Hepatoprotective effect of Pinostrobin against Thioacetamide-induced liver cirrhosis in rats

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Abstract

Pinostrobin was used in traditional medication for management of numerous syndromes. In the current study, histology, immunohistochemistry, and hepatoprotection effects of Pinostrobin were assessed against thioacetamide (TAA) hepatotoxicity in rats. Thirty rats were arbitrarily separated into five groups. Group 1 was intraperitoneally (i.p) injected with distilled water 3 times/week and fed (po) daily with 10% Tween 20 for 2 months. Group 2-5 were i.p. injected with 200 mg/kg TAA thrice weekly for 8 weeks and fed with 10% Tween 20, 50 mg/kg silymarin, 30 and 60 mg/kg of Pinostrobin daily for 8 weeks, respectively.

Experimental groups fed groups showed that Pinostrobin significant reduction in liver index and hepatocyte proliferation with much lesser cell injury. These groups were significantly down-regulated the PCNA and α-SMA. The liver homogenate exhibited increased antioxidant enzymes (SOD and CAT) activities accompanied with decline in malondialdehyde (MDA) level. The serum level of bilirubin, total protein, albumin and liver enzymes (ALP, ALT, and AST) were restored to normal and were comparable to that normal control and silymarin with TAA treated groups.

The hepatotoxic group showed a significant rise in serum liver biochemical markers together with a considerable decrease in protein and albumin level compared to the normal group. The hepatotoxic group displayed decreased catalase and superoxide dismutase activities while increased lipid peroxidation.

Pinostrobin decreased level of TNF-a, IL-6 and increased the level of IL-10. Acute toxicity with a higher dose of 500 mg/kg Pinostrobin did not manifest any toxicological signs in rats.

Macroscopy of hepatotoxic liver exhibited irregular, rough surface with micro and macro nodule. Histopathology stained by Hematoxylin and Eosin, and Masson Trichrome showed there was inflammation and infiltration of lymphocytes, focal necrosis, fibrosis, and bile duct propagation. Pinostrobin fed group had expressively reduced TAA toxicity in gross and histology as designated by fewer disturbances of hepatic tissue, slight fibrosis, and low-grade cells infiltration. Immunohistochemical staining designated that pinostrobin significantly down-regulated the expression of proliferation cellular nucleus antigen (PCNA) and alpha-smooth muscle actin (α-SMA) in the liver. Thus, the findings of this study presented that the hepatoprotective effect of this plant may be due to a reduction in toxicity, inhibition of hepatocytes proliferation, down-regulation of PCNA and α-SMA, decreased enzyme markers; and increased protein and albumin increased endogenous enzymes and reduced lipid peroxidation level.

1. Introduction

Pinostrobin is a natural flavonoid found in diverse various medicinal plants which include Lauraceae, Zingiberaceae, Fabaceae, and Polygonaceae [1, 2]. Pinostrobin is known to have a spectrum of pharmacological effects [3], including antiulcer [4], antiplatelet, antimutagenic [5], anti-oxidant, anti-inflammatory, and anti-cancer properties [4, 6-8], antimicrobial [9], antiparasitic properties [10] and peptic ulcer therapy [11].
Although liver is a highly crucial organ for cleansing, liver diseases can be the greatest health complications [12]. Cirrhosis, hepatocellular carcinoma, viral hepatitis, and alcoholic hepatitis are the most common liver diseases, all of which are closely linked to jaundice [13]. In scientific literatures, numerous revisions well-known helpful influence of uncountable medicinal plants defensing liver from hepatotoxic damage of TAA in laboratory animals [14-22]. The most common hepatoprotection agent is silymarin, which is herbal substance extracted from seeds of *Silybum marinus* plant [23]. The latter is used broadly as a therapeutic supplement to for aimed at liver disease symptoms such as hepatitis, fatty acid infiltration and cirrhosis resulted from toxic chemical and alcohol effect [23]. Several studies have used silymarin as a reference medication for hepatoprotection against TAA hepatotoxicity [21, 24-30]. TAA increases oxidative stress and attracts free radicals, which causes damage to proteins, lipids, and DNA [31, 32].

TAA makes hepatic cells impairment subsequent its breakdown to thioacetamide sulphene and sulphone, which is caused by a hazardous trail that includes Bio-transformation involving the CYP4502E1 enzyme [33]. Several studies by different co-researchers evidenced TAA have been used in the early stages of liver fibrosis [21, 27, 34-40].

The effectiveness of rodent tuber herb since traditional rights necessity verified to aid progress novel medicines functioning in contradiction of liver syndromes. However, there was no study found on the compound's hepatoprotective properties. This study aims to assess hepatoprotective action of pinostrobin on TAA-persuaded liver injuries in rats.

2. Material And Methods

2.1 Thioacetamide

TAA obtained from Sigma-Aldrich, Switzerland, and then liquefied in 10% Tween 20 also mixing well until complete dissolved. At that time, 200 mg/kg body mass inserted i.p rodent three times weekly for 8 weeks. TAA induced vicissitudes in together biological besides morphology structures comparable to that of humanoid liver cirrhosis [25].

2.2 Silymarin

Silymarin is a reference drug (International Laboratory, USA) used in research as a standard medicine. Silymarin was melted in 10% Tween 20, and then gavage to rats in a dose of 50 mg/kg [35, 41].

2.3 Pinostrobin

Pinostrobin was purchased from Sigma-Aldrich Chemical Co., (USA). Pinostrobin was dissolved in 10% Tween 20 and given to rats in doses of 30 and 60 mg/kg. (5 mL/kg) [14].
Acute toxicity study and experimental animals

Thirty (18 males and 18 females) healthy Sprague Dawley rats (6–7 weeks old, weighed between 180 and 210 g) were acquired from the Experimental Animal House, Cihan University-Erbil. The rats were given standard rat pellets diet and tap water ad libitum and located in separate caged with wide-mesh wire bottom to prevent coprophagia. The rats were kept in cages one week for adaptation. The acute toxicity study was used to determine a safe of pinostrobin. The rats were allocated similarly into 3 groups; vehicle (10% Tween 20, 5 mL/kg), 30 mg/kg and 60 mg/kg of the pinostrobin (5 mL/kg). Prior to the dosing, the rats were fasted overnight (food but not water). Food was withdrawn for a further 3 to 4 h after dosing. The animals were observed for 24-48 h after the administration of the pinostrobin for the beginning of clinical or toxicological signs. Mortality, if any, was reported over a period of 2 weeks. The animals were sacrificed then by giving an overdose of xylazine and ketamine anesthesia on the 15th day. Blood samples were collected by intracardial puncture and serum was separated for biochemical parameters analysis. Histological and serum biochemical parameters were determined following standard methods (Gwaram et al., 2016, Salga et al., 2017)

2.4 Experimental animals for hepatoprotective activity

Sprague Dawley rats were obtained from Animal House Unit Department of Medical Microbiology, Cihan University-Erbil. Rats weight approximately 180 - 200 grams were housed individually via wide-mesh wire bottoms to avoid coprophagia throughout the experimental time, at 25°C ± 2°C temperature, approximate moisture 55-65% and 12 hours exposure light/dark rotation. All the rats were fed on tape water and standard pellet. The experiment was planned and approved by the Ethics Commission for Animal Research. Human care for whole experimental animals was applied and followed the Guide for Maintenance and usage of laboratory Animals which produced by the National College of Knowledge and issued through national Institute of health. Thirty healthy adult male Sprague Dawley rats were arbitrarily alienated into five clusters with six rats respectively. Rats’ clusters divisions besides treatment protocol were determined following the method of [35, 37] with a few modifications; Group 1 (normal), which was treated by distilled water (5 mL/kg) i.p. injection for thrice a week, and 10% Tween 20 (5 mL/kg) via oral administration every day for two months. Group 2 (hepatotoxic) inoculated i.p. (200 mg/kg) of TAA three times a week and daily oral administrated by 10% Tween 20 (5 mL/kg) for two months. Group 3 (reference drug) given TAA (200 mg/kg) i.p. injection three times weekly, followed by regular administration Silymarin (50 mg/kg) for 2 months. Collection 4 and 5 were received TAA (200 mg/kg) i.p injection thrice/week for 2 months, and daily oral administration of pinostrobin with 30 mg/kg, (group 4) and 60 mg/kg (group 5) for 2 months, respectively.

After the last treatment on end of experimental time (two months), all animals fated 24 hours and then processed for general anesthesia using ketamine and xylazin 30 mg/kg (100 mg/mL), 3 mg/kg (100
mg/mL) [42, Mahmood et al., 2004]. Blood withdrawn from intracardial puncture and store it in gel-activated tube for liver functions test [43, Omer et al., 2017].

2.5 Biochemical parameters (liver function test)

Blood in clot-activator tubes were separated by centrifugation for 15 min at 2500 rpm. A spectrophotometer is used to measure alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, and total protein in addition to albumin. Biochemical parameters were assessed in the Erbil Hospital Laboratory [42, Salga et al., 2017].

2.6 Macroscopic appearance of liver

Liver assessment was done by opening the rat’s abdominal and thoracic cavities. The livers showed significant macroscopic proof of pathological changes. Also, other organs showed pathological grossly lesions, but excluded from current study. All livers were separately washed in cold saline and checked for any gross pathological abnormalities by taking microscopic images [24].

2.7 Histopathology of liver tissue

2.7.1 H & E stain & Masson Trichrome stain

Liver samples washed in cold saline, cut 2 cm cubic, fixed in 10% phosphate buffered formalin. Leica, Germany tissue processor machine was used to process the specimens. and embedded in paraffin. Five µm thickness slices routinely stain by H&E [42, Mahmood et al., 2007] in addition, the Masson trichrome stain [44]. Nikon microscope (Y-THS, Japan) used to evaluate the liver slides for histopathological change and characteristic areas were photographed.

2.7.2 Immunohistochemistry (PCNA)

Immunostaining, PCNA was achieved as formerly designated by [37] and [16]. The propagation directory of PCNA-stained liver slices was determined by counting the proportion of labeled cells per 1000 liver cells, and the number of mitotic cells was expressed as the mitotic index [14].

2.8 Liver tissue homogenate for endogenous (CAT, SOD) enzymes and oxidative Stress (MDA)

Neutral ice-cold phosphate buffer saline 10% (w/v) was used to wash rats’ livers. Teflon homogenizer used to homogenize liver samples (all steps done on ice), then at 4500 rpm centrifugation for 15 min at 4°C cell debris were detached. Supematant collected to verify antioxidant activity via superoxide dismutase (SOD) and catalase (CAT) analyze kits (Cayman Chemical Company, USA) (Omer et al., 2017). Malondialdehyde (MDA) is a marker of cellular oxidative stress. MDA, assay kits was used to assess the levels of thiobarbituric acid reactive substance (TBARS, Cayman Compony) [45].
Assessment of inflammatory cytokines

Valuation of TNF-α, IL-6 and IL-10 in liver tissue homogenate was accomplished utilizing marketable ELISA kit from (Cusabio Biotech Co. China). Briefly, liver homogenate was centrifuged at 3000 g for 15 minutes and supernatant was used for recognition of cytokine levels using commercial enzyme-linked immunosorbent assay kit. The assess was achieved according to the constructor’s procedure stated in Rat TNF-α ELISA Kit (109331), Rat IL-6 ELISA Kit (84597) and Rat IL-10 ELISA Kit (84236). Cytokine concentrations were designed using standard purified recombinant cytokines.

2.9 Statistical analysis of data

Data analyses were showed as mean ± SE, One-Way ANOVA using Tukey post hoc assessment, SPSS software, and version 24. The p values statistical meaning at p<0.05.

3. Results

3.1 Liver biochemical markers

Hepatotoxic effect of TAA was significantly increased (p 0.001, mean ± SE) ALT, ALP, total bilirubin, and AST levels indicate liver damage (Table 1). Moreover, the TAA group showed significant decreases (P 0.001, mean ± SE) in total protein and albumin comparing with normal control group, demonstrating acute hepatocellular injury. Pinostrobin and silymarin treated groups are significantly dropped (P 0.001, mean ± SE) enzyme levels of ALT, ALP, total bilirubin and AST. Furthermore, total protein and total albumin values were elevated (P 0.001, mean ± SE) in Pinostrobin and silymarin treatments in comparison with TAA control group. Hence, Pinostrobin revoked the hepatotoxic effect of TAA via reinstating typical liver activities. Pinostrobin effectively prevented TAA-induced hepatotoxicity at dosage of 30 mg/kg, whereas slightly affected at dosage of 60 mg/kg.

Table 1: The effects of pinostrobin on liver biochemical parameters in rats with TAA-induced hepatotoxicity. The effects of Pinostrobin or silymarin on serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities, as well as total bilirubin, albumin, and protein levels. The data are presented as mean ± SE (n = 6 per group). Significant difference from the normal control group at *p < 0.001, Significant difference from the TAA control group at #p < 0.001.
| Groups                      | ALP IU/L  | ALT IU/L  | AST IU/L  | T.Bilirubin (µM/L) | T. Protein g/L | T. Albumin g/L |
|----------------------------|-----------|-----------|-----------|-------------------|----------------|---------------|
| Normal Control             | 70.3±0.6  | 30.4±0.6  | 63.9±0.7  | 1.2±0.01          | 73.2±0.7       | 32.9±0.5      |
| TAA + NS                   | 193±1.1*  | 130.3±0.5* | 172.5±0.5* | 5.1±0.07*        | 45.8±0.6*      | 13.2±0.2*     |
| Silymarin + TAA (50mg/kg) | 62.4±0.6# | 28.1±0.9# | 62.5±0.6# | 1.4±0.01#        | 68.1±0.7#      | 29.4±0.6#     |
| TAA + LD (30mg/kg)         | 58.2±0.8# | 22.3±0.6# | 55.4±0.5# | 1.9±0.03#        | 60±0.8#        | 22.1±0.6#     |
| TAA + HD (60mg/kg)         | 54.3±0.5  | 25±0.4#   | 58.7±0.7# | 1.7±0.05#        | 64.8±0.4#      | 25.6±0.6#     |

3.2 Gross appearance of liver

The morphological changes of liver in all groups (Figure 1 (GA)) were evaluated and showed that the normal control group liver had a smooth surface with regular lobes (Figure 1, GA (A)). TAA-induced hepatotoxicity group liver showed an irregular surface with many macro and micronodules (Figure1, GA (B)). The TAA + silymarin-treated group had a smooth surface that was similar to the control group (Figure1, GA (C)). TAA + Pinostrobin 30 mg/kg and TAA+ Pinostrobin 60 mg/kg groups were exhibited liver with smooth surface and closely maintain the liver normal architectural structure and shape (Figure 1, GA (D, E)).

3.3 Histopathological examination of hepatocyte sections

Histopathological changes of liver sections stained with hematoxylin and eosin are shown in Figure 1 (H&E). Liver slides of normal group display typical hepatocytes architecture, preserved cytoplasm, distinguished nucleus and nucleolus with distinct regular plates of liver cells separated by sinusoidal capillaries and central vein (Figure 1, H&E (A)). Liver sections from TAA group were showed irregular hepatocyte architecture resulted from the presence of reforming nodules. Moreover, liver section was divided via fibrous septa stretching from central vein to portal area. Hepatocytes presented sever damage, necrosis and extensive propagation of bile duct, congested central vein, fatty changes, and granulocytes and monocytes which are presented surround the central vein due to the inflammation (Figure 1, H & E stain (B)).

Silymarin + TAA, low and high dose of pinostrobin +TAA groups were illustrated relative protection from hepatocyte-disruptions induced by TAA. The hepatic cellular compositions showed a reduced amount of damage with a slight fibrotic septum.

Insignificant penetration of lymphocytes was observed in these liver sections groups. Moreover, the histopathological sections demonstrated remarkable regenerative parenchymal nodules, which are boarded with fibrous tissue as well as noteworthy growth in the cells-fat storing, bile ducts and Kupffer cells (Figure 1, H&E (C-E)). To assess tissue fibrosis, the liver tissues were stained with Masson's
trichrome. Collagen deposition was not detected in the normal control liver section (Figure 1, MT (A)). TAA
group was shown bile duct regeneration with notable dense fiber septa and increased collagen fiber
accumulation around a congested central vein, which is referred to as severe fibrosis in the hepatic tissue
(Figure1, MT (B)). The silymarin, 30 mg/kg and 60 mg/kg of pinostrobin clusters illustrated a reduction in
number of fibrous septa and regeneration nodules. In addition, the collagen fibers in all these three
groups were observed to be homologous, which indicated to the hepatoprotection activity of pinostrobin
extract (Figure 1, MT (C-E)).

3.4 Immunohistochemical Staining of Liver Sections

The effect of pinostrobin on hepatocyte proliferation after TAA-induced liver injury was observed through
immunohistochemical analysis of PCNA appearance in the liver parenchyma using anti-PCNA antibody
(Figure 2). Hepatocytes in the normal control cluster showed no PCNA staining, indicating that no cell
renewal was taking place. In comparison, hepatocytes from the TAA control group had upregulated PCNA
appearance and an increased mitotic directory, showing proliferation to restore the severe liver tissue
impairment caused by TAA.

Liver tissues treated with 30 mg/kg pinostrobin, 60 mg/kg Pinostrobin, or silymarin had condensed
hepatocyte renewal compared to the TAA controls, as designated by abridged PCNA countenance and a
significant decrease of the mitotic index. by condensed PCNA appearance and a substantial discount of
the mitotic index. pinostrobin had an excellent outcome on PCNA labeling and mitotic index in a dose-
dependent method had an outstanding effect on PCNA labeling and mitotic index in a dose-dependent
method. The effect on PCNA labeling had an outstanding and mitotic index in a dose-dependent method.

Effects pinostrobin on endogenous antioxidant enzymes in TAA-induced liver cirrhosis in rats

Hepatotoxic group revealed significantly lower SOD and CAT activities, comparison to normal group
(Figure ). Experimental groups fed pinostrobin exhibited significantly restored the depletion of SOD and
CAT level to normal values (Table ). Rats treated with pinostrobin 60mg/kg had significantly higher SOD
and CAT values than that pinostrobin 30mg/kg and ulcer hepatotoxic control groups, respectively. The
MDA levels were significantly lower (23.645±2.19) in rats treated with p-Cymene 500mg/kg when
compared to 29.45±3.17 and 88.816±6.04 of G2 ulcer control and G4 p-Cymene groups, respectively. The
Normal control and Omeprazol treated rats showed non-significant changes in their SOD, CAT, and MDA
profiles. The prostaglandin E2 was significantly higher (32±2.36) in G5 group in compare to 11.83±1.32,
7.16±1.16e, and 22±2.60 of G1, G2, and G4 groups, respectively.

3.4 Effect of Pinostrobin on TNF-α, IL-6, and IL-10 in TAA-induced liver cirrhosis in rats

Pinostrobin showed an immune-modulatory influence in liver tissue homogenate by dropping the level of
TNF-α and IL-6, and augmented in level of IL-10 (Figure 5).
Table 2. Effect of Pinostrobin on TNF α, IL-6 and IL-10 on TAA-induced liver cirrhosis in rats.

| Animals group  | TNF α (pg/ml) | IL 6 (pg/ml) | IL 10 (pg/ml) |
|----------------|---------------|--------------|---------------|
| G1 Normal      | 83.84±2.31a   | 160.5±3.61b  | 242.16±3.31e  |
| G2 TAA control | 540.33±6.37e  | 340±5.40d    | 106.83±3.31a  |
| Silymarin 50mg/kg | 129.6±5.24b   | 140±5.40a    | 210.16±3.31d  |
| Pinostrobin 30mg/kg | 261±4.28d  | 181±4c       | 174.5±3.27b   |
| Pinostrobin 60mg/kg | 189±4.28c | 163.8±2.92b | 189.66±4.36c |
| f-ratio        | 8804.68       | 2522.050     | 1213.90       |

4. Discussion

In the present study, hepatotoxic group was related with visible increase in activities of liver markers in blood circulation such as ALP, ALT, AST, and bilirubin level. Similarly, numerous academics reported increase in liver function markers [17, 26, 37]. The increase in liver function biomarkers imitates hepatocellular dysfunction. With the consistence of the results of current study increased in liver markers activities and bilirubin level in hepatotoxic group were previously reported by several researchers [18, 35, 43, 46].

These values were meaningfully reduced to near-normal levels after feeding with pinostrobin. With the consistency of our findings, several coworkers used various plant extracts to show reduced liver function enzyme activities and bilirubin levels, which have been previously reported elsewhere [14, 15, 18, 21, 34, 47, 48].

The hepatoprotective achievement may be due to of its effect against cells leakage and injury of hepatocytes covering. TAA specified to burden with RNA initiative from nucleus to cytoplasm, starting exterior injury which results in rise statement of serum liver pointers [43, 49].

In the current study, total protein and albumin quantities in serum were obviously reduced in TAA control group. Though, silymarin or pinostrobin feeding groups bring back these values to closely normal level. With the agreement of the results of our investigation enormous numbers of scientists displayed that rat's
gavage silymarin or various plant extracts brought the albumin and protein to almost normal levels [15, 20, 21, 25, 46, 50, 51] reported that ethanolic leave extracts of *Garuga pinnata* can serve as promising herbal medicine for the treatment of both acute and chronic hepatotoxicity due to the presence of flavonoids rutin.

Outcomes of the existing research showed a decline collagen deposition in pinostrobin fed groups in tissue sections stained with Masson’s trichrome dye. Analogous to the results of current study many investigators used innumerable plant extracts confirmed reduction of collagen fibers compared to TAA control group [14, 16, 21, 38, 41].

A recent research reported that acacetin and pinostrobin inhibit malignant breast epithelial cell adhesion and focal adhesion formation to attenuate cell migration [8]. Sopanaporn et al., (2020) reported that pinostrobin suppresses the Ca2+ reported that acacetin and pinostrobin inhibit malignant breast epithelial cell adhesion and focal adhesion formation to attenuate cell migration. Moreover, pinostrobin suppresses the Ca2+-signal-dependent growth arrest in yeast by inhibiting the Swe1-mediated G2 cell-cycle regulation [7]. pinostrobin can be considered as a potential drug for breast cancer [52]. The flavonoids from *B. rotunda* may be considered as promising Alzheimer’s disease preventative agents through inhibition of β-amyloid formation [53].

Histopathological (H & E staining) and Masson’s Trichrome staining, and immunostaining displayed the repressing effect of feeding with pinostrobin, which could be owing to its capability to prevent hepatocyte propagation, as designated by down-regulation of PCNA staining. Similarly, Shreef et al., (2021) exposed that green tea potentially inhibited the progression of liver cirrhosis, down-regulation of PCNA proliferation [48]. Jadaun et al., (2019) stated that pinostrobin inhibits proliferation and induces apoptosis in cancer stem-like cells through a reactive oxygen species-dependent mechanism [54].

The outcomes of the existing study exhibited that normal liver group or silymarin treated collections demonstrated down-regulation of PCNA, suggesting the absence of cell regeneration. Up-regulation of PCNA countenance hepatocytes was observed in a hepatotoxic set, exemplifying comprehensive construction, imaginable exertion to reconstruction tissue impairment [55, 56].

Otherwise, rats fed with silymarin or pinostrobin dramatically reduced cell proliferation by means of a lessening in PCNA stain. In scientific literatures, huge numbers of remedial plants with hepatoprotective potential have been noticeable by quite a lot of co-authors [57-60].

In present study, Endogenous enzymes, SOD and CAT, in liver tissues homogenate significantly decline in hepatotoxic group as compared to normal cluster. Both enzymes become flagging by free radical’s resulting liver weakening [61]. Meanwhile, pinostrobin expressively elevated concentration of serum CAT and SOD by self-protective liver from the injurious influence of free radicals compared to TAA control group. Matching outcomes have been described formerly by uncountable researchers [18, 27, 35, 41, 48,
62, 63]. Similarly, Hajrezaie et al., (2015), presented that biochanin a flavonoid increase SOD in gastric homogenate of rats and decreased the release of MDA. Panduratin significantly increase the SOD aand CAT activity and decreased MDA level [18].

MDA as a lipid peroxidation marker is usual injurious process [61, 64]. MDA level elevated in tissue improved lipid peroxidation [65]. Rise MDA initiating damages and tragedy of antioxidant protection to block the expansion of additional free radicals [66]. Existing search exhibited TAA yield increase in MDA quantity has been promisingly reduced by pinostrobin feeding. Parallel results have been previous reported by various academics elsewhere [18, 24, 37, 46, 67, 68].

Drop of hepatic SOD and CAT activities in hepatotoxic group might possibly explain elevated MDA. TAA created liver fibrosis in rodents. Nonetheless, rat's gavage with pinostrobin could dramatically accelerate the recovery of the liver injuries suggestively prevent the impact of TAA intoxication. These results are likewise consistent with former studies stated by abundant inventers using diverse medicinal plants [18, 21, 27, 69].

Sidahmed et al., (2015) demonstrated that zerumbone reduced the level of MDA in gastric homogenate [70]. Devkota et al., (2021) showed Flavonoids from the leaves and twigs of Lindera sericea potent free radical scavenging and α-glucosidase inhibitory activities [71]. Taha et al., (2012) demonstrated that Turnera diffusa possesses anti-ulcer activity, which could be attributed to lipid peroxidation inhibitory, immunomodulatory and anti-oxidant mechanisms of arbutin flavonoid [72]. Shareef et al., (2021) showed that green tea potentially inhibited the progression of liver cirrhosis, prevented oxidation of hepatocytes, recovered SOD and CAT enzymes, condensed MDA and reduced cellular inflammation [48].

TAA induces inflammatory response that initiates a dynamic chain of immune responses associated with the release of vast amounts of inflammatory cytokines such as TNF-α and IL-6, which in turn produce increased quantity of ROS (Wei et al., 2003). TNF-α, being a major proinflammatory cytokine produced by macrophages, attracts neutrophils to the site of gastric mucosal injury Martin and Wallace, 2006; Kishimoto, 2005). IL-6 is another important proinflammatory cytokine shown to mediate immune response and acute inflammation. IL-6 activates granulocytes and agranulocytes, which in turn trigger a stress response in injured tissue (Mei et al., 2012); Sabat et al., (2010) suggested that IL-10 has the ability to suppress inflammatory response and inhibit TNF-α production. Previous reports showed that ethanol was able to increase proinflammatory cytokines and decrease anti-inflammatory cytokines in gastric tissue.56,57 Our results were in agreement with these observations, where exposure to ethanol showed elevated TNF-α and IL-6 and decrease in IL-10 levels, compared to normal controls. However, MECE pretreatment inhibited depletion of IL-10 and elevation of TNF-α and IL-6 levels, which shows its anti-inflammatory effect on ethanolinduced gastric ulcer in the rat. This also concurs with the earlier histological finding where less inflammatory responses were observed in MECE-treated rats with gastric ulcer.
5. Conclusion

The current study found that pinostrobin had a significant hepatoprotective effect in reducing TAA toxicity in rats, as evidenced by biochemical liver parameters, endogenous enzymes, histology, and immunohistochemistry. Pinostrobin intensely raises the CAT & SOD activities, whereas significant reduction of hepatic MDA. The hepatoprotective effect of pinostrobin could be attributed to its ability to inhibit hepatocyte multiplication, reduce oxidative stress and lipid peroxidation, down-regulate PCNA, and possess antioxidant and free radical scavenger properties.

Declarations

Ethics Announcement

1. The current experiment was authorized through conscience team for animal investigation, Faculty of Science, Cihan University-Erbil, and Ethic No. ERB, 115, 11/03/2019. All animals for duration of trials, obtained human attention in accordance with principles set forth “Director for the Maintenance and Use of research laboratory Animals” which was organized by the Nation-wide School of Sciences has issued by the national Institution of healthiness.

2. This manuscript has not been published in whole or in part elsewhere.

3. The manuscript is not currently being considered for publication in another journal.

4. All authors have read and approved the manuscript.

Author contributions

All authors contributed to the study conception and design. S.H.S and M.A.A: conducting the experiments, data collection and analysis, manuscript preparation. M.H.A. and P.Y.A: analysis and interpretation of the data, reviewed drafts of the paper. N.F.S.A., S.O.M. and A.S.M.J.: contributed reagents and materials, interpretation of the data, reviewed drafts of the paper.

Conflict of interest

The authors declare no conflict of interest.

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**Figures**
Figure 1

Histopathological examination of liver tissue sections. (H&E) Hematoxylin and Eosin and (MT) Mason Trichrome stains are presenting histopathological sections and (GA) Gross appearance of liver from (A) normal group, (B) TAA group, (C) silymarin group, (D) Pinostrobin low dose group, and (E) Pinostrobin high dose group. Stained liver sections were examined under a Nikon microscope (Y-THS, Japan). 20X magnification.
Figure 2

Immunostaining analysis of liver tissue sections. (PCNA) stains. (A) Normal group, (B) TAA group, (C) Silymarin group, (D) Pinostrobin low dose group, and (E) Pinostrobin high dose group. Stained liver sections were examined under a Nikon microscope (Y-THS, Japan). 20X magnification.
Figure 3

Effects of pinostrobin on antioxidant enzyme activities (SOD and CAT) and MDA level in the liver. Data are expressed as mean ± SEM. Means among groups (n=6 rate/group) show significant difference, *P < 0.5 compared to TAA control group, and **P <0.01 compared to normal control group.
Figure 4

Alpha-smooth muscle actin (α-SMA) in the liver. (A) Normal group, (B) TAA group, (C) Silymarin group, (D) Pinostrobin low dose group, and (E) Pinostrobin high dose group. Stained liver sections were examined under a Nikon microscope (Y-THS, Japan). 40X magnification.
Figure 5

Effect of Pinostrobin on TNF α, IL-6 and IL-10 on TAA-induced liver cirrhosis in rats. (A) Normal group, (B) TAA group, (C) Silymarin group, (D) Pinostrobin low dose group, and (E) Pinostrobin high dose group