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Hepatoprotective effect of methanolic extract of *Iris florentina* L. on paracetamol-induced liver toxicity in rats

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Abstract: Despite of plethora of research on hepatoprotective potential of medicinal plants, there is still need to discover potential plants with hepatoprotective activity. *Iris florentina* L. is a medicinal plant with traditional claims but ignored investigation regarding its hepatoprotective effects. The current study is aimed to investigate the hepatoprotective potential of *I.florentina* L. methanolic extract on paracetamol (PCM)-induced liver injury. The phytochemical and HPLC screening was done which showed the presence of potential constituents including flavonoids and phenols. For investigating the hepatoprotective effect of *I.florentina* L. methanolic extract, rats were given five different treatments for seven consecutive days. The normal control (group 1) was administered with normal saline, group 2 (Diseased) received paracetamol and group 3 (Standard) was given silymarin as reference drug. In group 4 and 5 (Treated), *I.florentina* L. methanolic extract (250 and 500 mg/kg) were administered. Different serum biomarkers and histopathological studies were performed to assess the recovery caused by PCM in comparison to diseased group. The treatment of *I.florentina* methanolic extract significantly improve the serum biomarkers and restored the hepatic injury towards normal, indicating the hepatoprotective potential. Thus, we can conclude that *I. florentina* have significantly reversed the damage caused by paracetamol in hepatotoxic rat model due to their potential phytochemical constituents.

Keywords: Liver, hepatoprotective, *Iris florentina*, flavonoids, phenolic compounds, paracetamol.

INTRODUCTION

The liver is an important organ in the body that aids in the metabolism of lipids, proteins and carbohydrates. It also performs a variety of functions such as glycogen storage, detoxification, and synthesis of vitamins (A, B_{12} and D), hormones (angiotensinogen), growth factors (IGF_1), and biochemical required for digestion such as bile (Wolf, 1999). As liver is involved in drug metabolism, therefore, it is highly vulnerable to drug-induced toxicity. Paracetamol (PCM) is an antipyretic and analgesic drug which is widely used for medicinal and therapeutic purposes; however, it is also known to cause liver damage. Different studies reported that toxic concentrations of PCM have altered the function and morphology of the liver mitochondria (Myers *et al*., 1988). Moreover, in mice the treatment of PCM has also been reported to cause oxidative stress in mitochondria (Jaeschke, 1990), opening of mitochondrial-permeability transition pore (Ramachandran *et al*., 2011), lysis of outer membrane and matrix swelling in rat models (Placke *et al*., 1987). Thus, it is one of the widely utilized liver-injury models for investigating drug-induced hepatotoxicity.

Herbal medicines play a significant role for the treatment of different hepatic disorders. The traditional medicine system uses variety of medicinal plants, their extracts and formulations to treat hepatic problems, due to the lack of reliable and effective liver protecting drugs in the modern medicine (Girish *et al*., 2009). Various studies have been conducted for the drug development; however due to the adverse effects of modern therapeutics, natural therapies are believed to be safe and effective alternative treatments for liver toxicity. Plants have extensively been studied for evaluating their anti-oxidant potential. Numerous studies reported that plant extracts can protect against drug-induced liver toxicity due to their anti-oxidative activity (Shahjahan *et al*., 2004; Sheweita *et al*., 2001). Around 600 commercial medications having hepatoprotective activity are available in the market worldwide. Hepatoprotective potential has been found in around 170 phyto-constituents extracted from 110 plant species belonging to 55 different families (Franchesca *et al*., 2010; Nirmala *et al*., 2012).

Among different medicinal herbs, Iris genus has also been reported for its therapeutic properties. There are around 300 species of genus Iris that are found all over the world (Kossak, 2012). Many species of Iris are used for ornamental purposes; however, they also possessed the potential to treat different inflammations, cancer, viral and bacterial infections (Orhan *et al*., 2003; Wang *et al*., 2010). Different phytochemical studies of this genus have showed the isolation of various flavonoids, isoprenoids, isoflavonoids, and glycosides such as xanthones, stilbene and quinones (Kossak, 2012; Kukula-Koch *et al*., 2015). These isolated bioactive compounds have been reported

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to possess anti-neoplastic, anti-oxidant, anti-tuberculosis, anti-bacterial, anti-plasmodial, anti-inflammatory, molluscicidal and phytoestrogenic properties (Kukula-Koch *et al*., 2015).

Iris florentina belongs to Iris family and has been used as a decongestant and expectorant medicinally. However, hepatoprotective potential of *I. florentina* has not been explored *in vivo*. Therefore, present study is aimed to investigate the hepatoprotective potential of methanolic extract of *I. florentina* against paracetamol-induced hepatotoxicity.

MATERIALS AND METHODS

Plant material and chemicals

Plants of *I. florentina* L. (aerial part) were collected from the Thal desert, Punjab, Pakistan, identified by an expert taxonomist of same university and voucher specimen via no "16602506" was submitted. All the reagents and materials used in this study were of the analytical-grade and were purchased from Sigma Chemical Co.

Test animals

This study was conducted on 40 male albino rats (120-130 gm) taken from the animal house located at the Department of Pharmacology, Faculty of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan. Before experimentation, these rats were housed in controlled environmental conditions (relative humidity: 55%, room temperature: 25-27°C and 12 h dark/12 h light cycle) for five to six days and were given standard food pellet and water. All rats were given humane care according to the guidelines of "Guide for the Care and Use of Laboratory Animals" (NRC, 1996) with the permission of Ethical committee for utilization of laboratory animals for research via letter no "EC/09-PHL-2019-2021".

Preparation of Iris florentina L. Extract

The fresh leaves of *I. Florentina* were collected and shade dried following pulverization. Approximately 1 kg was air-dried following maceration with methanol: water (70:30) and was filtered after seven days. It was concentrated at 40˚C in the rotary evaporator for three days and was stored in airtight container at 2-4˚C.

Phytochemical analysis

Preliminary phytochemical analysis of methanolic extracts of *I. Florentina* was performed. According to standard procedures, phytochemical tests were conducted to determine the presence of different components (Aleem & Janbaz, 2018).

HPLC-based quantification of phytochemicals

The powdered extract of *I. Florentina* (10 mg) was diluted with methanol (10 mL) to achieve 1 mg/mL final concentration following sterilization using the syringe filter (0.45 µM). Before HPLC analysis, each standard (1 mg) was diluted in methanol (1 mL) and were filtered using the syringe filter (0.45 µM). These prepared standards and samples were subjected for HPLC analysis using a binary system having a PDA detector linked to the system processor. For the evaluation of the data, the system recruited Empower software having standard certification. The pressure was kept at 2500 psi. The solvents HPLC was performed on a reverse phase using C-18 column with a wavelength range of 200-600 nm. Using the binary state of the gradient mechanism, a flow rate of 1ml/min was kept throughout the experiment. Different solvent combinations (20:80, 50:50, 80:20 and 20:60) of water and methanol were used, respectively. Finally, the experiment was carried out at a 50:50 solvent ratio to get the optimum peak resolution. Several phenolic acids and flavonoids were employed as standards in order to determine the constituents. The peaks were evaluated through comparing the standard retention time (RT) to the RT of various peaks obtained from extracts HPLC analysis (Alam, 2015; Viktorova *et al*., 2019).

PCM-induced hepatotoxicity

Albino rats were separated into 5 different groups of treatments having eight animals in each group, and the following treatments were given orally for seven consecutive days. Group 1 (normal control) received normal saline, group 2 (diseased control) received 2 g/kg dose of paracetamol, group 3 received silymarin (standard) at the dose of 100 mg/kg along with paracetamol while group 4 and 5 (Treated) were administered with 250 mg/kg and 500 mg/kg of methanolic extract of *I. florentina*, respectively after half hour of paracetamol treatment. Blood sample was taken from the retro-orbital vein after 24 hours of last dose, following clotting at room temperature for 1hour. Centrifugation was done at 2500 rpm for 15 minutes at 30°C for separating serum. After that, the serum was collected and examined for different biochemical parameters (Madkour and Abdel-Daim, 2013).

Liver function test

After centrifugation, the serum was examined for biochemical markers such as Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (ALP), Total Bilirubin (TB), Albumin Serum Test (ALB) and Lactic Acid Dehydrogenase (LDH). The activity of serum transaminases was determined using the Reitman and Frankel method (1957). The ALB and ALP were measured using the method by Rahmioglu (2009).

Histopathological studies

All the treated mice were sacrificed, and their livers were excised following cleaning with saline solution and were placed into 20% formalin solution for one week. After that, the tissues of liver were dehydrated using ethanol solutions, and were cut into sections of 5 µm following embedding in paraffin. These embedded tissues were

stained with eosin-haematoxylin dye and examined using a photomicroscope.

STATISTICAL ANALYSIS

All of the data was presented as Mean ± Standard Deviation. Graph-Pad Prism-5 software was used to conduct the statistical analysis, which included one-way ANOVA and Dunnett's multiple comparison tests taking $P<0.05$ as significant value.

RESULTS

Phytochemical analysis

The preliminary phytochemical investigation showed that tannins, alkaloids, flavonoids, terpenoids, phenols, steroids, glycosides and saponins were found in the methanolic extract of *I. florentina*. Furthermore, the HPLC analysis of 250 mg/kg methanolic extract of *I. florentina* showed the presence of ascorbic acid, amino acid (leucine), flavonoids (rutin and quercetin) and phenolic compound (pyrogallol) as shown in Fig. 1A. The maximum retention time was observed by pyrogallol (13.61 min) followed by leucine (11.18), quercetin (7.76) and rutin (6.22) while minimum time was observed by ascorbic acid (1.25 min).

The ascorbic acid and pyrogallol showed maximum absorbance (AU) in 250 mg/kg methanolic extract of *I. florentina*. However, the 500 mg/kg methanolic extract of *I. florentina* showed the presence of another phenolic compound (caffeic acid) along with the same phytochemicals present in 250 mg/kg extract (Fig. 1B). The retention time observed in 500 mg/was same as in 250 mg/kg extract. The ascorbic acid rutin and leucine showed maximum absorbance as compared to other compounds.

Liver function analysis

The results of liver function analysis showed that paracetamol treatment significantly increased enzymes of serum markers including ALP, ALT, AST, TB and LDH as compared to control group (treated with normal saline). Moreover, as paracetamol-induced hepatic injury reduces protein production, therefore, paracetamol group showed lower levels of ALB than the control group. However, the methanolic extract of *I. florentina* showed hepatoprotective effect for all these serum markers in a dose-dependent manner as shown in Fig. 2 and table 1. The maximum hepatoprotective effect was observed with 500 mg/kg methanolic extract of *I. florentina*.

Histopathological observations

In group 1 (control group), histopathological observations revealed that the central vein is encircled by cells hepatic cord giving a normal architecture (Fig. 3A). Moreover, hepatocyte nuclei appeared to be regular having a normal nucleolus and chromatin material. The sinusoidal gaps and cytoplasm also showed regular patterns. The liver tissues of PCM-treated rats (group 2) showed massive cell necrosis, fatty changes and vascular degeneration (Fig. 3B). The chromatin material of group 2 animals was also dense. Rats treated with silymarin and paracetamol had almost normal tissues of liver in their liver sections (Fig. 3C).

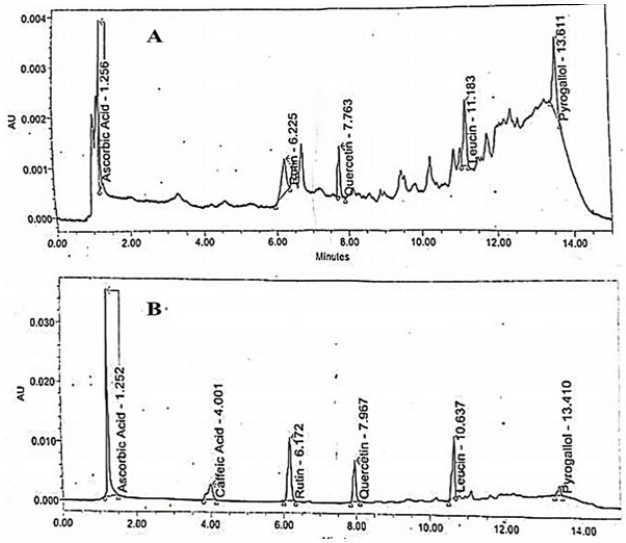


Fig. 1: HPLC Chromatogram of (A) 250 mg/kg and (B) 500 mg/kg methanolic extract of *I. florentina*. The x-axis shows retention time while y-axis shows maximum absorbance of different phytochemicals.

Hepatocytes, hepatic nuclei, sinusoidal spaces and cytoplasm also showed normal patterns. Rats given paracetamol and 250 mg/kg methanolic leaf extract of *I. florentina* showed few inflammatory cells near the central vein and no necrosis in their liver tissue (Fig. 3D). However, few areas showed vascular degeneration and condensed chromatin. The liver tissues of rats administered with 500 mg/kg methanolic leaf extract of *I. florentina* and paracetamol showed reduced inflammatory cell infiltration, hepatocytes regeneration across the central vein, and almost normal architecture of liver (Fig. 3E), suggesting that the methanolic extract (500 mg/kg) of *I. florentina* has hepatoprotective activity.

DISCUSSION

Liver diseases are global health issue primarily caused by different chemical compounds, viruses, and metabolic disorders (Graier *et al*, 2009; Negi *et al*., 2008). PCM-induced hepatotoxicity is one of the most frequently utilized screening methods to test the hepatoprotective nature of different medicinal plant extracts (Ahmed *et al*., 2011; Lima *et al*., 2019). Apart from any other metabolites, plants are mostly known for their protective effects due to their phenylpropanoid derivatives, like polyphenols. Numerous species of the *Iris* genus are

being used in the traditional medication due to its biologically active compounds. Many secondary metabolites characterized and isolated from the different *Iris* spp. have displayed antioxidant, antibacterial, anti-inflammatory, immunomodulatory and anticancer properties (Burcu *et al*., 2014; Kostić *et al*., 2019; Mocan

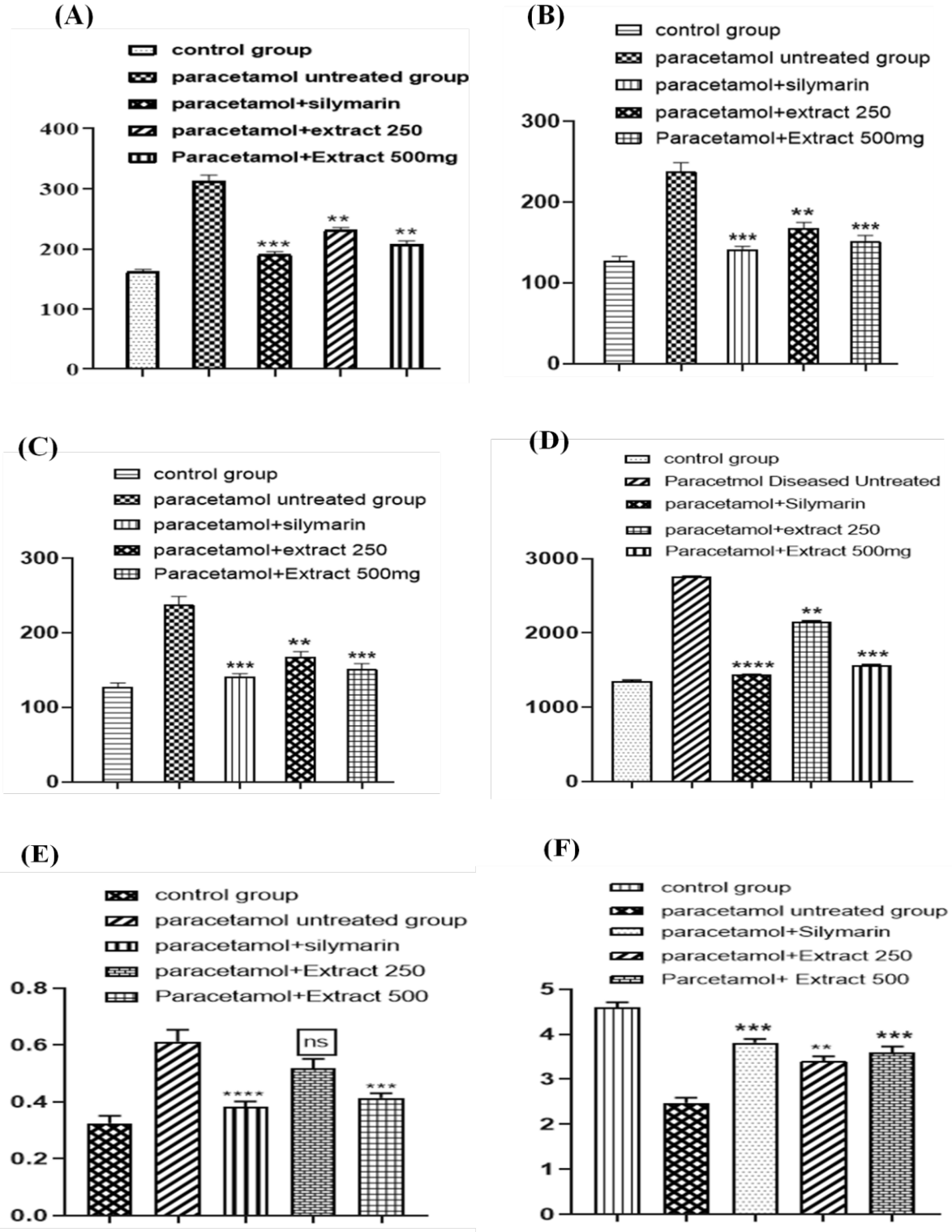
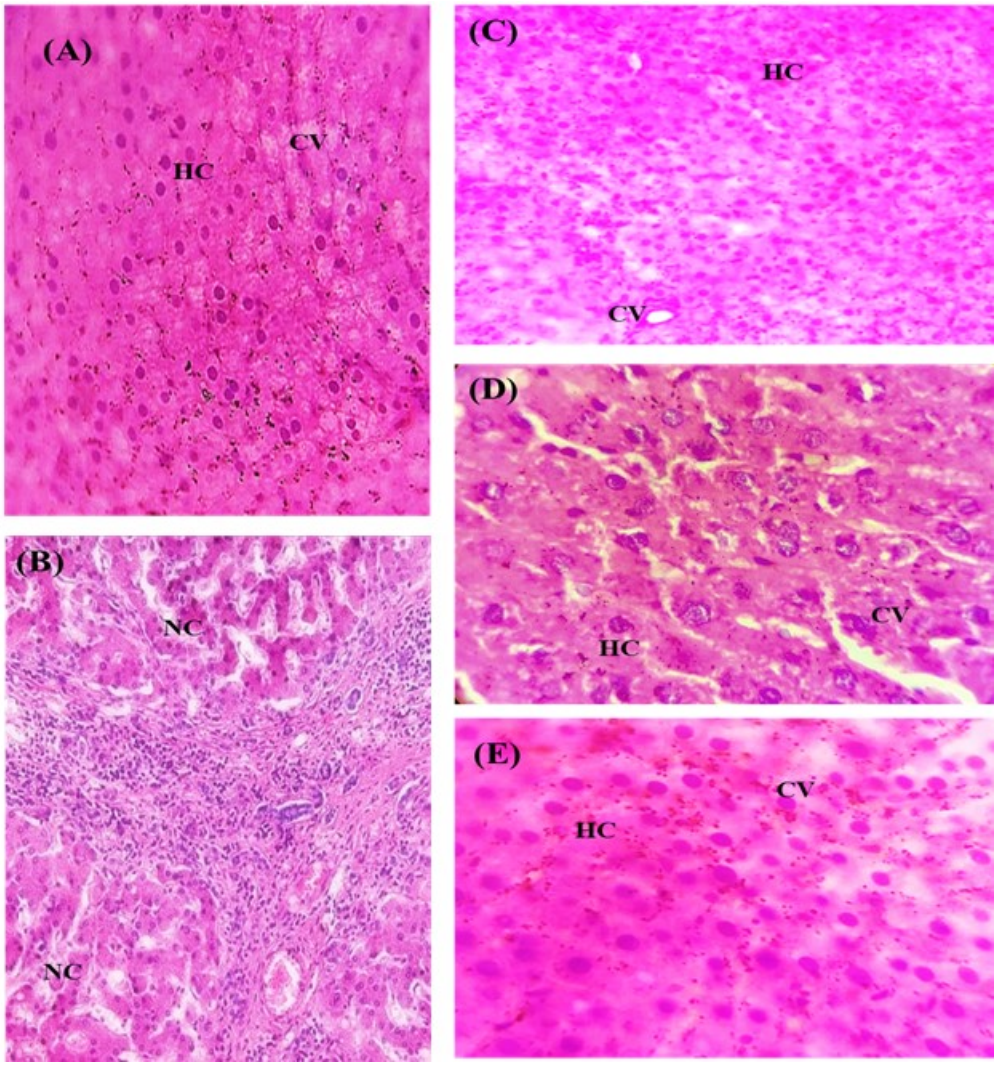


Fig. 2: Effect of *Iris florentina* extract on biochemical markers of liver in PCM-induced hepatotoxic rats (A) ALP; (B) ALT; (C) AST; (D) LDH; (E) TB; and (F) ALB. Bar shows mean values ± Standard deviation. *P =< 0.05, **P = < 0.01, ***P = < 0.005 and ***P = < 0.001 compared to paracetamol control (Group 2).

et al., 2018). However, limited literature review is available for the hepatoprotective activity of this genus, particularly *Iris florentina* plant. This study explored the potential metabolites of the *I. florentina* and investigated the impacts of its methanolic extracts on PCM-induced liver injury in albino rats.

Table 1: Effect of varying concentrations of *I. florentina* extract on different biochemical markers of liver in PCM-induced hepatotoxic rats. (Group1: Normal control; Group 2: Diseased control; Group 3: Standard/ Reference; Group 4: 250 mg/kg of methanolic extract of *I. florentina;* Group 5: 500 mg/kg of methanolic extract of *I. florentina*)

	Treatment groups				
Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
ALP (IU/L)	161.84±4.06	313.02±9.60	191.08±4.21***	231.5±4.01**	208.16±5.11**
ALT (IU/L)	45.18±3.75	110.06±7.30	54.44±2.53***	77.26±3.62**	67.54±5.01***
AST (IU/L)	127.78±4.96	237.64±11.12	140.78±4.47***	167.86±6.91**	151.66±7.05***
LDH (IU/L)	1349.6±14.29	2754.4±8.12	1436.2±6.07****	1728.35±428.19**	1560.2±13.83***
TB (mg/dL)	0.324±0.03	0.612±0.04	0.382±0.02****	0.518±0.03	0.414±0.02***
ALB (mg/dL)	4.596±0.11	2.472±0.12	3.808±0.08***	3.404±0.09**	3.596±0.13***



A. Saline-treated rats (Normal control Group); **B.** Paracetamol-treated (Diseased control) rats; **C.** Silymarin + Paracetamol (Standard) treated rats; **D.** 250 mg/kg *I. florentina* extract + Paracetamol; and **E.** 500 mg/kg *I. florentina* extract + Paracetamol

Fig. 3: Liver sections of PCM-treated albino rats showing Hepatic Cells (HC), Central Vein (CV), and Necrotic Cell (NC) stained with eosin-hematoxylin dye.

To the best of our knowledge, this is the first report on analysis of phytochemical components in *I. florentina* methanol extracts identified using the standard protocols and HPLC approach. The *I. florentina* extracts of this study have similar chemical profile to what has previously been reported. According to Kukula-Koch *et al*., (2015), (iso) flavonoids are the major class of polyphenolics in several *Iris* species and different compounds from this group were identified in this study. The hepatoprotective properties of *I. florentina* are due to its different polyphenolic contents (Hajimahmoodi *et al*., 2008; Moodi *et al*, 2020). Phenolic compounds are largely comprised of flavonoids and phenolic acids, which serve as primary antioxidant components found in natural products. These components stop the initiation step of the lipid oxidation reaction by donating electron to the radicals (Singh *et al*., 2002).

In present study, paracetamol treatment induces hepatic injury in albino rats, resulting in a significant increase in serum bio-markers. Among the indication of PCM-induced hepatic damage is an increase in the serum enzymatic levels such as AST, ALT, ALP, TB and LDH (Janbaz and Gilani, 2000). This indicates loss of cell membrane functional integrity and cellular damage in the liver (Drotman, 1978). Such hepatocyte injury, i.e., cell necrosis, as presented in the fig. 3B, resulted in lower protein production, leading to reduction in ALB levels from 4.59 to 2.47 IU/L. The increased serum enzyme production in the blood can be linked to submissive/central necrosis of the hepatocytes, which leads to serious hepatic injury. Furthermore, treatment with methanolic leaf extract of *I. florentina* at 250 mg/kg and 500 mg/kg significantly reduced the increased levels of serum enzymes, indicating that the extract helped prevent liver damage. The treatment of *I. florentina* methanolic extracts showed dose-dependent activity, *I. florentina* methanolic extract at 500 mg/kg showed more promising results than 250 mg/kg, as shown in table 1 and fig. 1 for measured intensities of various serum enzymes. This was confirmed by the decreased histopathological injuries in fig. 3D and 3E. The hepatoprotective potential of *I. florentina* methanolic extracts (fig. 3E and 3F) can be attributed to the presence of different phytochemicals such as polyphenols (Hajimahmoodi *et al*., 2008; Moodi *et al*, 2020). Different studies reported *in vitro* free-radical scavenging potential of various *Iris* species extract including assays of DPPH-radical scavenging, hydroxyl-radical scavenging and superoxide-radical scavenging (Askin *et al*., 2018; Amin *et al*., 2021), suggesting that *I. florentina* methanolic extract has the ability to decrease cellular oxidative damage stress. Thus, the hepatoprotective activity of I. *florentina* leaves extract may be due to efficient radical scavenging system.

Hence, it can be concluded that *I. florentina* methanolic extract has hepatoprotective activity against PCM-induced hepatotoxicity in albino rats due to different phytochemicals such as flavonoids, sterols, saponins and phenols and these antioxidative phytochemicals of *I. florentina* may contribute to its hepatoprotective effects.

CONCLUSION

From the above stated data of our research, it can be concluded that the methanolic extract of *I. florentina* exhibited a significant hepatoprotective activity against paracetamol induced liver damage, which was confirmed by biochemical and histopathological studies. There is still a need to find out the active principle responsible for the hepatoprotective activity and to elucidate the mechanism of action.

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