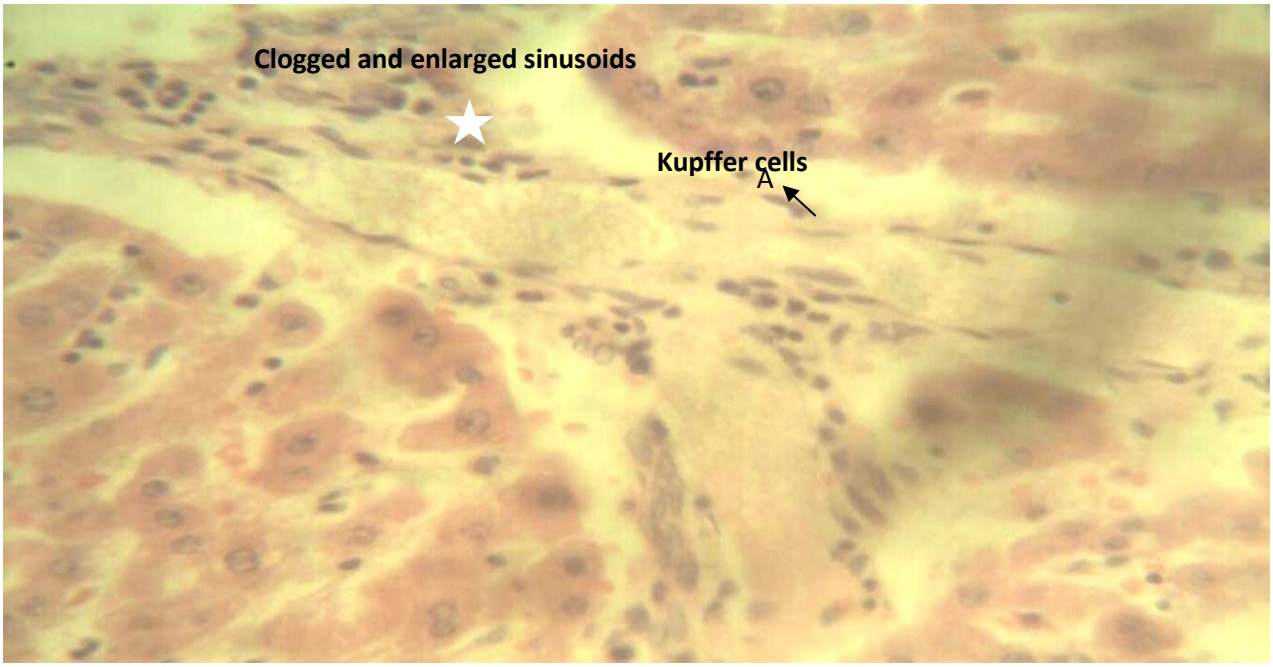

Plate V: The Photomicrograph shows liver of wistar rat induced with paracetamol and treated with 400 mg/kg *T. tetraptera* seed extract (1). Kupffer cells hyperplasia,(A) which is attached to the walls of enlarged sinusoid which participated in the removal of spent erythrocytes and other particulate debris from circulation. This function appeared to be impeded due to presence of dilated/or enlarged and clogged sinusoids which may make the erythrocytes removal impossible. (2). (H&E. mag.100X).

The liver of rat induced with 2 g/kg paracetamol and treated with 100 mg/kg of *T. tetraptera* seed extract showed the presence of (1) proliferated focal inflammatory cells (A) which is an indication of a response to parenchyma cell death with causes ranging from infectious agent, exposure to toxicants, tissue anoxia etc, (2) enlarged sinusoid (B) with distortion of hepatic cords, (3) Presence of binucleated hepatocytes. The result is suggestive of damage to the liver architecture. The liver of albino rat induced with 2 g/kg paracetamol and treated with 200 mg/kg *T. tetraptera* seed extract showed (1) massive liver degeneration especially the hepatic cords which appeared destroyed. (2) severe (chronic) proliferation of predominace diffused inflammatory cells (A). The sequence of liver damage appears to progress as follows; swelling/vacuolation of hepatocytes, cytoplasmic swelling and eccentric displacement of the nucleus followed by necrosis of hepatocytes (B), while liver of rat induced with paracetamol and treated with 400 mg/kg *T. tetraptera* seed extract indicated (1). Kupffer cells hyperplasia, (A) which is attached to the walls of enlarged sinusoid which participated in the removal of spent erythrocytes and other particulate debris from circulation. This function appeared to be impeded due to presence of dilated/or enlarged and clogged sinusoids which may make the erythrocytes removal impossible. Although liver issues can be life-threatening and are known to have a high mortality rate, there is no complete treatment or prevention. Alternatively, herbal products and pure compounds are under consideration for the preventive and therapeutic outcomes in liver diseases (Kumar *et al.*, 2009).The study has shown that oxidative damage of the liver architecture could be treated by neutralizing it with antioxidants (Ighodaro and Akinloye, 2018).

Conclusion

The study has shown that paracetamol induced hepatotoxicity caused changes in liver architecture while present evidence suggests that *T. tetraptera* seed

extract could ameliorate against the changes in liver architecture caused by paracetamol induced hepatotoxicity. Experimental evidence from this study is suggesting that *T. tetraptera* seed extract rich food in the diet to ameliorate liver hepatotoxicity. Further studies using other *T. tetraptera* as ameliorative agent on other tissue should be carried out.

Acknowledgements:

The authors thank the AB Sigma laboratories, Port Harcourt for using the Laboratory for some analysis. We are also grateful to staffs of animal house and the Medical Laboratory Services Department of Madonna University Teaching Hospital for using their facilities.

Conflict of Interest:

The authors declare that no known conflict of interest or personal relationships that have influenced the work reported in this paper.

Source of Funding:

This study was self-funded.

Authors' Contributions:

HOI: Conceptualized the study, Design, AO.: Supervision. Project administration, wrote, reviewed and edited the manuscript original draft preparation. Methodology, data analysis. KEU: Laboratory analysis. All the authors have read and approved the final version of the manuscript.

Article History

Received: 06th June, 2025.

Accepted: 08th January 2026.

Published online: 30th March 2026

Reference

Adeyemi, O. S., Akanji, M. A., & Oguntoye, S. O. (2014). Ethanolic seed extract of *Tetrapleura tetraptera* protects against paracetamol-induced hepatotoxicity in rats. *Journal of Intercultural Ethnopharmacology*, **3**(4):149–155.

Adjanohoun, E.J., Aboubakar, N., Dramane, K., Ebot, M.E., Ekpere, J.A., Enow-Orock, E.G., Fadojutimi, H.O., Gbile, Z.O., Kamanyi, A.,&Kamsu-Kom, J. (1996). Traditional Medicine and Pharmacopoeia: Contribution to Ethnobotanical and Floristic Studies in Cameroon. CNPMS. *Journal of pharmacology and pharmacy*, **29**(3):17–25.

Akinmoladun, A.C., Obuotor, E.M.,&Farombi, E.O. (2015). Evaluation of antioxidant and hepatoprotective properties of ethanol extract of *Tetrapleura tetraptera* fruit in rats. *Journal of Medicinal Food*, **18**(10): 1124-1131.

Al-Harbi, N. O. Imam, F. Nadeem, A. Al-Harbi, M. M. Iqbal,M.&Ahmad,S. F. (2014). Carbon tetrachloride-induced hepatotoxicity in rat is reversed by treatment with riboflavin," *International Immunopharmacology*, **21(**2):383– 388.

Edeoga, H.O., Okwu, D.E.,&Mbaebie, B.O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*, **4**(7): 685-688.

Hussain,S.,Ullah,F.,Sadiq A., etal., (2019a). Cytotoxicity of *Anchusaarvensis* against HepG-2 cell lines: Mechanistic and computational approaches. *Current Topics in Medicinal Chemistry*, **19** (30): 2805–2813.

Hussain,S.,Ullah,F.,Ayaz M.et al., (2019b). In silico, cytotoxic and antioxidantpotentialofnovel ester,3-hydroxyoctyl-5-transdocosenoate isolated from anchusaarvensis (L.) M. Bieb. against HepG-2 cancer cells. *Drug Design, Development and Therapy*, **13**: 4195–4205.

Ighodaro O. M.,&Akinloye, O. A.(2018). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defence grid. *Alexandria Journal of Medicine*, **54**,(4):287–293.

James, L.P., Mayeux, P.R.,& Hinson, J.A. (2003). Mechanisms of acetaminophen hepatotoxicity and their translation to the human pathophysiology. *Advances in Pharmacology*, **60:** 203-222.

Kaplowitz, N. (2005). Idiosyncratic drug hepatotoxicity. *Nature Reviews Drug Discovery*, **4**(6): 489-499.

Kumar,P. V., Sivaraj,A., Elumalai, E.,&Kumar,B. S. (2009).Carbon tetrachloride-induced hepatotoxicity in rats-protective role of aqueous leaf extracts of *Cocciniagrandis*. *International Journal of Pharmaceutical Technology Research*, **1 (**4):1612–1615.

Larson, A.M., Polson, J., Fontana, R.J., Davern, T.J., Lalani, E., Hynan, L.S., Reisch, J.S., Schiødt, F.V., Ostapowicz, G., Shakil, A.O.,& Lee, W.M. (2005). Acetaminophen induced acute liver failure: results of a United States multicenter, prospective study. *Hepatology*, **42**(6): 1364-1372.

Nishikawa H. and Osaki, Y. (2015). Liver cirrhosis: evaluation, nutritionalstatus,and prognosis. *Mediators of Inflammation*, **2015**:9. Article ID 872152,

Olayinka, E. T., Ore, A., Adeyemo, O. A., Ola, O. S., Olotu, E. J.,& Olaoye, I. F., (2016) Amelioration of paracetamol-induced hepatotoxicity in rats by the ethanol extract of the fruit pulp of *Lagenaria breviflora* (Cucurbitaceae). *Pharmaceutical Biology*, **54**(5):858–865.

Owolabi, M. S., Adebayo, J. O., Ademiluyi, A. O., Akinyemi, A. J., Oguntoye, O. S., & Oguntoye, S. O. (2016). Phytochemical and Antioxidant Properties of *Tetrapleura tetraptera* and *Parkia biglobosa* Seeds Extracts. *Journal of Complementary and Alternative Medical Research*, **1**(1):1-10.