HEPATOPROTECTIVE EFFECTS OF TALINUM TRIANGULARE AQUEOUS EXTRACT ON PARACETAMOL-INDUCED LIVER DAMAGE IN WISTAR RATS: A HISTOMORPHOLOGICAL STUDY

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 aims to evaluate the potential of *Talinum triangulare* aqueous extract in protecting and repairing liver damage induced by paracetamol through histomorphological analysis in Wistar rats. **Method:** Thirty-five Wistar rats were divided into five groups: a negative control, a positive control (paracetamol only), and three treatment groups receiving paracetamol followed by 100, 200, or 300 mg/kg body weight of *T. triangulare* extract for seven days. The experiment lasted for 14 days. **Result:** Histomorphological examination showed significant liver deterioration in the untreated group (Group 2), whereas Groups 3, 4, and 5 treated with Talinum triangulare extract exhibited notable recovery. There was reduced necrosis, inflammation and improved hepatocyte regeneration. **Conclusion:** The findings indicate that *T. triangulare* extract exhibits significant hepatoprotective effects, suggesting its potential as a natural remedy for managing paracetamol-induced liver injuries. **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 [1,2,41]. 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% [4,41].

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 hepato-protective, 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: Paracetamol was purchased from a registered pharmaceutical store in Enugu. The drug was dissolved in water at an 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 Bucchi 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 selected doses were based on previous studies. 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 paracetamol for 7 days.

Group III: was administered 200 mg/kg body weight of paracetamol 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 paracetamol 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 paracetamol 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 5m 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.

Statistical Analysis: Spearman's correlation was used to compare the histological features among the groups.

RESULT

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

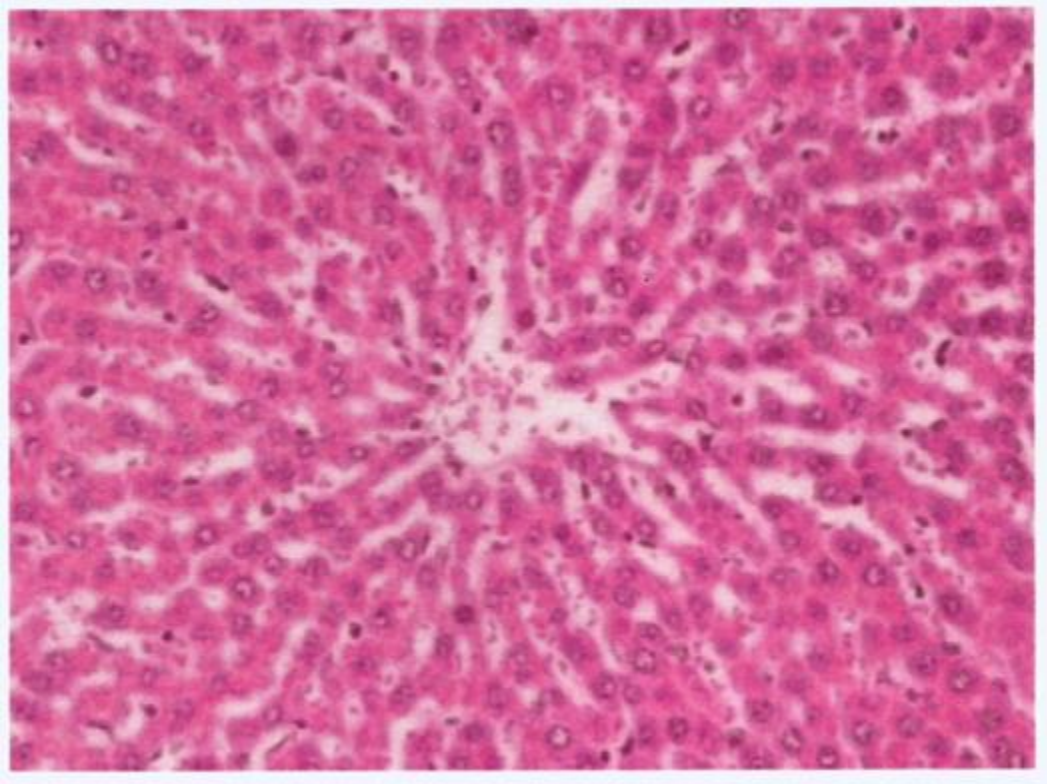

Figure 1: Group1 (Negative control): showing normal tissue morphology. (H&E X400). HC: Hepatocyte, CV: Central vein.

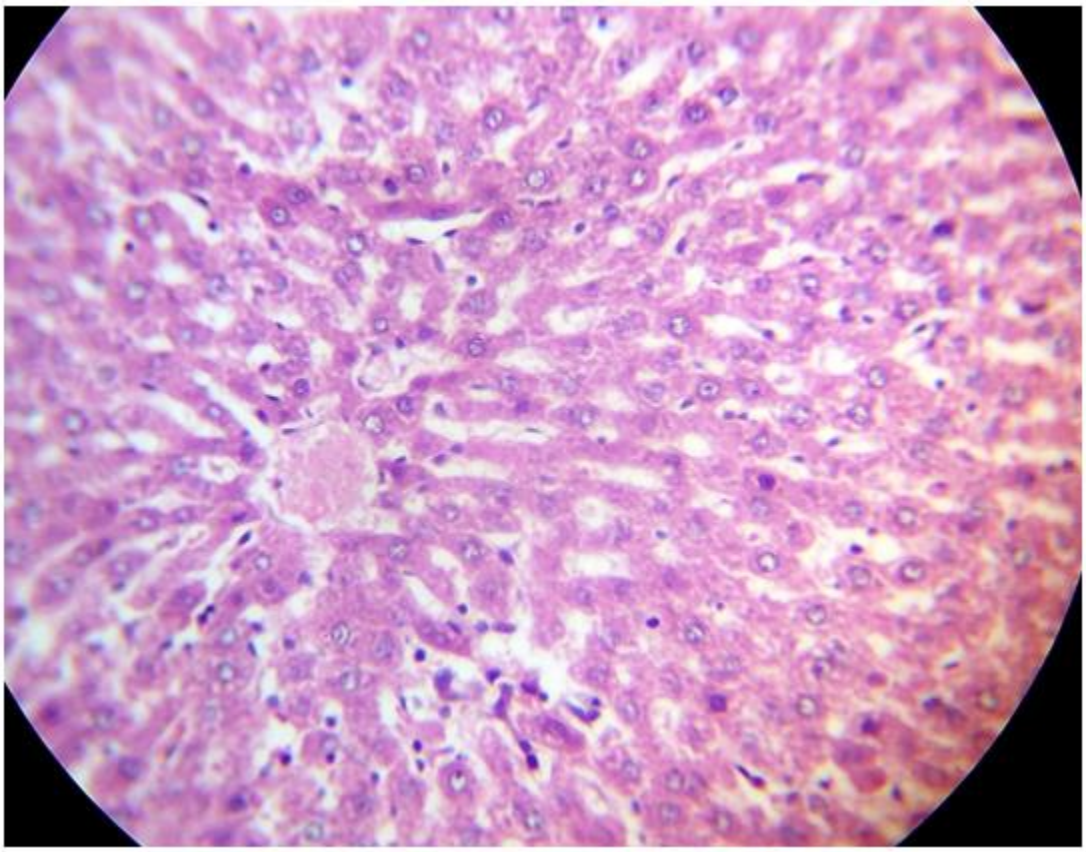

Figure 2: Group 2 (Positive control): Rat's liver tissue administered 200mg/kg body weight of paracetamol showed; hepatocyte deformation, a clogged and enlarged portal vein (CG), an expanded bile duct (EB), and an Area of Necrosis (NC).

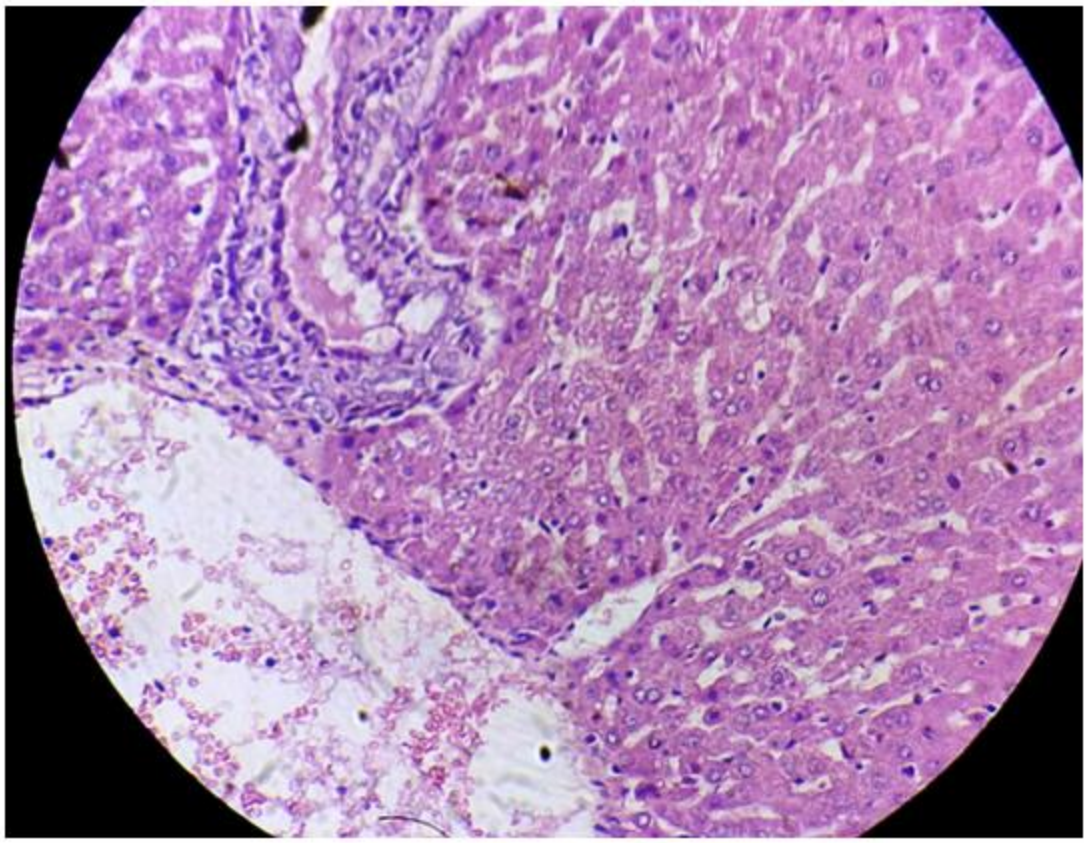

Figure 3: Group 3: Rat's liver tissue treated with paracetamol 200mg/kg followed by *T. triangulare* 100 mg/kg body weight extract showed; inflammatory cells infiltrated the periportal space in a moderate amount, and portal congestion was mild. (H&E X400)

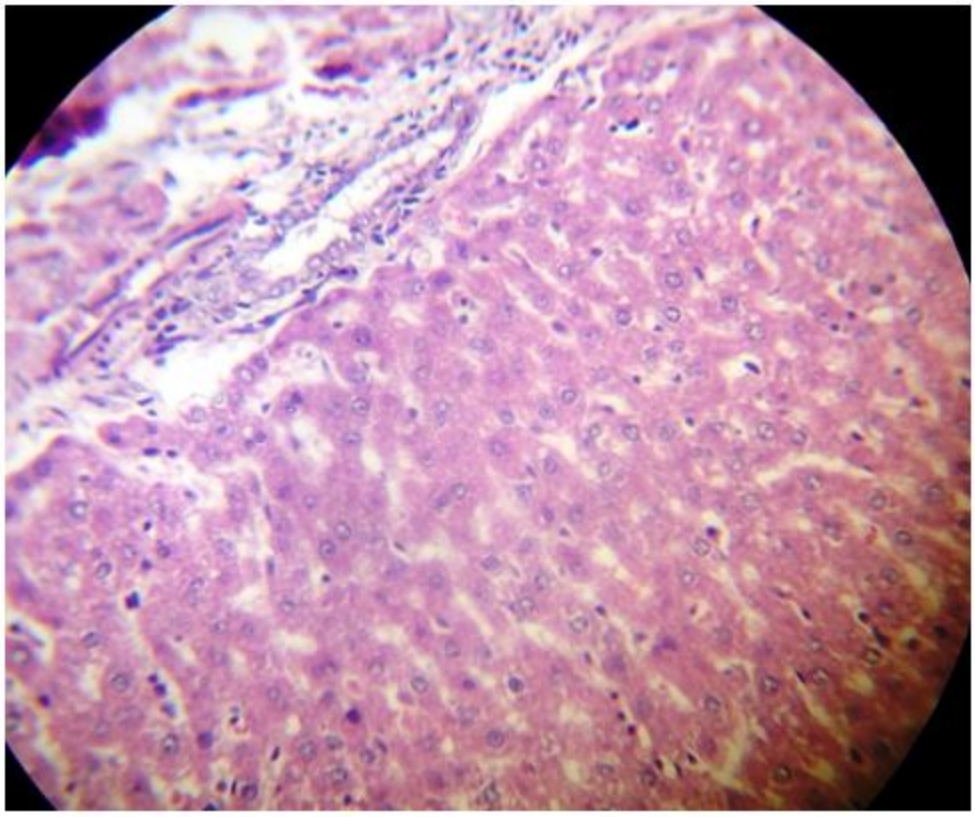

Figure 4: Group 4: Rat's liver tissue treated with paracetamol 200mg/kg followed by *T. triangulare* 200 mg/kg body weight extract showed; hepatocytes with significant diffuse vacuolar degeneration (VD) and minor portal congestion. Only a small percentage of hepatocytes appeared necrotic. (H&E X 400)

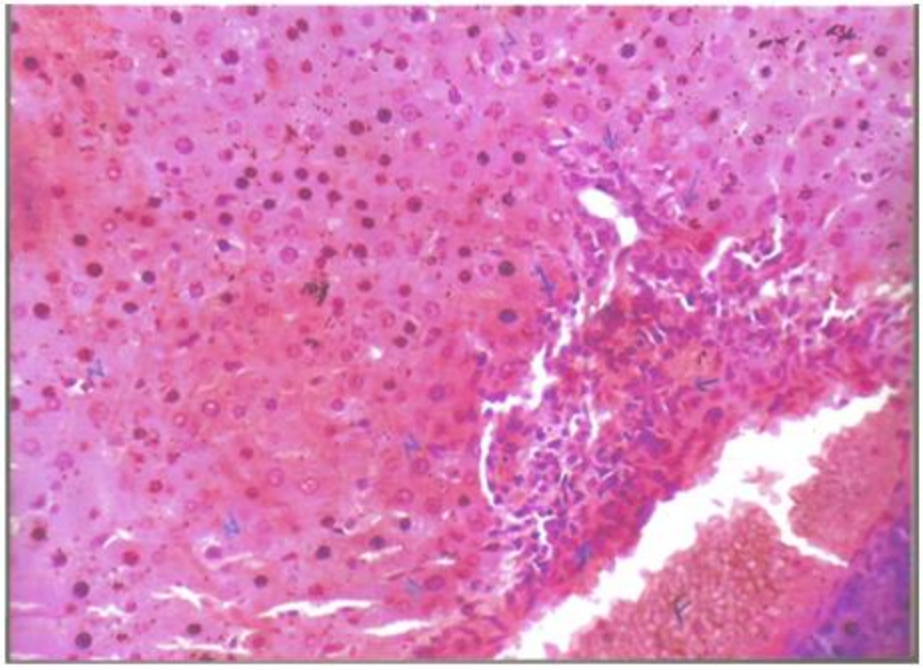

Figure 5: Group 5: Rat's liver tissue treated with paracetamol 200mg/kg followed by 300mg/kg body weight of *T. triangulare* extract showed; a moderate to high healing effect to periportal cellular infiltration by inflammatory cells and periportal fibrosis (PF) and a moderate regeneration of Hepatocytes.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

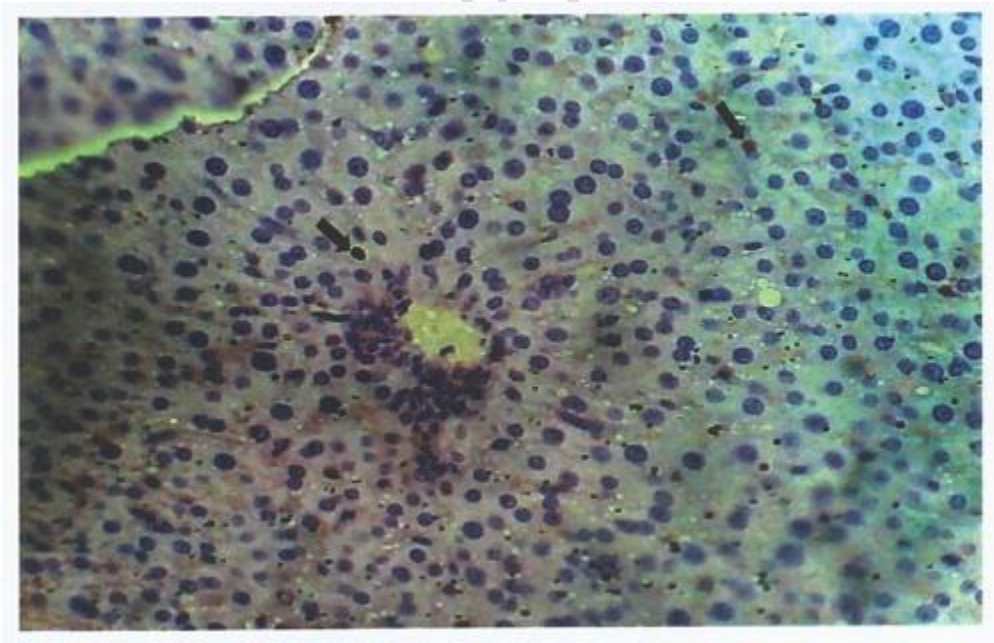

Plate 1: Immunohistochemistry of Liver section showing scanty expression of the marker Ki67

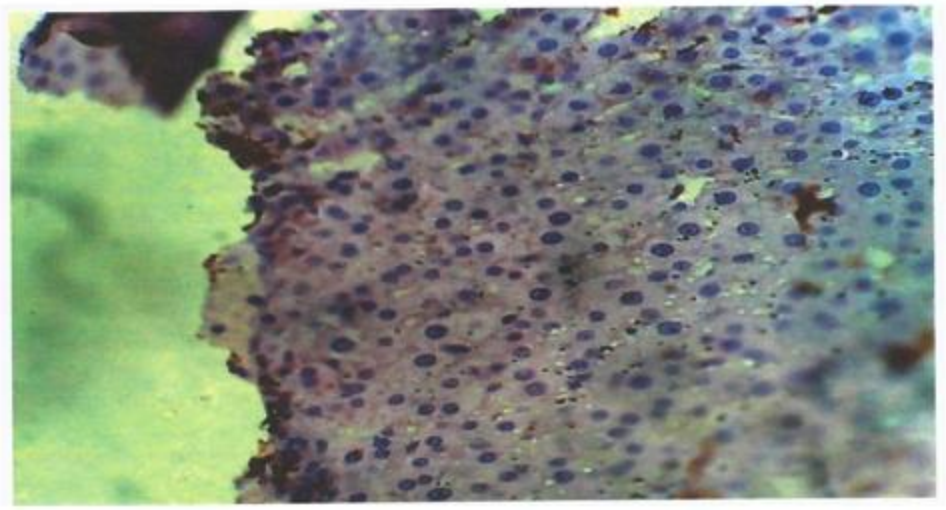

Plate 2: Immunohistochemistry of Liver section showing no expression of the marker Ki67

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]. The ability of *T. triangulare* to protect against liver toxicity can be attributed to its antioxidant activity as reported by several studies [27,28,29]. Similar hepatoprotective effect has been recorded from several studies on other natural plant extract such as *Cleome viscosa L*, resveratrol, curcumin among others [30,31,32]. Also, 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 [33,34,35]. 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. Low Ki67 marker expression is a key indicator of limited liver cell regeneration, reflecting the liver's ability to proliferate new cells [36]. As a nuclear protein associated with cell growth, Ki67 expression levels provide valuable insights into the liver's regenerative state after injury or surgery [37]. Prolonged liver damage and inadequate regeneration can lead to liver fibrosis. Research has shown that reduced Ki67 expression correlates with impaired liver cell proliferation, as seen in studies using Mettl3 knockout mice [38]. Furthermore, Ki67 expression levels can serve as prognostic indicators for liver cancer, with lower levels associated with less aggressive disease [39,40].

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.

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

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