

Original Research Article

Protective effect of *Parthenium hysterophorus* against carbon tetrachloride- and paracetamol-induced hepatotoxicity in rabbits

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Sent for review: 12 December 2017

Revised accepted: 19 February 2018

Abstract

Purpose: To investigate the possible hepatoprotective potential of *Parthenium hysterophorus* crude extract (Ph.Cr) against carbon tetrachloride (CCL₄)- and paracetamol-induced hepatotoxicity in rabbits.

Methods: Twenty rabbits were divided into five groups of four rabbits each. Group 1 served as normal control and received normal saline (5 mL/kg). Group 2 received normal saline followed by CCL₄ (0.75 mL/kg p.o dose) after 1 h. Groups 3 and 4 received Ph.Cr at doses of 15 and 30 mg/kg po, respectively, for 7 days followed by one dose of CCL₄, 2 h after the last extract dose (0.75 mL/kg, sc). Group 5 received silymarin as reference standard at a dose of 100 mg/kg orally for 7 days followed by one dose of CCL₄ (0.75 mL/kg, sc), 2 h after the last drug dose. The effect of the extract on potassium (K⁺)-induced contractions in isolated rabbit jejunum was also evaluated. At the end of the study, the animals were sacrificed and their liver architecture examined microscopically.

Results: Pre-treatment of rabbits with Ph.Cr reduced ALT, ALP and TB levels ($p < 0.05$, $p < 0.01$, $p < 0.001$) dose dependently. Hepatoprotective data indicate that Ph.Cr markedly reduced CCL₄- and paracetamol-induced toxicity by preserving the histological architecture of the liver tissue at near normal. In isolated rabbit jejunum tissue, Ph.Cr relaxed high K⁺ (80 Mm)-induced contractions in a concentration-dependent (0.03 - 10 mg/mL) manner like that caused by silymarin.

Conclusion: In the light of the results obtained, *Parthenium hysterophorus* possesses hepatoprotective activity against CCL₄- and paracetamol-induced hepatic damage, possibly mediated via its antioxidant and Ca⁺⁺ antagonist mechanisms.

Keywords: *Parthenium hysterophorus*, Toxins, Hepatoprotection, Ca⁺⁺ antagonist, Silymarin

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INTRODUCTION

The liver is a vital organ of human body responsible for biotransformation of many

endogenous and exogenous substances. It plays a significant role in removal of many drugs and toxins from the body. Changes in hepatocytes biochemistry and generation of reactive oxygen

species results in disturbances of these functions [1]. Numerous medicinal plants are used for the treatment of liver disorders. Because of more effectiveness, less consequences of side effects and low cost, natural remedies are now considered to be safe and effective alternative for treating hepatotoxicities.

Parthenium hysterophorus is an annual herb belongs to the family *Asteraceae* and commonly known as Carrot weed, Congress weed, Star weed and Chatak Chandani. It is native to North and South America, but usually it is found in many other parts of the world and is a constant flourishing component of the flora [2]. *Parthenium hysterophorus* exhibits analgesic activity in neuralgia, antipyretic tonic and as febrifuge. Its root decoctions have shown significant activity in hepatic amoebiasis. The plant has been reported to exhibit promising antimicrobial, antioxidant, free radical scavenging and lipoprotective activities [3]. In this study, hepatoprotective action of *Parthenium hysterophorus* was observed against carbon tetrachloride (CCl₄) and paracetamol-induced hepatotoxicity in rabbits.

EXPERIMENTAL

Plant material and extraction

Whole plant of *Parthenium hysterophorus* was collected in the month of August 2015, from various areas of Islamabad and Rawalpindi, Pakistan. The plant was authenticated by Dr Mushtaq Ahmad, a taxonomist at Department of Plant Sciences, Quaid-a-Azam University, Islamabad, and a voucher specimen (no. 175200) was submitted to the same Department's Herbarium. The plant material (5.2 kg) was dried under shade and crushed into coarse powder. The crushed material was allowed to soak for 7 days in 3 L of 70 % aqueous-ethanol at room temperature, stirring continuously at regular intervals. The extract was filtered via a Whatmann filter paper no. 1 and concentrated in a rotary evaporator. The obtained thick semi-solid paste of *Parthenium hysterophorus* crude extract (Ph.Cr) was dissolved in 5 % dimethyl sulphoxide (DMSO). The concentrated dried crude extracts were weighed to calculate the percentage yield.

Chemicals

Chemicals used in this study with sources are specified: CCl₄, ethanol, potassium chloride (KCL), verapamil (Sigma Chemicals Company, USA), assay kits for alanine transaminase (ALT), alkaline phosphatase (ALP) and total bilirubin (TB) (Merck, Germany), silymarin (Abbott

Laboratories), paracetamol (olive laboratories, Islamabad), DMSO (Labscanasia Company, Thailand), methyl cellulose (Colorcon USA), and olive oil (Marhaba Laboratories, Lahore) 1 % of methyl cellulose (50 mg/mL) and (20 % v/v) of olive oil were employed for the suspension of paracetamol and CCl₄ respectively. Chemicals employed in the study were of good analytical grade.

Animals

Healthy rabbits (local breed) of either sex, weighing between 1.5 - 2 kg were obtained from the local market. The animals were housed under hygienic conditions at 23 - 25 °C in the Animal House of Riphah Institute of Pharmaceutical Sciences, supplied with standard diet and water *ad libitum*. All the experiments on animals were performed with a prior permission from Riphah Institute of Pharmaceutical Sciences Ethical committee (ref no. REC/RIPS/2015/004) and fulfilled guidelines of Institute of Laboratory Animal Resources, Commission on Life Sciences University, National Research Council [4].

Preliminary phytochemical screening

Preliminary phytochemical analysis was carried for detection of different classes like alkaloids, tannins, saponins, proteins, steroids and flavonoids according to standard procedures with modifications [5]. Dragendorff's and Mayer's reagent detected alkaloids. Yellow color was detected with sodium hydroxide which indicates the positive test for flavonoids. Cream yellow color was appeared with lead acetate which specified the presence of tannins. Amino acids were detected using ninhydrin reagent, blue color indicated presence of amino acids steroids were considered positive, if plant material treated with chloroform and sulfuric acid subsequently produced red coloration. Bubbles appearance upon vigorous shaking of diluted samples indicated presence of saponins.

Induction of hepatic injury

Administration of CCl₄ subcutaneously at a dose of 0.75 mL/kg suspended in olive oil 1:1 induced hepatic injury [6] in the first model, while 2 g/kg dose of paracetamol was used to induce hepatotoxicity in the second model [7]. The saline group (normal control) received normal saline at a dose of 5 mL/kg.

Hepatoprotective study

The models of CCl₄ and paracetamol-induced hepatic injury were used to evaluate

hepatoprotective activity. In first model, five groups of rabbits consisted of four animals each. Group 1 served as normal control and received normal saline (5 mL/kg). Group 2 received normal saline followed by CCL₄ (0.75 mL/kg p.o dose) after 1 h. Groups 3 and 4 received Ph.Cr at doses of 15 and 30 mg/kg po, respectively, for 7 days followed by one dose of CCL₄, 2 h after the last extract dose (0.75 mL/kg, sc). Group 5 received silymarin as reference, standard at dose of 100 mg/kg orally for 7 days followed by one dose of CCL₄ (0.75 mL/kg s.c), 2 h after the last drug dose [6]. In paracetamol induced hepatotoxicity, five groups of rabbits were made each containing four animals. All these groups were treated similar to CCL₄ induced hepatotoxicity for a period of 7 days. After respective drug treatments administration on the 7th day, all rabbits of group 2, 3, 4 and 5 were challenged with paracetamol at dose of 2g/kg orally [7].

Assessment of hepatic injury

Hepatotoxicity was assessed by measuring alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin (TB) levels in serum along with changes in histopathology of liver. From all groups blood samples were collected, 24 and 12 h after CCL₄ and paracetamol administration respectively. The blood (3 mL) was drawn from jugular vein of rabbits using sterile disposable syringe and were allowed to clot in sterile gel tubes at room temperature for 45 minutes. Serum was separated through centrifugation at 3000 rpm for 30 minutes and was then biochemically analyzed for the evaluation of ALT, ALP and TB spectrophotometrically, using Merck diagnostic kits [6].

Isolation of rabbit jejunum

Rabbits were sacrificed after fasting for 24 h. Intestinal segments of approximately 2 cm in length were suspended in tissue bath containing 10 mL of Tyrode's solution maintained at 37 °C, bubbled with 95 % oxygen in carbon dioxide [8]. Tyrode's solution composition in mM was KCl 2.7, NaCl 136.9, NaHCO₃ 11.9, MgCl₂.6H₂O 0.5, CaCl₂ 1.8, NaH₂PO₄.2H₂O 0.32 and glucose 5.05. Intestinal responses were recorded via force transducer coupled to a Transbridge and Power Lab 4/25 data acquisition system (AD Instrument, Sydney Australia).

Determination of Ca⁺⁺ antagonist effect

High K⁺ (80mM) was employed for evaluation of calcium channel blocking (CCB) action, in order

to depolarize the isolated jejunum preparations [9]. The control response percentage arbitrated by K⁺ was estimated by intestinal preparations relaxation, pre-contracted with K⁺ (80 mM).

Statistical analysis

Data are expressed as mean ± standard error of mean (SEM, n= number of experiment). Statistical comparison between various groups was analyzed using one-way analysis of variance (ANOVA) followed by Tukey post-hoc test with the aid of GraphPad program. *P* < 0.05 was considered significant.

RESULTS

Phytochemical profile

Ph.Cr showed positive tests for alkaloids, flavonoids, saponins, tannins, steroids, amino acids and carbohydrates.

Effect of Ph.Cr on CCL₄-induced hepatic damage

Ph.Cr dose-dependently (15-30 mg/kg) prevented CCL₄-induced raise in levels of ALT, ALP and TB (Table 1). In saline treated group, ALT, ALP and TB values were 52.50 ± 2.10 IU/L, 156.50 ± 5.62 IU/L and 0.70 ± 0.04 mg/dL respectively. CCL₄ (0.75mL/kg) treatment raised levels of ALT, ALP and TB to 257.80 ± 6.82 IU/L, 305.50 ± 20.90 IU/L and 1.40 ± 0.09 mg/dL respectively (*p* < 0.001 vs. saline group). In Ph.Cr (15mg/kg) pretreated group, ALT, ALP and TB levels reduced to 167.30 ± 4.32 IU/L, 194.80 ± 4.11 IU/L (*p* < 0.001 vs. CCL₄ group) and 1.09 ± 0.04 mg/dL (*p* < 0.05 vs. CCL₄ group) respectively. In Ph.Cr (30 mg/kg) pretreated group, ALT, ALP and TB levels decreased to 86.50 ± 3.79 IU/L, 169.00 ± 5.7 IU/L and 0.85 ± 0.02 mg/dL respectively (*p* < 0.001 vs. CCL₄ group). In silymarin (100 mg/kg) pretreated group, ALT, ALP and TB values were to 80.00 ± 2.94 IU/L, 164.30 ± 4.90 IU/L and 0.76 ± 0.04 mg/dL respectively (*p* < 0.001 vs. CCL₄ group) as shown in Table 1. Histopathologies were further performed, which collaborated the results. In saline group, normal hepatic cells are characterized by having well-defined cell linings, prominent nucleus and central vein (Figure 1A). Liver histopathology of CCL₄ treated group showed hepatocyte necrosis, vacuolization of cytoplasm, hepatocytes distoration and fatty degeneration (Figure 1B). These histopathological changes were partially prevented by Ph.Cr pretreatment at dose of 15 mg/kg, showing mild portal inflammatory infiltrate with bile canalicular dilatation (Figure 1C). Ph.Cr

pretreatment at 30 mg/kg showed normal histopathology with intra-hepatic cholestasis only with absence of any congestion and focal necrosis (Figure 1D). Silymarin (100 mg/kg) pretreated group, showed normal hepatocytes with nucleus and central vein surrounded by reticular fibers (Figure 1E).

Effect of Ph.Cr on paracetamol-induced hepatic damage

Ph.Cr exhibited significant effect in protecting liver against paracetamol-induced hepatotoxicity (Table 2). In saline treated group, ALT, ALP and TB values were 66.50 ± 10.81 IU/L, 169.00 ± 11.23 IU/L and 0.66 ± 0.61 mg/dL respectively. Paracetamol (2 g/kg) treatment raised levels of ALP, ALP and TB to 289.50 ± 8.85 IU/L, 306 ± 8.60 IU/L and 1.44 ± 0.07 mg/dL respectively ($p < 0.001$ vs. saline group). In Ph.Cr (15 mg/kg) pretreated group, ALT, ALP and TB levels were reduced to 186.00 ± 7.15 IU/L, 239.90 ± 6.66 IU/L ($p < 0.001$ vs. paracetamol group) and 1.11 ± 0.68 mg/dL ($p < 0.01$ vs. paracetamol group) respectively. In Ph.Cr (30 mg/kg) pretreated group, ALT, ALP and TB levels decreased to 109.30 ± 5.70 IU/L, 209.50 ± 4.97 IU/L and 0.81 ± 0.40 mg/dL respectively ($p < 0.001$ vs. paracetamol group). In silymarin (100 mg/kg) pretreated group ALT, ALP and TB values were 81.50 ± 4.05 IU/L, 181.30 ± 4.90 IU/L and 0.71 ± 0.02 mg/dL respectively ($p < 0.001$ vs. paracetamol group) as shown in Table 2. In the saline group, normal hepatic cells are characterized by having well-defined cell linings, prominent nucleus and central vein. (Figure 1 A). In paracetamol treated group, histopathology of liver tissue showed necrosis, ballooning of cytoplasm, hepatocytes distortion and hematoma of central vein (Figure 2B). In case of Ph.Cr (15 mg/kg) pretreatment, liver tissue showed centrilobular canaliculi dilatation, with mild intracellular cholestasis (Figure 2C). In case of Ph.Cr (30 mg/kg) pretreatment, liver tissue showed mild intracellular cholestasis only with absence of any necrosis and fatty degeneration (Figure 2D). Silymarin (100 mg/kg) pretreated group had a normal histopathology with well-defined cell linings, prominent nucleus and central vein surrounded by reticular fibers

showing lack of any necrosis and fatty degeneration (Figure 2E).

Effect of Ph.Cr on K^+ (80 mM)-induced contractions

In isolated rabbit jejunum preparations, Ph.Cr and verapamil caused inhibition of K^+ (80 mM)-induced contractions with respective EC_{50} values of 0.50 mg/mL (0.41-0.60 mg/mL, 95% CI, $n = 3$) and 0.18 μ M (0.13 - 0.23 μ M, $n = 3$), as presented in Figure 3.

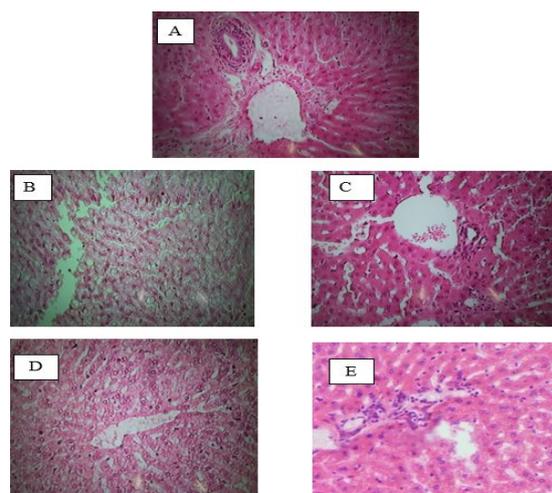


Figure 1: Representative photomicrographs (40 \times magnification, hematoxylin and eosin staining), showing protective effect of *Parthenium hysterophorus* crude extract (Ph.Cr) and silymarin against carbon tetrachloride (CCL_4)-induced histopathological changes in rabbit liver. **A:** saline (5 mL/kg) treated group: tissue showing normal central vein, plates of hepatocytes, composed of portal tract surrounded by hepatocytes. **B:** CCL_4 (0.75 mL/kg) intoxicated group: showing areas of hepatocyte necrosis, distortion of hepatocytes and hematoma of central vein as well as infiltration of the lymphocytes and kuffer cells around central vein. **C:** Ph.Cr (15 mg/kg) pretreated liver tissue, showing mild portal inflammatory infiltrates, bile canalicular dilatation with absence of hepatocyte necrosis. **D:** Ph.Cr (30 mg/kg) pretreated liver tissue, showing intra hepatic cholestasis only having normal central vein, plates of hepatocytes with absence of hepatocyte necrosis. **E:** silymarin (100 mg/kg) pretreatment shows normal histopathology with well-defined central vein, plates of hepatocytes with absence of any necrosis

Table 1: Inhibitory effect of crude extract (Ph.Cr) *Parthenium hysterophorus* and silymarin against carbon tetrachloride (CCL_4)-mediated increase in alanine transaminase ALT, ALP and total bilirubin TB levels of rabbits

Treatment	ALT (IU/L)	ALP (IU/L)	TB (mg/dL)
Saline (5 mL/kg)	52.50 ± 2.10	156.50 ± 5.62	0.70 ± 0.04
CCL_4 (0.75 mL/kg)	$257.80 \pm 6.82^{\#}$	$305.50 \pm 20.90^{\#}$	$1.40 \pm 0.09^{\#}$
Ph.Cr (15 mg/kg) + CCL_4 (0.75 mL/kg)	$167.3 \pm 4.32^{***}$	$194.8 \pm 4.11^{***}$	$1.093 \pm 0.047^*$
Ph.Cr(30 mg/kg) + CCL_4 (0.75 mL/kg)	$86.50 \pm 3.79^{***}$	$169.0 \pm 5.70^{***}$	$0.85 \pm 0.02^{***}$
Silymarin (100 mg/kg) + CCL_4 (0.75mL/kg)	$80.00 \pm 2.94^{***}$	$164.30 \pm 4.90^{***}$	$0.76 \pm 0.04^{***}$

Values are mean \pm SEM ($n = 4$). $^{\#}p < 0.001$ vs. saline group, $^*p < 0.05$, $^{***}p < 0.001$ vs. CCL_4 treated group, one way ANOVA with Tukey post-hoc test. IU=International Unit

Table 2: Inhibitory effect of *Parthenium hysterophorus* crude extract (Ph.Cr) and silymarin against paracetamol-mediated increase in alanine transaminase (ALT), alkaline phosphatase (ALP) and total bilirubin (TB) levels of rabbits.

Treatment	ALT (IU/L)	ALP (IU/L)	TB (mg/dL)
Saline (5 mL/kg)	66.50 ± 10.81	169.00 ± 11.23	0.66 ± 0.61
Paracetamol (2 g/kg)	289.50 ± 8.85 [#]	306.00 ± 8.60 [#]	1.44 ± 0.07 [#]
Ph.Cr (15 mg/kg) + paracetamol (2 g/kg)	186.00 ± 7.15 ^{***}	239.90 ± 6.66 ^{***}	1.11 ± 0.68 ^{**}
Ph.Cr (30 mg/kg) + paracetamol (2 g/kg)	109.30 ± 5.70 ^{***}	209.50 ± 4.97 ^{***}	0.81 ± 0.40 ^{***}
Silymarin (100 mg/kg) + paracetamol (2 g/kg)	81.50 ± 4.05 ^{***}	181.30 ± 4.90 ^{***}	0.71 ± 0.02 ^{***}

Values are mean ± SEM (n=4). [#]p < 0.001 vs. saline treated group, ^{**}p < 0.01, ^{***}p < 0.001 vs. paracetamol treated group, one way ANOVA with Tukey post-hoc test. IU = International Unit

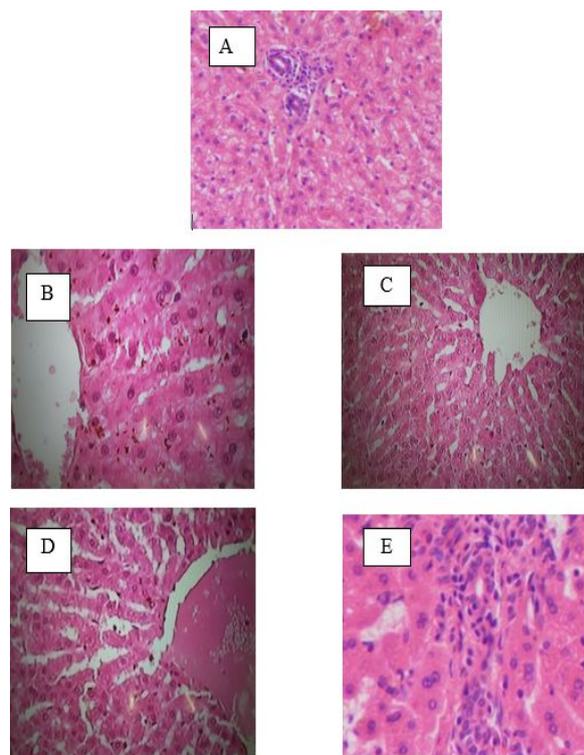


Figure 2: Representative photomicrographs (40× magnification, hematoxylin and eosin staining), showing protective effect of *Parthenium hysterophorus* crude extract (Ph.Cr) and silymarin against paracetamol-induced histopathological changes in rabbit liver. **A:** saline (5 mL/kg) treated group: showing normal central vein, plates of hepatocytes, composed of portal tract surrounded by hepatocytes. **B:** paracetamol (2 g/kg) intoxicated group: shows areas of hepatocytes necrosis, distortion of hepatocytes and hematoma of central vein as well as infiltration of the lymphocytes and kuffer cells around central vein. **C:** Ph.Cr (15 mg/kg) pretreated liver tissue, showing centrilobar, canalicular dilatation with mild intracellular cholestasis. **D:** Ph.Cr (30 mg/kg) pretreated liver tissue, showing well defined central vein, plates of hepatocytes and absence of necrosis with mild intracellular cholestasis. **E:** silymarin (100 mg/kg) pretreatment shows nearly normal histopathology with absence of any necrosis and have normal central vein with plates of hepatocytes.

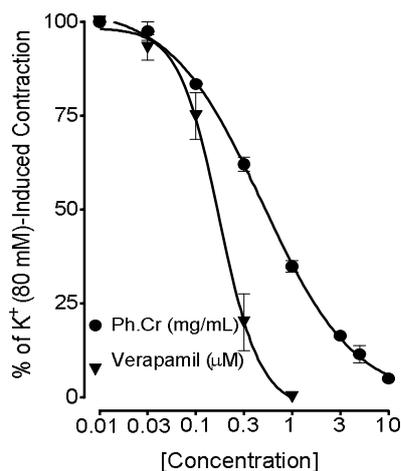


Figure 3: Concentration-response curves showing inhibitory effect of *Parthenium hysterophorus* crude extract (Ph.Cr) and verapamil on K⁺ (80 mM) induced contractions in isolated rabbit jejunum preparations. Values shown represent the mean ± SEM (n = 4)

DISCUSSION

Paracetamol and CCl₄ are the most commonly used hepatotoxicants in experimental models to investigate hepatoprotective effects [10]. Biochemical markers in blood along with histopathological assessment of liver sections shows the degree and type of liver damage, while restoration of serum enzyme levels towards its respective normal values by the test drug treatment defines its hepatoprotective potential. Carbon tetrachloride and paracetamol intoxicated groups showed increased level of biochemical markers: ALT, ALP and TB along with damaged liver architecture. Halogenated free radicals are formed by CYP2E1 as a result of CCl₄ metabolism [11]. While paracetamol is metabolized to *N*-acetyl-*p*-benzoquinone imine (NAPQI), a toxic reactive intermediate, through different cytochrome P-450 (CYPs) isozymes i.e., CYP1A2 [12], CYP2D6 and CYP3A4 [13]. Hepatotoxic doses of paracetamol deplete normal levels of glutathione results in widespread production of reactive free radicals, causing severe damage to the liver [14]. The

most remarkable pathological characteristics of CCL₄-induced hepatotoxicity are cirrhosis, fatty liver and necrosis [15]. This hepatotoxicity mediated by reactive species can be managed effectively by agents which possess antioxidant, anti-lipid peroxidation and free radical scavenging activities [16].

In this study, an attempt was made to investigate hepatoprotective activity of *Parthenium hysterophorus* and to elucidate the underlying mechanisms involved in this protection. Ph.Cr significantly attenuated CCL₄ and paracetamol hepatotoxic effects in a dose dependent fashion. These effects were comparable to silymarin, a reference hepatoprotective agent [17].

This ability of the plant extract shows that restoration of these biochemical markers may be due to its potential cytochrome P-450 inhibitory effects. Moreover, the potential of the plant extract to reduce level of enzymes could be attributed to its ability of prevent peroxidative degradation of endoplasmic reticulum membrane lipids. This phenomenon could be achieved via the antioxidant activity of Ph.Cr as earlier reported [3]. Apart from these, the presently evaluated hepatoprotective activity of Ph.Cr could be linked to other possible mechanisms. Studies suggest that xenobiotics activate ligand-activated transcription factors like peroxisome proliferators activated receptor- α (PPAR- α) which are highly expressed in hepatocytes and play a crucial role in fatty acid oxidation [18].

Interestingly it has been reported that flavonoids and saponins which are present in Ph.Cr also activates PPAR- α system contributing in prevention of CCL₄ and paracetamol-induced hepatotoxicity [19,20]. This mechanism of activating PPAR- α system could be attributed to role of flavonoids and saponins present in *Parthenium hysterophorus*, playing role in prevention of CCL₄ and paracetamol-induced hepatotoxicity. Alkaloids, flavonoids and saponins are phytochemical constituents present in Ph.Cr having free radical scavenging, antioxidant and lipid peroxidation inhibition abilities.

In isolated tissue experiments, Ph.Cr caused relaxation of high K⁺-induced contractions, likewise verapamil, a standard Ca⁺⁺ antagonist [21], suggesting CCB effect of *Parthenium hysterophorus*. At elevated concentration (> 30 mM), K⁺ causes contraction of smooth muscles by opening L-type Ca⁺⁺ channels, causing a contractile effect through influx of extracellular Ca⁺⁺. Any substance triggering high K⁺-induced contraction inhibition is said to be an inhibitor of

Ca⁺⁺ influx [22]. Excessive calcium ions in hepatocytes cytosol causes changes in cell plasma membrane integrity along with its organelles, leads to deterioration of DNA and activates phospholipases, proteases and endonucleases, which causes degradation of essential proteins [23]. There are special binding sites for calcium channel blockers (CCBs) in the inner mitochondrial membrane, which in turn inhibit calcium influx, resulting in reduce production of macrophage related cytokines.

The hepatoprotective action of *Parthenium hysterophorus* may be attributed to the existence of flavonoids, tannins and saponins, as such chemicals of such classes have been reported to demonstrate hepatoprotective, antioxidant and Ca⁺⁺ channel blocking activities [4,20,24].

CONCLUSION

The findings of this study indicate that *Parthenium hysterophorus* exhibits hepatoprotective activity by attenuating elevated levels of biochemical markers, possibly mediated through its antioxidant and the observed Ca⁺⁺ antagonist potential.

DECLARATIONS

Acknowledgement

The authors are thankful to Riphah Academy of Research and Education (RARE), Islamic International Medical College Trust for partial financial support of the study.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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