Journal of Complementary and Alternative Medical Research

10(4): 16-28, 2020; Article no.JOCAMR.60783
ISSN: 2456-6276

Methanol Extract of *Chasmanthera dependens* Stem Mitigates against Mechanisms Involved in Piroxicam-induced Liver Damage in Rat

Tijani Stephanie Abiola[1*], Olori Ogaraya David[1] and Farombi Ebenezer Olatunde[2]

[1]Department of Biochemistry, Bowen University of Iwo, Nigeria.
[2]Drug Metabolism and Toxicology Research Laboratories, Department of Biochemistry, College of Medicine, University of Ibadan, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors TSA and OOD performed animals' experiments, data acquisition, the decision to publish and manuscript preparation. Author FEO provided some of the chemicals and reagents used and revised the manuscript and provided valuable advices. Author TSA designed the experiments and interpreted the results. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JOCAMR/2020/v10i430170
Editor(s):
(1) Dr. Sachin Kumar Jain, IPS Academy College of Pharmacy, India.
Reviewers:
(1) Shiara Martins de Souza, Universidade Federal de Alfenas, Brasil.
(2) Tanveer Ahmad Dar, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/60783

Original Research Article

Received 28 June 2020
Accepted 03 September 2020
Published 19 September 2020

ABSTRACT

Chasmanthera dependens has been claimed by tradition healers as a therapeutic agent in many diseases including hepatotoxicity. This study sought to evaluate the possible mechanisms involved in the hepatoprotective potential of tannin-rich extract of *Chasmanthera dependens* stem (TRECDS). Thirty two male Wistar rats (100- 130 g) were divided into four groups of eight rats per group labelled as group 1,2,3 and 4. The rats were treated orally for ten days consecutively. Group 1 served as control group and received normal saline, group 2 rats received 40 mg/kg piroxicam alone, groups 3-4 were treated with 40 mg/kg piroxicam and 200 and 400 mg/kg TREDS respectively concomitantly. All the experimental rats were fed with standard rat chows. Twenty four hours after, blood was collected to obtain serum; liver was excised to prepare homogenate and histology staining under

**Corresponding author: E-mail: tijaniabiol@yahoo.com;*

pentobarbital sodium anaesthesia. Liver function test (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT)) and oxidative stress (superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and lipid peroxidation (LPO)) biomarkers were assessed. Pro-inflammatory cytokines (tumor necrosis factor-α (TNF- α), interleukin-1β (IL-1β)) and apoptotic markers; caspase-3 (CASP-3) and caspase-9 (CAPS-9), cytochrome-c (CYT-c)) were also assessed.
The results showed that piroxicam administration caused hepatic damage as was evident in the histological assessment with increased serum activities of liver function markers, levels of pro-inflammatory cytokines and apoptotic markers. TRECDS also showed significant attenuation of the oxidative stress by decreasing the LPO level and increasing the activities of SOD, CAT and GSH level.
Oral administration of TRECDS also restores the morphological structure of the liver in a dose-dependent manner.
Conclusion: Oral administration of TRECDS exhibited protective potential against piroxicam-induced hepatotoxicity.

Keywords: Chasmanthera dependens; hepatoprotective; piroxicam; oxidative stress.

1. INTRODUCTION

Piroxicam is an acidic carboxamide oxicam derivative of the non-anti-inflammatory drugs (NSAIDs) with low solubility and high permeability. It is commonly used as an antipyretic, anti-inflammatory and analgesic drug [1] used in management of arthritis, postoperation, musculoskeletal diseases, atherosclerosis, colic, mastitis, autoimmune diseases, and dysmenorrhea [2,3]. Piroxicam also offers protection against cerebral ischemia [4] and has hypotensive and sedative effects [5]. Despite its widespread use, it has many adverse effects such as severe gastrointestinal toxicity, renal and hepatic injury and cerebrovascular damage [6,7,8]. The toxicity mechanism of action of piroxicam involves inhibition of cyclooxygenase enzyme that catalyze prostaglandin synthesis, thus reducing prostaglandin synthesis [9], generation of reactive oxygen species and lipid peroxidation resulting in oxidative stress and induction of apoptosis [10,11].

Liver is a central organ in the body and plays remarkable roles including detoxification and elimination of toxic substances from the body, thus controlling and preserving homeostasis of the body. Liver is highly vulnerable to the toxic agents such as drugs. Drugs, when metabolized generate reactive oxygen species (ROS) as by-products. These (ROS) when generated in excess at cellular level may cause destruction to tissue proteins, nucleic acids and membrane lipids and subsequent cellular damage [12]. Because of its role in drug metabolism, the liver is predisposed to toxic injury and since piroxicam is metabolized in the liver, there is a probability of liver injury by piroxicam or its metabolites.

Inflammation is an intricate defensive response of the body against harmful agents, such as drugs [13]. Nonetheless, it must be regulated to prevent the advent of pathological disorders related to the immune system [14]. Inflammation increases native blood flow, vasodilation, release of proinflammatory cytokines and fluid extravasation [15]. Early reaction of inflammation is characterized by activation of local cells with liberation of proinflammatory cytokines and chemokines, resulting in the recruitment of neutrophils [16]. Activation of neutrophils is associated with an up-regulated inflammatory response with increased expression of nuclear factor kappa B (NF-kB), which controls the transcription of pro-inflammatory cytokines including TNF-α and IL-1β.

Enhancement of caspase-3 activation and considerable epithelial cell apoptosis are important pathological events during NSAIDs-induced cytotoxicity [17]. The amplification and propagation of the cell death signaling cascade has also been linked to TNF-α in which it activates a family of specific cysteine proteases known as caspases [18].

Thus, compounds capable of modulating oxidative stress, inflammatory and apoptotic pathways may contribute to reduction of critical mediators in these pathological events. Thus, researchers have focused more on medicinal plants due to their multiple therapeutic functions with less side effects and presence of different phytoconstituents [19,20]. Many of these phytoconstituents have been identified and have

been reported to have therapeutic potentials. *Chasmanthera dependens* is widely distributed and usually planted in home gardens [21]. *Chasmanthera dependens* is rich in alkaloids and contains the quaternary protoberberine alkaloids and the non-phenolic quaternary alkaloids, steroids, oleic acid, tannin and phenol [21]. *Chasmanthera dependens* is commonly used in traditional medicine. It is used topically on sprained joints and bruises [22], has also been reported to possess analgesic and anti-inflammatory effects in laboratory animals [23,24] and as a remedy for venereal discharges or as a general tonic for physical or nervous weakness in inflammatory and exhausting diseases but there are no scientifical evidence on its hepatoprotective activity. Thus, this study investigated the possible hepatoprotective potential of tannin-rich extract of *Chasmanthera dependens* stem (TRECDS) and its mechanism of action on piroxicam-induced liver damage in rats.

2. MATERIALS AND METHODS

2.1 Chemicals and Drug

Piroxicam (Hingbo Dahongying pharmaceutical Co. Ltd, China). The reagent kits for albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin (BIL), gamma glutamyl transferase (GGT) were purchased from Randox Laboratories Limited, UK. Glutathione (GSH), thiobabituric acid (TBA), trichloroacetic acid (TCA), epinephrine were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA), Elisa kits for rats tumor necrosis factor-α (TNF- α), interleukin-1β (IL-1β) levels were purchased from Ray Biotech, Inc., caspase-3 (CASP-3) and caspase-9 (CAPS-9), cytochrome-c (CYT-c) were purchased from Cusabio Biotech Co., Ltd,China. All other chemicals used were of analytical grade and were obtained from the British Drug House (Poole, Dorset, UK).

2.2 Plant Materials

2.2.1 Plant collection and authentication

Fresh stems of Chasmanthera dependens were obtained in a local garden at Oke Oba area in Iwo along Ibadan-Iwo express road, Osun state, Nigeria. Iwo is situated on latitude 7.6292˚N and longitude 4.1872˚E.The plant was identified and authenticated by Esimekhuai, D.P.O. at University of Ibadan with voucher number UIH - 22478.

2.2.2 Preparation of the plant powder and extract

The stems were chopped into pieces and air dried at room temperature. The dried samples were pulverized with an electric blender (Bajaj bravo 3 jars mixer grinder/blender, India). Weighed and kept in an air-tight container prior to extraction.

The tannin-rich extract of Chasmanthera dependens stem was prepared by extracting 1 kg of powdered Chasmanthera dependens with 2.5 Litres of 70% acetone for 72 hours at room temperature with intermittent shaking and filtered with cheese cloth. The filtrate obtained was subjected to further extraction using equal volume of diethyl ether in separating funnel and shaken vigorously, until complete separation. The tannin-rich layer the lower layer was then collected and evaporated under reduced pressure using rotary (Edward Vacuum Co-operation, Crawley, England) at 40 ^{o}C. The aliquot obtained was freeze dried to obtain a dark brown extract of 20.17% w/w referred to as tannin-rich extract of Chasmanthera dependens stem (TRECDS). The dried extract was stored in an air tight container at -20^{o}C and used for this study.

2.3 Experimental Animals

Thirty two male Wistar rats (6 weeks 100-120 g ± 5 g) were obtained from the Department of Biochemistry University of Ibadan, Ibadan, Nigeria, were used in this study. The rats were housed in plastic cages placed in a well-ventilated rat house at the Department of Biochemistry, Bowen University, Iwo, Nigeria, to acclimatize for fourteen days prior to the commencement of the experiment, provided standard rat chows (Ladokun Feeds, Nigeria) and water ad libitum throughout the experiment and subjected to natural photoperiod of 12-h light/12-h dark cycle.

2.4 Experimental Design

The research design comprises concomitant oral treatment of the rats with piroxicam and TRECDS for 10 days according to the procedure described previously [26]. Rats were randomly divide into four groups of eight rats per group. Group 1 served as the control received 0. 9% v/w of normal saline in distilled water. Group 2 rats were treated with 20 mg/kg piroxicam (PIRO), group 3 rats received 20 mg/kg piroxicam + 200 mg/kg body weight of TRECDS and group 4 rats

received 20 mg/kg piroxicam + 400 mg/kg body weight of TRECDS. All treatments were administered once daily.

Twenty four hours after the last doses of the extract and/or piroxicam, rats were anesthetized with pentobarbital sodium (35 mg/kg, i.p.) and blood sample was collected by retro-orbital vein using glass capillary tube from each rat from each overnight-fasted rats into plain tubes for serum preparation. The rats were then euthanized and liver of each rat was harvested, washed with ice-cold phosphate buffer saline (PBS; pH 7.4) to remove blood stain. The liver was dried and weighed. Portions from each liver were cut for histological assessment and the rest used for the preparation of homogenate.

2.5 Preparation of Serum and Liver Homogenate

The blood sample was centrifuged using a cooling centrifuge (Sigma 3-18KS, Sigma, Germany) at 3000 rpm for 10 minutes to obtain serum. The liver (1 g) of each rat was minced and homogenized (WiseTis® HG-15A, DAIHAN Scientific Co. Ltd., Korea) in PBS. The homogenate was centrifuged at 10,000 g for 10 minutes at 4^{o}C to obtain supernatant. The serum and the supernatant were stored at -20 ^{o}C and used for biochemical analyses.

2.6 Determination of Liver Function Markers

The liver function indices were evaluated using commercially available kits from Randox Laboratory Limited (UK). Serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) were determined according to the manufacturer instructions.

2.7 Estimation of Liver Oxidative Stress Biomarkers

The oxidative markers were evaluated by assessing superoxide dismutase (SOD) activity according to the method described by Misra and Fridovich [27] and catalase (CAT) activity was determined using H_2O_2 as a substrate according to the method described by Sinha [28] and levels of reduced glutathione (GSH) according to the method described by Jollow et al. [29] and concentration of malondialdehyde (MDA) as measurement of lipid peroxidation according to method of Farombi [30] and the result was expressed as micromoles of MDA per milligram protein. Protein concentration was determined according to the method of Lowry et al. [31].

2.8 Quantitative Assessments of Tumor Necrosis Factor Alpha (TNF- A) and Interleukin-1beta (IL-1β) Levels

Serum levels of TNF-α and IL-1β were evaluated using antibody sandwich Enzyme- Linked Immunosorbent Assay (ELISA) kits based on their manufacturers' instructions. Briefly, 100 µL of samples or standards were added into the wells already pre-coated with antibody specific for rat TNF-α or IL-1β and incubated for 2 h at 37 °C. Unbound substances were removed and 100 µL of biotin-conjugated antibody specific for rat TNF-α or IL-1β was added to the well. After washing, 100 µL of avidin conjugated Horseradish Peroxidase (HRP) was added to each well and incubated for 1 h at 37 °C, the unbound components were washed away. Then, 90 µL of TMB substrate solution was added to each well and incubated for 15-30 minutes at 37 °C to give a blue color proportional to the amount of TNF-α or IL-1β bound. The reaction was terminated by adding stop solution to each well to obtain a yellowish color that was measured at 450 nm using a microplate reader (Cytation 5, BioTek Instrument, USA). All of the experiments were performed in triplicate, and the results were presented in pg/ml.

2.9 Determination of Serum Cytochrome C, Caspase-9 and Caspase-3 (Cyt C, Casp-9 and Casp-3) Activities

Serum Cyt c, CASP-9 and CASP-3 activities were measured by enzyme-linked immunosorbent assay (ELISA) using rat specific Cyt c, CASP-9 and CASP-3 assay kits (Cusabio Biotech Co., Ltd, Wuhan, China) according to the manufacturer's instructions. The assays rely on a quantitative sandwich enzyme immunoassay technique and the final colored product was measured at 450 nm by a microplate reader (Cytation 5, BioTek Instrument, USA). All of the experiments were performed in triplicate, and the results were presented in pg/ml.

2.10 Histopathology Assessment

Liver biopsies were processed for histology according to Songur et al. (32). Briefly, liver specimens were fixed in 10% neutral buffered formaldehyde solution for 48 hours. After

dehydration procedures, the samples were blocked in paraffin. Sections of 4-5 μm were cut by a microtome and stained with hematoxylin and eosin (H&E). All slides were coded before examination with light microscope (Olympus CH; Olympus, Tokyo, Japan) by pathologists who were blinded to control and treatment groups and photographed using a digital camera.

2.11 Statistical Analysis

Data were expressed as mean ± standard error of mean (SEM) for 8 rats. Statistical analysis was carried out using one-way analysis of variance (ANOVA) to compare the experimental groups followed by Duncan's test to identify significantly different groups (SPSS for Windows, version 22, SPSS Inc., Chicago, Illinois). Graphical construction was performed using Graphpad® Prism 7 (Graphpad Software, La Jolla, CA) and $p < 0.05$ were considered statistically significant.

3. RESULTS

3.1 TRECDS Alleviates the PIRO-Induced Liver Damage

The activities of the serum ALT, AST, ALP and GGT were evaluated in the rats following administration of PIRO for ten days. The results (Fig. 1), PIRO significantly increased the enzyme activities compared to the control group. But, simultaneous administration of TRECDS at different doses significant ($p < 0.05$) attenuated the observed increase in the activities of ALT, AST, ALP and GGT in the rats' serum particularly at 400 mg/kg b.w.

3.2 TRECDS Ameliorates PIRO-Induced Liver Oxidative Stress in Rats

The activities of SOD and CAT and levels of GSH and MDA were assessed in the liver of rats (Fig. 2). The SOD and CAT activities and level of GSH were significantly decreased with concurrent increase in the level of MDA in the liver of rats following administration of PIRO for 10 consecutive days when compared to the control group. However, simultaneous administration of TRECDS revealed significant ($p < 0.05$) increased activities of SOD and CAT and GSH level while decreased level of MDA was observed in a dose-dependent manner.

3.3 TRECDS Ameliorates the PIRO-Induced Elevation of TNF- α and IL-1β in the Serum of Rats

The serum levels of TNF- α and IL-1β as markers of inflammation were determined after administration of PIRO to rats. The levels of these pro-inflammatory cytokines were significantly ($p < 0.05$) elevated in the PIRO-alone treated group compared to the control group (Fig. 3). However, administration of TRECDS simultaneously with PIRO to rats decreased the levels of these cytokine and again the 400 mg/kg b. w. value in both parameters were similar to the normal control ($p < 0.05$).

3.4 TRECDS Alleviates the PIRO-Induced Increase in Serum Cyt-c, CASP-9 and CASP-3 Activities

The serum activities of Cyt-c, CASP-9 and CASP-3 were determined after the administration of PIRO to rats (Fig. 4). The activities of these intrinsic apoptotic markers were significantly increased when compared to the control group. Concomitant administration of TRECDS with PIRO to rats decreased the activities of Cyt-c, CASP-9 and CASP-3 significantly ($p < 0.05$). The decreases following TRECDS were dose-dependent.

3.5 TRECDS Mitigates the PIRO-I nduced Histological Damage in Rats Liver

Histological examination of rats' liver was performed by Haematoxylin and Eosin staining following PIRO and TRECDS administration for ten days. The result on the effect of TREDS on piroxicam-induced liver damage is presented in Fig. 5. In control rats, the hepatic structures were clear and intact (Fig. 5A) with no lesions. Hepatic sinusoids and interlobular bile ducts in portal areas were normal. The PIRO alone group (Fig. 5B), showed hepatic sinusoids dilation and inflammatory cells infiltration. Diffuse vacuolar degeneration and narrowing of hepatic lobules around the hepatic sinus. Nuclear fragmentation of some of the hepatocytes (black arrows) as evident of apoptotic process. However, concomitant administration of different doses of TREDS to rats revealed offset the pathological changes observed in the PIRO alone group. The 400 mg/kg of TREDS group particularly showed intact structures of the hepatic lobule and no obvious infiltrates of inflammatory cells.

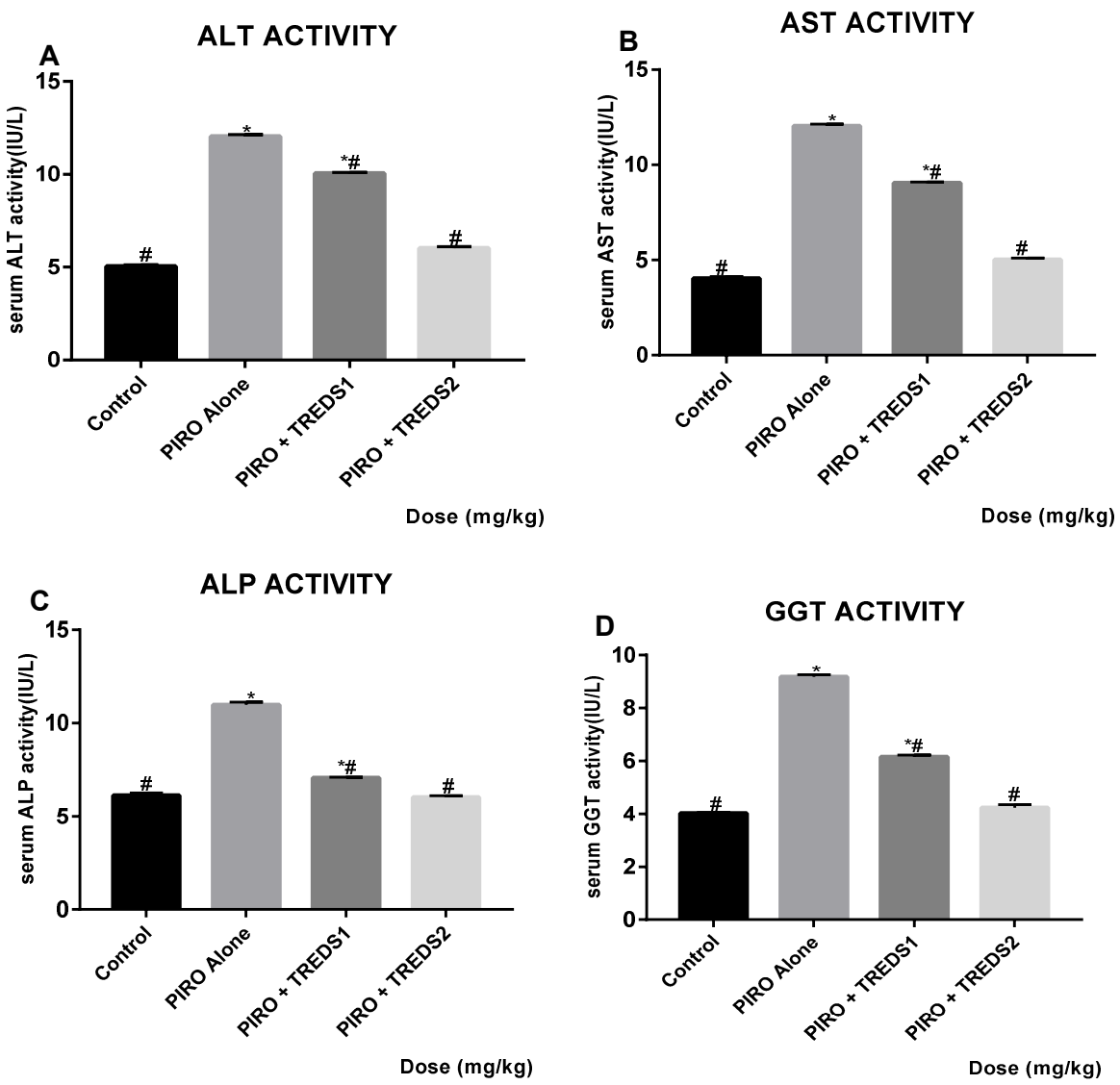

Fig. 1. TRECDS alleviate PIRO-induced increased liver activities of: (A) ALT; (B) AST; (C) ALP and (D) GGT in rats. Each bar represents mean ± SEM. (n = 8)

$^{*}p < 0.05$ is significant when compared with the control group, $^{\#}p < 0.05$ is significant when compared with the piroxicam alone group. PIRO: 20 mg/kg piroxicam; TRECDS1: 200 mg/kg of tannin-rich extract; TRECDS2: 400 mg/kg of tannin-rich extract

4. DISCUSSION

The liver is the major organ in drug metabolism and this predisposes the liver to damage. Enzymes and other components that are localized in the liver are used to assess liver damage. Quantitative measurements of these markers give an idea of the location and extent of the damage [32]. Commonly used markers of hepatic damage are ALT, AST, ALP and GGT that are released into the blood stream [33]. The liver is the main target organ in NSAID intoxication [11,34]. Alkaline phosphatase (ALP) is an indicator enzyme for evaluating the integrity of the hepatobiliary system and flow of bile into the small intestine [35] while gamma glutamyl transferase is a specific biomarker of hepatobiliary injury. These two enzymes help to ascertain the occurrence of bone or liver injury and their raised level is indicative of liver or bile duct disease.

In this study, rats that received piroxicam alone had elevated activities of ALP and GGT, this is in accordance with result of Sobeh et al., [36], but co-administration with different

doses of TREDS decreased the level of this enzyme.

In this study piroxicam caused liver damage as shown by increased serum activities of the ALT, AST, ALP and GGT accompanied by histological changes. The elevations in these biochemical markers can be as a result of lipid peroxidation of the membrane of hepatocytes caused by piroxicam that made the liver to lose it integrity, thus aiding the leakage of both intracellular and membrane bound enzymes into the bloodstream. Our results conformed to the previous studies of Sahu; Sobeh et al., [11,36]. However, concurrent administration of TRECDS with PIRO decreased the activities of these enzymes probably due to its ability to protect the membrane of hepatocytes from lipid peroxidation.

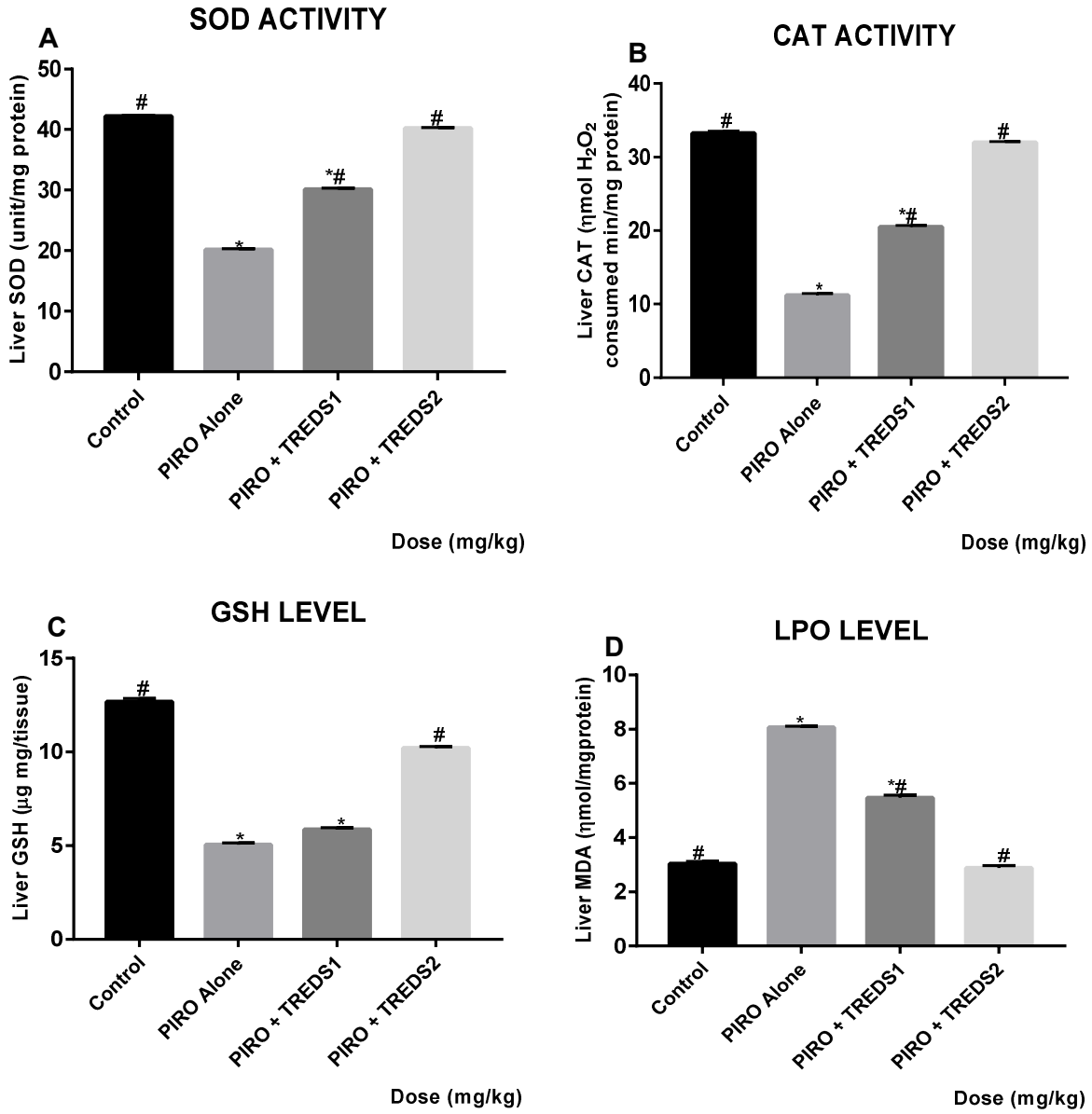

Fig. 2. TRECDSA mitigates PIRO-induced decrease in liver activities of SOD (A) and CAT (B) and level of GSH (C) and increase in (D) MDA level of rats. Each bar represents mean ± SEM. (n = 8)

$^{*}p < 0.05$ is significant when compared with the control group, $^{\#}p < 0.05$ is significant when compared with the piroxicam alone group. PIRO: 20 mg/kg piroxicam; TRECDS1: 200 mg/kg of tannin-rich extract; TRECDS2: 400 mg/kg of tannin-rich extract

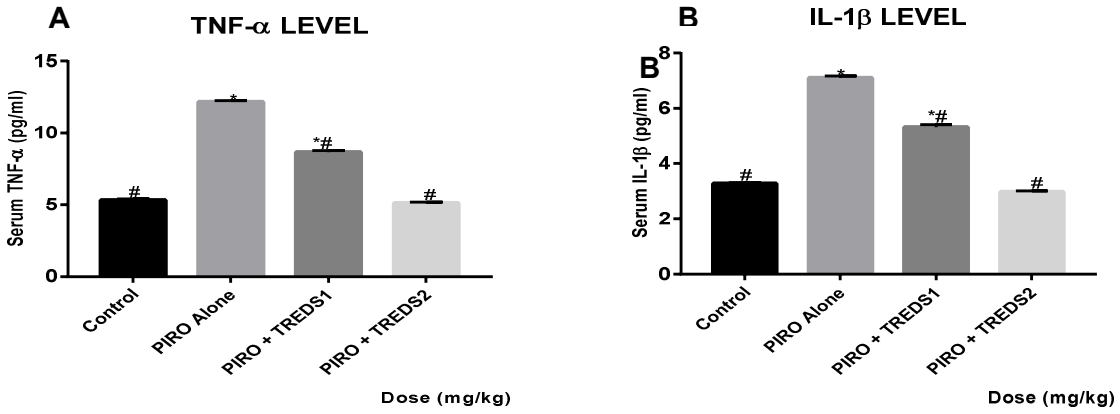

Fig. 3. TRECDS Mitigates PIRO-induced increase serum levels of: (A) TNF- α and (B) IL-1β in rats. Each bar represents mean ± SEM. (n = 8)

$^{*}p < 0.05$ is significant when compared with the control group, $^{\#}p < 0.05$ is significant when compared with the piroxicam alone group. PIRO: 20 mg/kg piroxicam; TRECDS1: 200 mg/kg of tannin-rich extract; TRECDS2: 400 mg/kg of tannin-rich extract

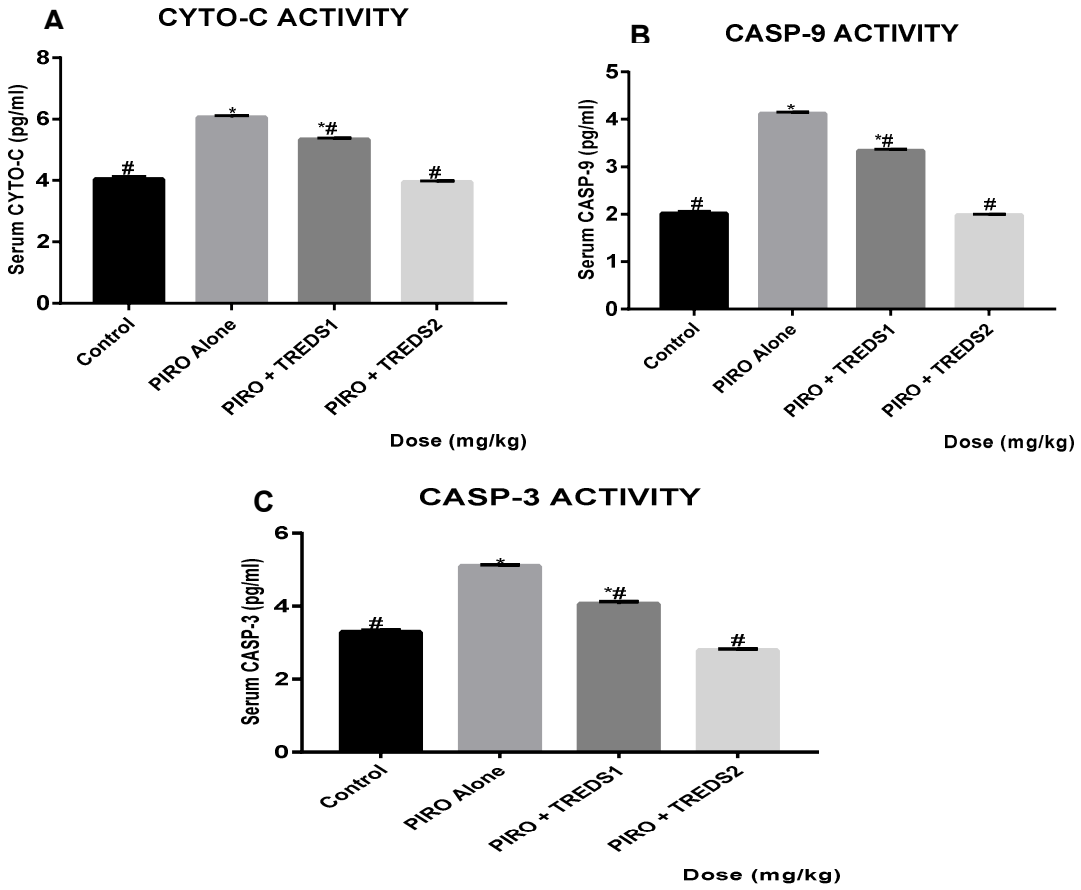

Fig. 4. TRECDS Mitigates PIRO-induced increase serum activities of: (A) Cyt-c; (B) CASP-9 and (C) CASP-3in rats. Each bar represents mean ± SEM. (n = 8)

$^{*}p < 0.05$ is significant when compared with the control group, $^{\#}p < 0.05$ is significant when compared with the piroxicam alone group. PIRO: 20 mg/kg piroxicam; TRECDS1: 200 mg/kg of tannin-rich extract; TRECDS2: 400 mg/kg of tannin-rich extract

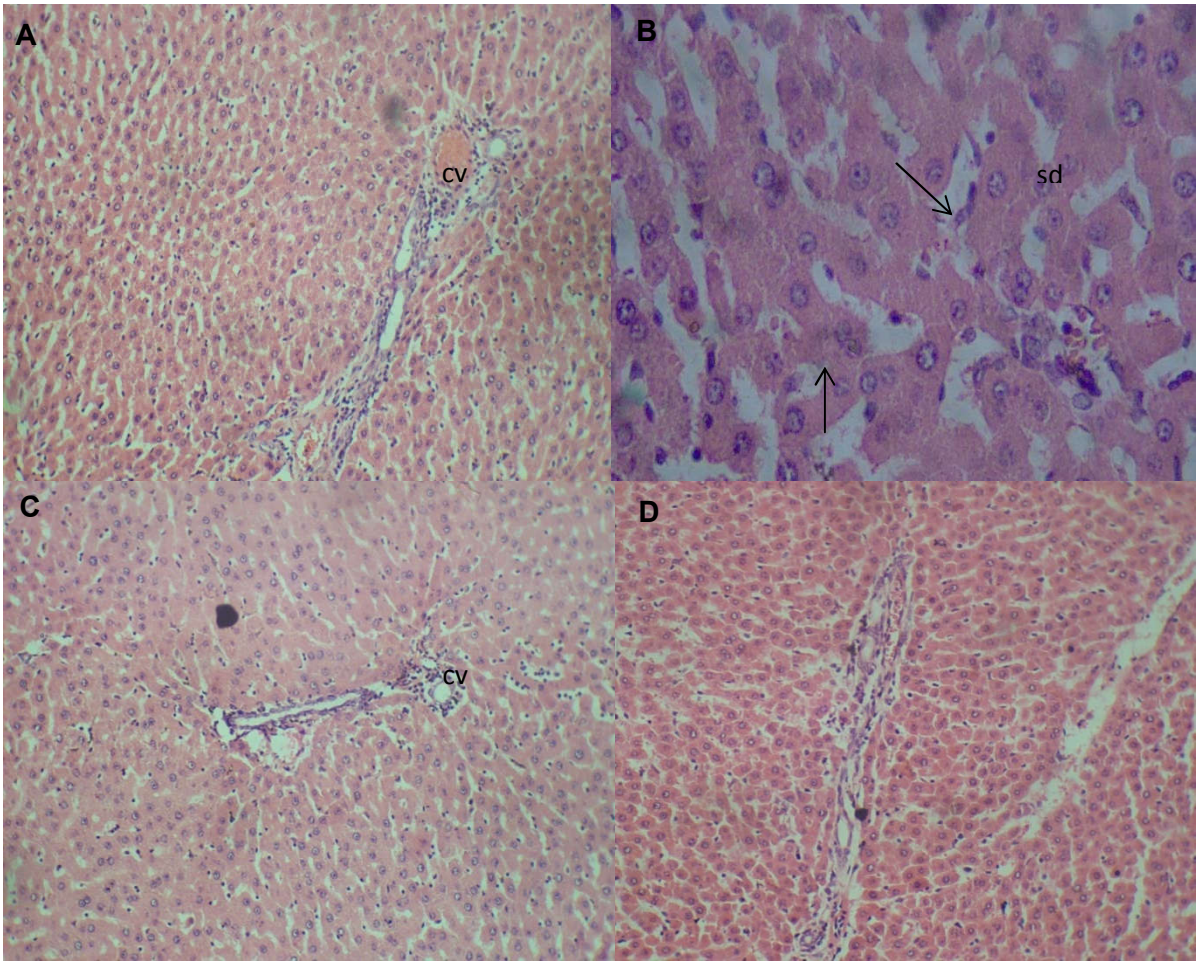

Fig. 5. Representative images of liver sections showing the effect of different doses of TRECDS on PIRO-induced liver damage in rats. (A) Control group shows no visible lesion; (B) PIRO alone group showing vacuolation, nuclear fragmentation and infiltration of inflammatory cells: (C) PIRO + 200mg/kg TRECDS showing almost intact structural architecture of the hepatocytes. (D) PIRO + 400 mg/kg TRECDS showing normal architectural arrangement of the hepatocytes, blood sinusoids and portal triad. (H& E X 100): cv: central vein; sd: blood sinusoid

The mechanism of piroxicam hepatotoxicity has been associated with its idiosyncratic reaction that involves defect in the mitochondrial synthesis of adenosine triphosphate and generation of the active metabolites of piroxicam that causes direct cytotoxicity, generation of reactive oxygen species, swelling of the mitochondria and oxidation of protein thiols amongst others [37,38]. Excessive generation of ROS result in lipid peroxidation and redox cycling that are implicated in hepatotoxicity leading to cell death due to oxidative stress [39]. Oxidative stress is triggered by alteration in the intracellular pro-oxidant to antioxidant ratio in favor of pro-oxidants resulting to depletion of the antioxidant status of the hepatic cells and subsequent cellular damage [40]. In our study, administration of PIRO to rats showed significant occurrence of lipid peroxidation as evident by elevated level of MDA and reduced GSH level with accompanied decreased activities of SOD and CAT. The results of our study agreed with the previous report of Abdeen et al., [10]. However, concomitant administration of TRECDS with PIRO decreased the MDA level and increased the GSH level and the activities of SOD and CAT suggesting that the extract has the potential to scavenge the ROS generated by PIRO and thus prevented the oxidative stress.

Studies have shown that inflammation and oxidative stress are interconnected phenomena

implicated in pathological disorders including liver disease [41]. It has been reported that ROS generation is increased during inflammatory events in the damaged tissue and stimulate the signaling pathway for inflammatory mediators like proinflammatory cytokines (TNF-α and IL-1β) and chemokines, resulting in inflammatory cell migration [42]. TNF-a is a key cytokine that exerts pleiotropic effects ranging from proliferative responses, inflammatory effects by increasing IL-1β production and modulation of immune responses, initiation of apoptotic responses, leukocyte activation, and tissue infiltration [43,44]. TNF-α is produced as a transmembrane or a membrane-cleaved circulating cytokine by various cell types such as macrophages, lymphocytes, cerebral microglia and astrocytes, and hepatic Kupffer cells. The hepatic inflammatory response is mediated by proinflammatory cytokines, especially TNF-α, the release of which is one of the first events in many types of liver injury, and further modulates the effects of other cytokines, such as IL- IL-1β [45]. The result of our study revealed elevated levels of TNF-α and IL-1β in PIRO alone group. This is agreement with the previous report of Hebatullah et al., [46]. But, oral administration with PIRO simultaneously decreased these pro-inflammatory cytokines and ameliorated the damage caused by piroxicam. This suggests that since TRECDS is capable of modulating oxidative stress, it can also intervene in inflammatory process by suppressing the pro-inflammatory cytokines.

Reactive oxygen species have been implicated to stimulate the mitochondrial permeability transition pore, the opening of this channel causes a loss in membrane potential, mitochondrial swelling with membrane rupture, cytochrome-C release, activation of caspases and apoptosis [47,48]. In this study, generation of ROS by piroxicam seems to activate the intrinsic apoptotic pathway via the release of cytochrome-C that activates the procaspase-9 and the subsequent activation of the executioner caspase-3. The enhancement of the apoptotic pathway observed can also be linked to increase TNF-α level [49]. This result conformed with the previous reports of Abdeen et al.,;de Cássia et al., [10,13]. Again, TRECDS administration at the same time with piroxicam abrogates this process and thus preserves the integrity of the liver.

The necrotic changes and inflammation observed in histological sections of the liver are typical observations in cell damage [50]. The assessment of the histological changes in the liver of rats treated with piroxicam alone and those treated simultaneously with TRECDS confirmed the biochemical results obtained in our study.

Overall, our results revealed that piroxicam-induced liver damage can be attributed to reactive oxygen species generation resulting in inflammatory process and subsequent intrinsic apoptotic pathway and that TRECDS has the capability to attenuate these processes probably due to the synergetic actions of the constituents.

5. CONCLUSION

TRECDS possesses hepatoprotective potential against piroxicam-induced liver damage and may be considered as a new therapeutic agent for hepatotoxicity.

ETHICAL APPROVAL

All the animals received humane care according to the principles stated in the ‘‘Guide for the Care and Use of Laboratory Animals’’ prepared by the National Academy of Science (NAS) and published by the National Institute of Health [25] and approval by the Bowen University Ethical Committee.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ACKNOWLEDGEMENTS

The authors thank Mr. Olayinka Olaleye for his assistance in the histological work and interpretations.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Trivedi M. Spectroscopic characterization of disulfiram and nicotinic acid after biofield treatment. J Anal Bioanal Tech. 2015;6:265.
2. Di Salvo A, Giorgi M, Nannarone S, Lee HK, Corsalini J, della Rocca G. Postoperative pharmacokinetics of meloxicam in horses after surgery for colic syndrome. J Vet Pharmacol Ther. 2018;41:369-373.
3. Nir RR, Nahman-Averbuch H, Moont R, Sprecher E, Yarnitsky D. Preoperative preemptive drug administration for acute postoperative pain: a systematic review and meta-analysis. Eur J Pain. 2016; 20:1025-1043.
4. Bhattacharya P, Pandey AK, Paul S, Patnaik R, Yavagal DR. Aquaporin-4 inhibition mediates piroxicam-induced neuro-protection against focal cerebral ischaemia/reperfusion injury in rodents. PLoS ONE. 2013;8(9):e73481. Available:https://doi.org/10.1371/journal.pone.0073481
5. Saganuwan SA, Orinya OA. Toxiconeurological effects of piroxicam in monogastric animals. J Exp Neurosci. 2016;10:121-128.
6. Laine L. NSAID-associated gastrointestinal bleeding: assessing the role of concomitant medications. Gastroenterol. 2014;147(4):730-733.
7. Möller B, Pruijm M, Adler S, Scherer A, Villiger PM, Finckh A. Chronic NSAID use and long-term decline of renal function in a prospective rheumatoid arthritis cohort study. Ann Rheum Dis. 2015;74(4):718-723.
8. Sriuttha P, Sirichanchuen B, Permsuwan U. Hepatotoxicity of nonsteroidal anti-inflammatory drugs: a systematic review of randomized controlled trials. Int J Heptagon 2018:1-13.
9. Steinmeyer J. Pharmacological basis for the therapy of pain and inflammation with non-steroidal anti-inflammatory drugs. Arthritis Res. 2000;2:379-985.
10. Abdeen A, Aboubakr M, Elgazzar D, AbdoM, Abdelkader A, Ibrahim S, Elkomy A. Rosuvastatin attenuates piroxicam-mediated gastric ulceration and hepato-renal toxicity in rats. Biomed Pharmacother. 2019;110:895-905.
11. Sahu CR. Mechanisms involved in toxicity of liver caused by piroxicam in mice and protective effects of leaf extract of Hibiscus rosa-sinensis L. Clin Med Insights Arthritis Musculoskelet Disord. 2016;9:9-13.
12. Wang SY, Jiao H. Scavenging capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals and singlet oxygen. J Agric Food Chem. 2000;48:677-684.
13. de Cássia da Silveira e Sá R, Andrade LN, de Sousa DP. "A review on anti-inflammatory activity of monoterpenes." Molecules. 2013;18(1):1227-1254.
14. Cássia da Silveira e Sá R, Andrade LN, de Sousa DP. Anti-inflammation activities of essential oil and its constituents from indigenous cinnamon (Cinnamomum osmophloeum) twigs. Bioresour Tech. 2008;99(9):3908-3913.
15. Nizamutdinova IT, Dusio GF, Gasheva OY, Skoog H, Tobin R, Peddaboina C,et al. Mast cells and histamine are triggering the NF-*κ*B-mediated reactions of adult and aged perilymphatic mesenteric tissues to acute inflammation. Aging. 2016;8(11):3065.
16. Stone MJ. Regulation of chemokine–receptor interactions and functions. Inter J Mol Sci. 2017;18(11):2415.
17. Slomiany BL, Piotrowski J, Slomiany A. Role of caspase-3 and nitric oxide synthase-2 in gastric mucosal injury induced by indomethacin: effect of sucralfate. J Physiol Pharmacol. 1999;50(1):3-16.
18. Kelany ME, Hakami TM, Omar AH. Curcumin improves the metabolic syndrome in high-fructose-diet-fed rats: role of TNF-α, NF-κB, and oxidative stress. Can J Physiol Pharmacol. 2017;95(2):140-150.
19. Adejuwon SA, Omirinde JO, Ebokaiwe AP, Aina OO, Adenipekun A, Farombi EO. Radiation-induced testicular injury and its amelioration by *Telfairia occidentalis*. Bri J Med Medical Res. 2014;4:7-18.
20. Channabasava GM, Chandrappa CP, Umashankar T. Gc-Ms study of two column fractions from methanol extracts of *Loranthus micranthus* and their *in-vivo* antidiabetic activity on alloxan-induced diabetic rats. J Diab Metabol. 2015;6:5-14.
21. Mosango D. *Chasmanthera dependens* Hochst. [Internet] Recordfrom. PROTA4U; 2008.
22. Ogunlesi M, Okei W, Ademoye M. Medicinal plants used in treating eye infections in Nigeria. In: Odugbemi T,

editor. A textbook of medicinal plants from Nigeria. University of Lagos Press, Lagos. 2008;299-317.
23. Morebise O, Awe EO, Makinde JM, Olajide OA. Evaluation of the anti-inflammatory and analgesic properties of *Chasmanthera dependens* leaf methanol extract. Fitoterapia. 2001;72:497-502.
24. Fatokun OT, Wojuola TE, Esievo KB, Kunle OF. Medicinal plants used in the management of asthma: a review. EJPMR. 2016;3(7):82-92.
25. Public Health Service, Policy on Humane Care and Use of Laboratory Animals, US Department of Health and Human Services, Washington, DC, USA; 1996.
26. Aithal GP, Day CP. Nonsteroidal anti-inflammatory drug-induced hepatotoxicity. Clin Liver Dis. 2007;11:563-75.
27. Misra HP, Fridovich I. The role of superoxide anion in the auto-oxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972;247:3170-3175.
28. Sinha AK. Colorimetric Assay of Catalase. Anal Biochem. 1972;47:389-394.
29. Jollow DJ, Mitchell JR, Zampaglione N, et al. Bromobenzene induced liver necrosis: protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacol. 1974;11:151-169.
30. Farombi EO, Tahnteng JG, Agboola AO, et al. Chemoprevention of 2-acetylaminofluorene-induced hepatotoxicity and lipid peroxidation in rats by kolaviron-a Garcinia kola seed extract. Food Chem Toxicol. 2000;38:353-541.
31. Lowry OH, Rosenbrough NJ, Farr AL, et al. Protein measurement with Folin phenol reagent. J Biol Chem. 1951;193:265-275.
32. Karimi-Khouzani O, Heidarian E, Amini SA. Anti-inflammatory and ameliorative effects of gallic acid on fluoxetine-induced oxidative stress and liver damage in rats. Pharmacol Rep. 2017;69:830–5.
33. Eidi A, Mortazavi P, Bazargan M, Zaringhalam J. Hepatoprotective activity of cinnamon ethanolic extract against CCl_4-induced liver injury in rats. Excli J. 2012;11:495.
34. Burukoglu D, Baycu C, Taplamacioglu F, Sahin E, Bektur E. Effects of nonsteroidal anti-inflammatory meloxicam on stomach, kidney, and liver of rats. Toxicol Ind Health. 2016;32:980–986.
35. Singh A, Bhat TK, Sharma OP. Clinical Biochemistry of Hepatotoxicity. J Clinic Toxicol. 2011;S4: 001.
36. Sobeh M, Mahmoud MF, Abdelfattah MAO, El-Beshbishy HA, El-Shazly AM, Wink M. Hepatoprotective and hypoglycemic effects of a tannin-rich extract from *Ximenia americana* var. caffra root. Int J Phythe Phytpharm. 2017;33:36-42.
37. Pandit A, Sachdeva T, Bafna P. Drug induced hepatotoxicity: A review. J Appl Pharm Sci. 2012;02(05):233-243.
38. Larrey D, Ursic-Bedoya J, Meunier L. Drug-Induced hepatotoxicity. In: Schiff ER, editor. Schiff 's diseases of the liver. 12th ed. New York: Wiley- Blackwell. 2018;740-773.
39. Patlevič P, Vašková J, Švorc P Jr, Vaško L, Švorc P. "Reactive oxygen species and antioxidant defense in human gastrointestinal diseases," Integra Med Res. 2016;5(4):250-258.
40. Ayala A, Muñoz MF, Argüelles S. "Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal," Oxid Med Cell Longev; 2014. Article ID 360438.
41. Ambade A, Mandrekar P, "Oxidative stress and inflammation: essential partners in alcoholic liver disease." Intern J Hepatol; 2012. Article ID 853175.
42. Hussain T, Tan B, Yin Y, Blachier F, Tossou MCB, Rahu N. "Oxidative stress and inflammation: what polyphenols can do for us?." Oxid Med Cell Longev; 2016. Article ID 7432797.
43. He J, Liang J, Zhu S, Zhao W, Zhang Y, Sun W. Protective effect of taurohyodeoxycholic acid from Pulvis Fellis Suis on trinitrobenzene sulfonic acid-induced ulcerative colitis in mice. Eur J Pharmacol. 2011;670:229-235.
44. Benchikh, F. Pharmacological Effects of Myrtus Communis L. on the Gastrointestinal Tract of Rats and Mice. Ph.D. Thesis, Department of Biology and Animal Physiology, Faculté des Sciences de la Nature et de la Vie, Université Ferhat Abbas Sétif 1, Setif, Algeria; 2018.
45. Liu J, Wang F, Luo H, Liu A, Li K, Li C, et al. Protective effect of butyrate against ethanol-induced gastric ulcers in mice by promoting the anti-inflammatory, anti-oxidant and mucosal defense mechanisms. Int. Immunopharmacol. 2016;30:179–187.

46. Hebatullah Samy Helmy, Ayman EEl-Sahar, Rabab HSayed, Rehab Nabil Shamma, Alaa Hamed Salama, Eman Maher Elbaz. Therapeutic effects of lornoxicam-loaded nanomicellar formula in experimental models of rheumatoid arthritis. Inter J Nanomed. 2017;12:7015-7023
47. Malhi H, Gores GJ, Lemasters JJ. Apoptosis and necrosis in the liver: a tale of two deaths? Hepatol. 2006;43:S31.
48. Wood KC, Gladwin MT. The hydrogen highway to reperfusion therapy. Nat Med. 2007;13:673.
49. Yan T, Wang H, Zhao M, Yagai T, Chai Y, Krausz KW, et al. Glycyrrhizin protects against acetaminophen - induced acute liver injury via alleviating Tumor Necrosis Factor *α*-mediated apoptosis**.** Drug Metab Dispos. 2016;44(5):720-731.
50. Thoolen B, Maronpot RR, Harada T, Nyska A, Rousseaux C, Nolte T, et al. Proliferative and nonproliferative lesions of the rat and mouse hepatobiliary system. Toxicol Pathol. 2010;38(7): 5S-81S.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/60783