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PRELIMINARY PROTECTIVE EVALUATION OF STEM BARK ETHANOLIC EXTRACT OF *SPHENOCENTRUM JOLLYANUM* AGAINST N-ACETYL-PARA-AMINOPHENOL (APAP)-INDUCED LIVER DAMAGE IN MALE WISTAR RATS.

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Abstract

The toxicity of paracetamol (APAP) overdose is one of the leading causes of liver injury that finally may lead to drug-induced acute liver failure. Global attention has been shifted towards the use of herbal plants as an alternative use to modern or pharmaceutical drugs, especially in rural communities. This study investigated the effects of ethanolic extract of *Sphenocentrum jollyanum* stem bark on N-acetyl-para-aminophenol (APAP)-induced liver damage in male Wistar rats. Thirty male Wistar rats were randomly divided into six (n=5) groups (A-F). Control (group A), 1000mg/kg of APAP only (group B), 700mg/kg of Stem bark of *Sphenocentrum jollyanum* (SBSJ) only (group C), 1000mg/kg of APAP + 700mg/kg of SBSJ (group D), 1000mg/kg of APAP + 350mg/kg of SBSJ (group E), 1000mg/kg of APAP for 14 days + 700mg/kg of SBSJ for the next 7 days. The administration was done via oral gavage daily for 14 days. The rats were sacrificed by cervical dislocation under mild anaesthesia and their liver was carefully harvested. Histological examination of the liver was done using Hematoxylin and Eosin stain. APAP causes significant histopathological damages on the histoarchitecture of the liver tissue with tissue tears, hepatocyte degeneration, necrosis and fibrosis while significant regenerative changes similar to control were observed following administration of stem bark of *Sphenocentrum jollyanum*. The result suggests that the stem bark of *Sphenocentrum jollyanum* has beneficial effects on APAP-damaged live

Keywords*: Sphenocentrum jollyanum*, liver, hepatoprotective, APAP

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Introduction

The liver is the critical organ that is essential for detoxification of deleterious materials among other functions including the regulation of various metabolic functions and maintains body homeostasis [1]. Maintaining the liver in healthy condition is therefore important for normal physiological function. The metabolism of xenobiotics by the liver renders it susceptible to drug-induced toxicity [2]. Worldwide, liver disease accounts for about 2 million deaths per year [3]. Drugs are major causes of liver disease which accounts for as much as 20% of acute liver failure in both paediatric and adult populations [4].

One clinically important drug that has been associated with liver injury is Paracetamol (PCM, N-acetyl-para-aminophenol, APAP), also known as Acetaminophen. APAP is the most commonly sold over-the-counter antipyretic and analgesic, is generally considered harmless at therapeutic doses. However, APAP overdose causes severe and sometimes fatal hepatic damage in humans and experimental animals [5]. In reality, APAP-induced hepatotoxicity is very much associated with the cause of acute liver failure in many countries [6]. APAP exert its toxicity majorly via the oxidative stress which is the imbalance between the antioxidant capacity of cells and the level of reactive oxygen species (ROS) [7]. ROS cause liver ischaemia, necrosis and apoptosis leading to alternations of gene expression and sever liver damage [8]. Regardless of great advances in modern medicine, there are no totally effective drugs that aid in regenerating hepatocytes and total protection to the organ. Therefore, it is necessary to search for alternative medicines that are more effective and less toxic in ameliorating drug-induced liver injury. One of the medicinal plants that have been reported to exert hepato-protective effects is *Sphenocentrum jollyanum* [9].

Sphenocentrum jollyanum Pierre (Menispermaceae) is a perennial plant that grows naturally along the west coast sub region of Africa with expanse from Cameroon across Nigeria to Sierra Leone [10]. The plant is distributed from Sierra Leone to Cameroon *via* Nigeria and is reputed against chronic wounds, cough, and other inflammatory conditions as well as tumours [11]. It is called “Akerejupon”in indigenous Yoruba language. It is also called “Ezeogwu” in Igbo language. It is called “Oban Abe” in Edo state, “Adurukokoo” or “Red medicine” in Akan language of Ghana, “Krakoo” in Asante language of Ghana, “Dangbo-Pobè-Niaouli” in Ewe, Eastern of Ghana and Orji-nkoro in Izzi language of Ebonyi State, Nigeria [12]. Phytochemical screening of the ethanolic extract of *Sphenocentrum jollyanum* showed the presence of certain antioxidant phytochemicals like phenols, tannins, terpenoids, steroids, flavonoids and alkaloids [12]. Earlier study has shown that the stem bark extract significantly attenuated liver damage in carbon tetrachloride (CCl4)-induced rats [13]. Further studies also revealed that this plant have anti-oxidant and

anti-inflammatory activities [9]. However, there is dearth of information on the effect of the plant extract on APAP induced liver damage. This study aimed to evaluate for the first time the hepato-protective effects of the stem bark of *Sphenocentrum jollyanum* on liver injury induced by APAP in adult male Wistar rats.

Methodology

Ethical statement

All protocols and treatment procedures for this research were done in compliance with the Institutional Animal Care and Use Committee (IACUC) guidelines and as approved by the Faculty of Basic Medical Sciences Ethics Review Committee, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

Experimental animals

Thirty (30) male wistar rats weighing (150-180g) were purchased at a private animal holding and acclimatized for 2 weeks at the animal house of the Department of Anatomy, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo state, Nigeria. The animals were kept in a well-ventilated cage at room temperature of 25^0C for12 hours light and 12 hours darkness. They were allowed free access to feed diet and water *ad libitum*.

Experimental design

The rats were randomly divided into six (n=5) groups (A-F). Control (group A), 1000mg/kg of APAP only (group B), 700mg/kg of Stem bark of *Sphenocentrum jollyanum* (SBSJ) only (group C), 1000mg/kg of APAP + 700mg/kg of SBSJ (group D), 1000mg/kg of APAP + 350mg/kg of SBSJ (group E), 1000mg/kg of APAP for 14 days + 700mg/kg of SBSJ for the next 7 days. The extract and the drug were prepared daily as the administration was done via oral gavage daily for 14 days. The rats were sacrificed by cervical dislocation under mild anaesthesia and their liver was carefully harvested.

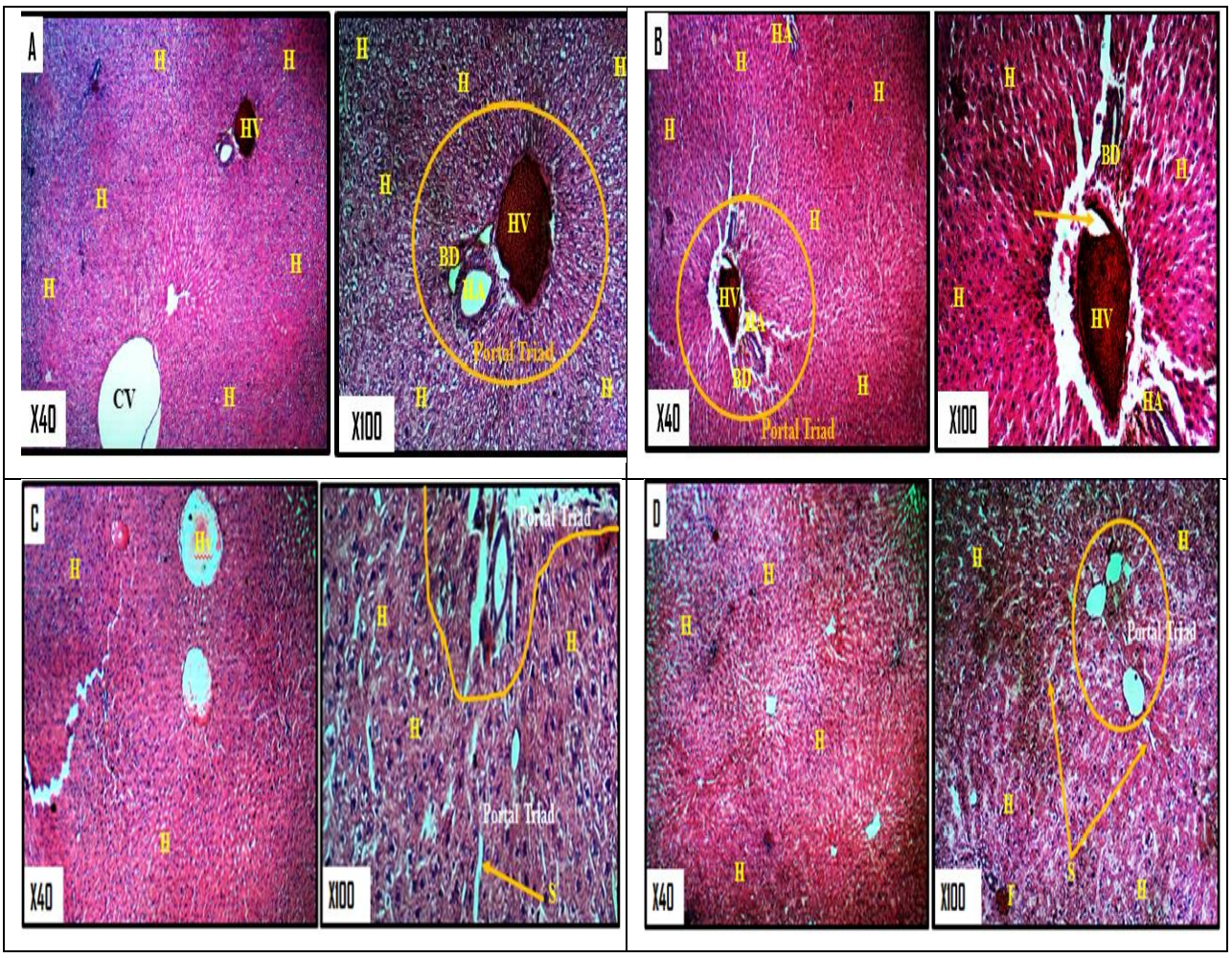
Preparation of plant extract

Fresh stems of *Sphenocentrum jollyanum* stems were collected from a farmland in Iwo, Osun state, Nigeria. The plant's stems were identified and authenticated by Professor A. J. Ogunkunle, (a Taxonomist) of the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria and a voucher sample (LHO 529) was deposited at the University herbarium [14]. The stems barks were peeled, air dried and pulverized into powder with an electric blender. Two hundred grams (200g) of dried and powered stem bark of *Sphenocentrum jollyanum* was macerated in 100% ethanol for 72 hours and filtered using a Buckner funnel and Whatman No.1 filter paper. The filtrate was concentrated under reduced pressure at 40^0C and stored in the extracting column at room temperature between 23^0C – 25^0C. It was allowed to siphon and then concentrated with the percentage yield was 47.26%, according to soxhlet method of extraction [15]

Histopathological examination

The tissues were processed and stained with haematoxylin and eosin as earlier described [16]. The micrographs of the stained sections were subsequently taken with the aid of an Olympus light microscope and snapped using AMSCOPE camera.

Results Histological findings



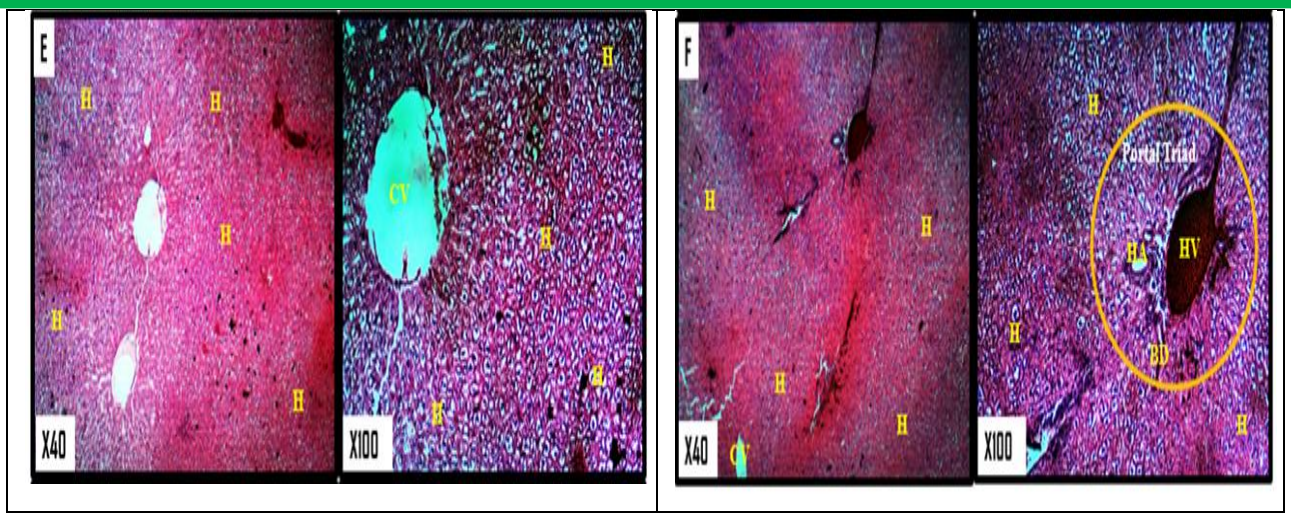


Figure 1 (X40 and X100, [H&E]): Photomicrograph of livers of male wistar rats in groups A to F showing the hepatocytes (H), portal triad (PT) comprised of hepatic vein (HV), hepatic artery (HA) and the bile duct (BD). Also observed across the micrographs are the central vein (CV) and branches of blood vessels. The (Control) group A shows intact cytoarchitecture with intact and well distributed hepatocytes. The Group B slide also shows mild infiltration of inflammatory cells. Red cells, hemorrhage, necrosis as well as fibrosis with some degenerative changes occurring in the liver are seen. Group C shows intact and improved cytoarchitecture with intact and well distributed hepatocytes. Group D shows mild infiltration degenerative changes occurring in the liver. Healing processes and some regenerative changes occurring are also seen. Group E also shows mild infiltration of inflammatory cells. Red cells, hemorrhage (H), necrosis (N) as well as fibrosis (F). Healing processes and some regenerative changes occurring in the liver are seen in Group E after the initial degeneration of hepatocytes by high doses of paracetamol. Groups E and F shows mild infiltration of inflammatory cells. Red cells, hemorrhage (H), necrosis (N) as well as fibrosis (F). Healing processes and some regenerative changes occurring in the liver are seen in Group F.

Discussion

The histological findings revealed that APAP causes significant histopathological damages on the histoarchitecture of the liver tissue with tissue tears, hepatocyte degeneration, necrosis and fibrosis as observed in the hepatic tissue (Fig 1B). The hepatic damage by APAP as seen by the histopathological alterations in this study correlates with earlier reported studies [5, 17, 4]. The hepatotoxicity of APAP has been implicated in functional suppression of immune cells, generation of reactive oxygen species (ROS), increased lipid peroxidation, depletion of tissue of antioxidant, alteration in membrane fluidity and permeability, enhanced rates of protein degradation and eventually cell death [1].

The ethanolic extract of stem bark of *Sphenocentrum jollyanum* (SBSJ) causes no histopathological damages on the architecture of the liver tissue; as there was no hepatic vacuolization, degeneration, inflammation or necrosis observed in the liver tissue (Fig 1C). This confirmed previous findings [18] that the extract has no toxic effect on the liver tissue morphology. However, the administration of the ethanolic extract of SBSJ healed and reversed the hepatic damages caused by APAP intoxication as observed in group D (1000mg/kg of APAP + 700mg/kg of ethanolic extract of SBSJ), group E (1000mg/kg of APAP + 350mg/kg of ethanolic extract of SBSJ) and group F (1000mg/kg of APAP for 2 weeks, withdrawn and subsequently treated with 700mg/kg of ethanolic extract of SBSJ for another 7 days), thereby suggesting that the ethanolic extract of SBSJ is effective in hepato-protection. This is in accordance with previous studies on the hepatoprotective efficacy of *Sphenocentrum jollyanum* against toxic substances, for example it significantly ameliorated liver damage in carbon tetrachloride (CCl4)-induced rats. [19, 9, 12]. The observed activity of the extract could be attributed to the phytochemicals such as saponins, tannins, alkaloids, terpenes and flavonoids which are present in the stem bark of *Sphenocentrum jollyanum* [20]. Also, the pharmacological activities of the extract like antioxidant and anti-inflammatory could also play a significant role in protecting the liver from harm [13].

Conclusion

This study showed that *Sphenocentrum jollyanum* extract demonstrated some regenerative changes indicating the progressive amelioration of damage caused by N-acetyl-para-aminophenol. However, further study is required on other biomarkers of liver damage induced by N-acetyl-para-aminophenol and the mechanistic hepatoprotective effect of *Sphenocentrum jollyanum* extract.

Competing interests

The authors declare they have no competing interest

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This research was self-funded.

Authors' contributions

TOA conducted the experiment and wrote the first draft of the manuscript. EAA designed and supervised the study. OIO reviewed and contributed to the manuscript writing. All authors read and approved the final manuscript.

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