Analgesic, antipyretic, anti-inflammatory, and hepatoprotective activities of *Pulicaria crispa* (Forssk.) Oliv. (Asteraceae)

Gamal A. Soliman[1,2], Mohd Nazam Ansari[1], Mohammed H. Alqarni[3],
Ahmed I. Foudah[3], Aftab Alam[3], Mohammed Ayman Salkini[3],
Hasan S. Yusufoglu[3,4*]

[1]Department of Pharmacology, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj, KSA, [2]Department of Pharmacology, College of Veterinary Medicine, Cairo University, Egypt, [3]Department of Pharmacognosy, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj, KSA, [4]Department of Pharmacognosy & Pharmaceutical Chemistry, College of Dentistry & Pharmacy, Buraydah Private Colleges, Buraydah 81418, Saudi Arabia

Some plants of the genus Pulicaria have been used in traditional medicines for treating back pain and inflammation. They possess various bioactivities such as antipyretic, analgesic, and hepatoprotective. This study aimed to investigate the potential analgesic, antipyretic, anti-inflammatory, and hepatoprotective activities of *Pulicaria crispa* (*P. crispa*) extract (PCE). Analgesic activity was evaluated using the hot plate and acetic acid-induced writhing tests. Antipyretic and anti-inflammatory activities were evaluated using rectal temperature and carrageenan-induced hind paw edema methods, respectively. CCl4-intoxication was used for hepatoprotective activity. Also, liver histopathology was assessed. PCE, at 500 mg/kg, exhibited significant analgesic, antipyretic, and anti-inflammatory effects. The increased serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and bilirubin of CCl4-exposed rats reflects their liver injury. PCE significantly decreased the elevated liver markers. The hepatoprotective effect of PCE was confirmed, as it successfully reversed the altered levels of total protein, malondialdehyde (MDA), and non-protein sulfhydryls (NP-SH) in the liver tissues of CCl4-exposed rats. Histopathological studies confirmed the hepatoprotective nature of PCE. Pretreatment of rats with PCE reduced the severity of CCl4-induced liver damage. These findings concluded that PCE possesses analgesic, antipyretic, anti-inflammatory, and hepatoprotective activities.

Keywords: Analgesic. Antipyretic. Anti-inflammatory. Hepatoprotective. *Pulicaria crispa.*

INTRODUCTION

Inflammation, pain, and pyrexia may result in several pathological conditions. Synthetic drugs are clinically the most important drugs used for the treatment of various inflammatory disorders; however, if used for a long duration, they may have severe toxic effects, including gastrointestinal ulceration, bleeding, renal impairment, etc. (Cryer, Duboisø,

1998; Griffin, Marie, 1998). Researchers worldwide are working to introduce new medicines, in an effort to develop effective, cheap, and harmless drugs (Vane, Botting, 1995). For many years, people in developing countries have believed that the traditional medicinal system plays an essential role as an alternative method in the health care system. This belief arose because the conventional medical system is considered a vital source of useful compounds, with potential therapeutic effects, that are almost free from side effects. Prescribers of medicinal plants use different medicinal plants and their preparations for the treatment of various disorders based only on their experience, without scientific knowledge (Muhammad, Saeed, Khan, 2012). It

*Correspondence: H. S. Yusufoglu. Department of Pharmacognosy & Pharmaceutical Chemistry, College of Dentistry & Pharmacy, Buraydah Private Colleges, Buraydah 81418, Saudi Arabia. E-mail: hasanagazar@hotmail.com, hasan.yusuf@bpc.edu.sa

Gamal A. Soliman, Mohd N. Ansari, Mohammed H. Alqarni, Ahmed I. Foudah, Aftab Alam, Mohammed A. Salkini, Hasan S. Yusufoglu

is anticipated that about one-quarter of approved modern medicines are derived from botanicals (Sahreen *et al*., 2014). Genus Pulicaria (family Asteraceae) includes about 100 species that are widely distributed in Europe, Asia, and Africa (Williams *et al*., 2003). The plants of genus Pulicaria have been used in traditional medicines to treat various ailments such as back pain, inflammation, menstrual cramps, intestinal disorders, dysentery, and diarrhea (Abdallah *et al*., 2019). They possess multiple bioactivities, such as cytotoxic, hepatoprotective, anti-inflammatory, cardioprotective, and nephroprotective (Abdallah *et al*., 2019). In conventional medicine, *Pulicaria crispa* is used for colds, cough, colic, excessive sweating, and carminative disorders (Elshiekh, Mona, 2015). Mandaville (1990) cited the name *Pulicaria undulata* as the primary name and *Pulicaria crispa* as a synonym. Other Pulicaria species that possess some of the standard therapeutic values include: *P. odora, P. paludosa, P. sicula, P. dysenteric, P. salviifolia*, and *P. vulgare* (Ezoubeiri *et al*., 2005; Sagitdinova *et al*., 1992). Previous phytochemical studies on Pulicaria species gave rise to the isolation of flavonoids and phenolics, mono-, di-, and sesquiterpenes, essential oils, and caryophyllene derivatives (Hussein *et al*., 2017). In our previous study, we reported the presence of alkaloids, phenols, flavonoids, and tannins in P. crispa extract (Foudah *et al*., 2015). The Pulicaria species has been reported to possess various activities such as anti-inflammatory, antileukemic, cancer chemo-preventive, and cytotoxicity (Al-Yahya *et al*., 1988). In scientific reports, *P. crispa* has been reported for cytotoxicity and antimicrobial activities (Kuete *et al*., 2013). Recently, the alcoholic extract of the aerial part of this plant was shown to possess in vitro antioxidant and antimicrobial activity (Foudah *et al*., 2016). However, because of the great importance of the plant extracts, in the tropics and traditional uses, we examined the effects of methanolic extract of aerial parts of the plant for analgesic, antipyretic, anti-inflammatory, and hepatoprotective activities in experimental animal models. As the plants of genus Pulicaria have been used in traditional medicines for treating back pain and inflammation, in addition to their antipyretic and hepatoprotective activities, the present work sought to assess the potential analgesic, antipyretic, anti-inflammatory, and hepatoprotective activities of Pulicaria crispa in experimental animal models.

MATERIAL AND METHODS

Preparation of *Pulicaria crispa* extracts (PCE) and selection of animals

The aerial parts of *Pulicaria crispa* were collected in early March 2015 from Al-Dubaia town, 25 km from Al-Kharj City. The collected plant was authenticated by taxonomist Dr. M. Atiqur Rahman from the College of Pharmacy, Medicinal, Aromatic and Poisonous Plants Research Center, King Saud University, Riyadh. A voucher specimen (PSAU-CPH-005-2013) is maintained in the herbarium of the College of Pharmacy, Prince Sattam Bin Abdul-Aziz University. *P. crispa* powder (1 Kg) was percolated with 2 L of 80% methanol. After percolation, the solvent was evaporated using a rotary evaporator. Further, the water content was evaporated by a deep freezer and then powdered and stored in a colored bottle for use as a test sample. Later, the freeze-dried powder of P. crispa extract (PCE) was examined for active metabolites using methods similar to those in our previous report (Foudah *et al*., 2015).

Laboratory animals

Swiss albino mice (25–30 g) and Wistar albino rats (150–200 g) of either sex used in this study were obtained from the Laboratory Animal Care Unit, College of Pharmacy, Prince Sattam Bin Abdul-Aziz University, Al-Kharj, Kingdom of Saudi Arabia. The animals were kept in well-ventilated and hygienic rooms, maintained under standard environmental conditions, and fed a standard rodent pellet diet and water ad libitum. The protocols were approved by the ethical committee of the College of Pharmacy, Prince Sattam Bin Abdul-Aziz University, Al-Kharj, approval number PHARM-2-3-2015. Equal numbers of male and female animals were used in all the groups in this study.

Acetic-acid-induced writhing test

Twenty albino mice (weighing 25-30 g) of either sex were arbitrarily divided into four groups of six mice per group using a method described by Dina

et al., 2010. All animals were fasted for 12 h and later treated as follows: group I (dose 10 ml/kg) mice were given normal saline (negative control group), groups II and III mice were administered PCE at doses of 250 and 500 mg/kg, respectively, and group IV received indomethacin at a dose of 4 mg/kg (positive control group). All treatments were given orally by using gastric gavage. One hour after administration of the drug and PCE, 1% glacial acetic acid (10 ml/kg) was given intraperitoneally (i.p.) to all the mice. The number of abdominal constrictions or writhes observed in each mouse was counted for 20 min and recorded. The percentage of protection against abdominal writhing was calculated using the formula.

$$\text{Analgesic activity (\%)} = \frac{\text{Mean writhing count } (C_g - T_g)}{\text{Mean writing count of } C_g} \times 100$$

Hot plate test

Swiss albino mice (weighing 25-30 g) of either sex used in this experiment were initially screened by placing each mouse individually on a thermostatically hot plate (IITC Life Sciences, Inc., USA) set at 55±1°C. Animals that failed to lick the hind paw or jump (nociceptive responses) within 20 s were discarded. Eligible animals were divided into four groups of six mice each and pre-treatment reaction time for each mouse was determined.

Mice in the different groups were then treated with normal saline (10 ml/kg, p.o.), PCE (250 and 500 mg/kg, p.o.), and indomethacin (4 mg/kg, p.o.). The time taken for either paw licking or jumping was recorded again after treatment at different periods. The response was determined at 30, 60, and 90 min. A post-treatment cutoff time of 30 s was used (Omisore *et al.*, 2004; Gupta *et al.*, 2005).

Antipyretic activity

Four groups of rats were injected subcutaneously with 15% yeast solution at a dose of 10 ml/kg body weight to induce pyrexia. The rectal temperature

of each animal was recorded before and 24 h after the yeast injection through gentle insertion a digital thermometer probe (TMP 812, Panlab Harvard apparatus, USA) intrarectal until a stable reading was obtained or for up to 20s. Thereafter, the test group was treated orally with PCE (250 and 500 mg/kg body weight). The control group was given normal saline (10ml/kg) and the standard reference was treated with 4 mg/kg of indomethacin. The post-treatment rectal temperature of each animal was recorded at 30, 60, and 120 min. Each result was calculated as the mean of three consecutive readings.

Anti-inflammatory activity

Twenty-four rats (weighing 150-200 g) of either sex were selected and randomly divided into four groups, each containing six rats, using a method described by Winter, Risley, Nuss, (1962). Each group of rats was injected with 0.05 ml of 1% carrageenan sodium salt into the right hind paw, under the plantar aponeurosis. Each rat of group I was treated with 10ml/kg normal saline, while groups II and III were treated with 250 and 500 mg/kg PCE. The positive control group IV was treated with 100 mg/kg phenylbutazone. All treatments were carried out 1 h before the carrageenan injection. Measurement of paw volume was carried out by the displacement technique using a calibrated glass tube immediately before and 3 h after the carrageenan injection. Inhibitory activity was calculated using the following formula:

Where 'a' is the mean paw volume of the test after carrageenan

$$\text{Inhibition (\%)} = \{1 - (\frac{a - x}{b - y})\} \times 100$$

Where 'a' is the mean paw volume of the test after carrageenan injection, 'x' is the mean paw volume of the test before carrageenan injection, 'b' is the mean paw volume of the control after carrageenan injection, and 'y' is the mean paw volume of the control before carrageenan injection.

Hepatoprotective activity

Hepatoprotective activity was evaluated in rats using a carbon tetrachloride (CCl4)-induced intoxication model. The rats were randomly divided into five groups (n=6); group I served as the normal control (normal saline), group II served as hepatotoxic (CCl4), group III served as a positive control (silymarin), and groups IV and V served as PCE-treated groups (250 and 500 mg/kg body weight). All the groups were administered with either normal saline or drug solutions for seven days. On the eighth day after the 24 h of the last dose of treatment, hepatotoxicity was induced in all groups except group I (normal control) through injection of a single dose of CCl4 at a dose of 1 ml/kg subcutaneously (s.c.) (1:1 dilution in groundnut oil) (Yusufoglu *et al.*, 2014). After the 24 h of CCl4 injection, blood samples were withdrawn through the retro-orbital venous plexus under light ether anesthesia. Blood samples were centrifuged at 5000 rpm for 20 min to separate serum. The blood samples were collected in dry tubes and allowed to rest for 30 min at room temperature. Serum obtained by centrifugation (5000 rpm for 20 min) was stored at $-20 \circ$C. Thereafter, animals were euthanized by decapitation and their liver tissues were excised for further biochemical analysis and histopathological studies. Samples of the liver were immediately frozen and stored at -70°C for biochemical analysis. The remaining portions were fixed in buffered formalin 10% for histopathological study.

The liver homogenate was prepared in a 0.25M sucrose solution for biochemical analysis and the ventral portion/s of the left lateral liver lobe was fixed in 10% neutral-buffered formalin solution for histopathological analysis.

Biochemical assays
Estimation of serum liver injury markers

Serum levels of ALT, AST, ALP, GGT, and total bilirubin were estimated using standard respective kits (Sigma-Aldrich, USA) and UV spectrophotometer (Model-150-200, Hitachi, Japan). All instructions supplied with the assay kits were followed.

Protein estimation

Estimation of total protein in liver tissues was carried out by the method of Lowry *et al.* (1951). Briefly, 0.1 ml of the supernatant was added to 1 ml of alkaline solution and mixed thoroughly. After an incubation of 30 min, the optical density of the mixture was recorded at 595 nm. The concentration of the total protein in liver tissue was calculated by using the standard curve of bovine serum albumin.

Lipid peroxidation assay

Lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA) via thiobarbituric acid (TBA) color reaction, using the method described by Buege and Aust (1978). In brief, 0.5 ml of the homogenate was mixed with 2.5 mL of TCA (10%, w/v), and the samples were centrifuged at 3000 rpm for 10 min. Afterward, 2 mL of each supernatant sample was transferred to a test tube, containing 1 mL of TBA solution (0.67%, w/v). The mixture was kept in boiling water for 10 min until a pink-colored solution was formed. The mixture was then cooled down immediately, and absorbance was measured at 532 nm by a spectrophotometer (UV-150-200, Hitachi, Japan). The concentration of MDA was calculated based on the absorbance coefficient of the TBA-MDA complex of 1.56×105 cm-1.M-1.

Estimation of nonprotein sulfhydryls (NP-SH).

Hepatic nonprotein sulfhydryls were measured according to the method of Sedlak and Lindsay (1968). The liver was homogenized in ice-cold 0.02 mmol/L ethylenediaminetetraacetic acid (EDTA). The absorbance was measured within 5 min of the addition of 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB) at 412 nm against a reagent blank.

Histopathological studies

For the histopathological study, a minute fragment of liver tissues was sited in a 10% formalin solution, for 1 h. The study was carried out by following the previous

method (Alqasoumi, 2010). The tissues were dehydrated by immersion in 80% isopropanol overnight and 100% isopropyl alcohol and, finally, in paraffin wax. A rotary microtome (Leitz 1512) was used for the section (3 μm) preparation, followed by subsequent staining with hematoxylin/eosin dye. Histological examination was carried out under a light microscope.

Statistical analysis

The results obtained were expressed as the mean ± SEM (standard error of the mean). The obtained data were analyzed using ANOVA (one-way analysis of variance), followed by Dunnett's multiple comparison test. All values of $p < 0.05$ were considered significant.

RESULTS

Acetic-acid-induced writhing test

The oral administration of higher doses of PCE (500 mg/kg) showed a significant reduction in the number of writhing episodes induced by acetic acid compared to the control. The inhibition of writhes was 29% after extract administration (Table I).

TABLE I - Antinociceptive effect of P. crispa extract (PCE) using acetic acid-induced abdominal writhing test in mice.

Treatment	Number of writhing (in 20 min)	% Inhibition
Control (AA)	30.33±1.81	-
AA + Indomethacin (4 mg/kg)	16.83±0.64*	44.51
AA + PCE (250 mg/kg)	27.50±1.11	9.33
AA + PCE (500 mg/kg)	19.66±0.88*	35.18

Values are expressed as Mean ± SEM (n=6).
* indicate significance compared to control group at p< 0.05 (Dunnett's test).

Hot plate test

The results of the hot plate test are shown in Table II. PCE produced a significant ($p < 0.001$) effect at the highest dose of 500 mg/kg, which peaked (85.83% inhibition) after the 60 min of extract administration. This effect was comparable to that produced by 4 mg/kg indomethacin (155.91%).

TABLE II - Analgesic efficacy of P. crispa extract (PCE) using the hot-plate test in mice

Treatment	Hot plate reaction time (seconds)			
	0	30 min	60 min	120 min
NC	13.5±0.21	13.4±0.98	12.7±0.87	13.1±0.79
Indomethacin (4 mg/kg)	12.7±0.36	30.7±1.72* (129.10%)	32.5±1.64* (155.91%)	33.7±1.65* (157.25%)
PCE (250 mg/kg)	12.3±0.26	15.0±0.83 (11.94%)	15.2±0.97 (19.69%)	15.5±0.94 (18.32%)
PCE (500 mg/kg)	13.4±0.22	21.5±1.26* (60.45%)	23.6±1.19* (85.83%)	23.8±1.57* (81.68%)

Values are expressed as Mean ± SEM (n=6).
* indicate significance compared to normal control (NC) group at p< 0.05 (Dunnett's test).
Values between brackets means % increase in pain threshold compared to NC group.

Antipyretic activity

The result of PCE against hyperthermia induced by yeast is shown in Table III. PCE showed significant protection against hyperthermia at doses of 250 and 500 mg/kg ($p < 0.001$). The standard, indomethacin (4mg/kg), also significantly decreased yeast-induced hyperthermia after 30-120 min ($p < 0.01$) of treatment.

TABLE III - Antipyretic effect of P. crispa extract (PCE) using yeast-induced hyperthermia in mice

Treatment	Rectal temperature (°C) after extract administration			
	0	30 min	60 min	120 min
NC	37.3±0.41φ	37.5±0.65φ	37.7±0.32φ	37.6±0.43φ
Hyperthermic control	38.9±0.43*	39.1±0.24*	38.9±0.41*	39.0±0.21*
Indomethacin (4 mg/kg)	39.1±0.51*	37.8±0.31φ	37.7±0.28φ	37.7±0.26φ
PCE (250 mg/kg)	39.2±0.65*	39.1±0.22*	38.8±0.22*	38.9±0.17*
PCE (500 mg/kg)	38.9±0.48*	38.2±0.28φ	37.9±0.18φ	37.9±0.32φ

Values are expressed as Mean ± SEM (n=6).

* indicate significance compared to normal control (NC) group at $p < 0.05$ (Dunnett's test).

φ indicate significance compared to hyperthermic control group at $p < 0.05$ (Dunnett's test).

Anti-inflammatory activity

The result of PCE against inflammation induced by carrageenan injection in the hind paw is shown in Table IV. PCE showed significant protection against inflammation at higher doses of 500 mg/kg ($p < 0.01$). The standard drug, phenylbutazone (PBZ) (100 mg/kg), also significantly ($p < 0.001$) decreased the volume of carrageenan-induced paw edema in the 3 h.

TABLE IV - Effect of P. crispa extract (PCE) on carrageenan-induced paw edema in albino rats

Treatment	Mean changes in paw volume (mL) 3 h after carrageenan administration	Reduction of paw swelling (%)
Carrageenan control	0.68±0.05	0.0
PBZ (100 mg/kg) + Carrageenan	0.21±0.01*	69.1
PCE (250 mg/kg) + Carrageenan	0.63±0.03	7.4
PCE (500 mg/kg) + Carrageenan	0.46±0.03*	32.4

Values are expressed as Mean ± SEM (n=6).

* indicate significance compared to carrageenan control group at $p < 0.05$ (Dunnett's test).

Hepatoprotective activity

The result of PCE against hepatotoxicity induced by CCl4 is shown in Table V. Treatment with a single dose of CCl4 in rats caused a considerable increase of AST (aspartate aminotransferase), ALT (alanine aminotransferase), GGT (gamma-glutamyl transferase), and ALP (alkaline phosphatase). Administration of silymarin (10 mg/kg) and PCE at the higher dose (500 mg/kg) for seven days before CCl4 injection significantly attenuated the serum markers of AST, ALT, GGT, and ALP in comparison to the toxic group (CCl4 only) and the results were found to be statistically significant. Moreover, a lower dose of PCE (250 mg/kg) showed no significant changes in AST, ALT, GGT, and ALP level in serum. Total bilirubin levels were significantly increased in the CCl4-treated rats as compared to the control group. Administration of the silymarin and PCE at both doses (250 and 500 mg/kg) led to a significant attenuation ($p < 0.05$) in their levels.

TABLE V - Effect of P. crispa extract (PCE) on the serum activity of liver marker enzymes and bilirubin in rats with CCl4-induced hepatotoxicity

Treatment	ALT (U/L)	AST (U/L)	GGT (U/L)	ALP (U/L)	Bilirubin (mg/dl)
NC	67.10±4.44ϕ	128.51±7.64ϕ	4.25±0.33ϕ	233.83±13.19ϕ	0.74±0.05ϕ
CCl4 control	241.50±8.80* (259.91%)	312.33±16.81* (143.04%)	14.18±0.72* (233.65%)	485.66±19.01* (107.70%)	3.52±0.20* (375.68%)
Silymarin (50 mg/kg) + CCl4	89.72±5.49*ϕ (-62.85)	147.10±8.67ϕ (-52.9%)	6.06±0.49*ϕ (-57.26%)	268.16±14.85ϕ (-44.78%)	1.05±0.09*ϕ (-70.17%)
PCE (250 mg/kg) + CCl4	223.16±9.81* (-7.59%)	298.50±14.16* (-4.43%)	13.16±0.86* (-7.19%)	445.33±17.87* (-8.30%)	3.04±0.24* (-13.64%)
PCE (500 mg/kg) + CCl4	155.83±8.07*ϕ (-35.47%)	156.00±9.04*ϕ (-50.05%)	8.25±0.68*ϕ (-41.82%)	289.66±15.11*ϕ (-40.36%)	1.38±0.08*ϕ (-60.80%)

Values are expressed as Mean ± SEM (n=6).

* indicate significance compared to normal control (NC) group at $p < 0.05$ (Dunnett's test).

ϕ indicate significance compared to CCl4 control group at $p < 0.05$ (Dunnett's test).

Values between brackets mean % changes.

The effect of PCE on hepatic total protein and NP-SH in CCl4-intoxicated rats is shown in Figures 1 and 2. Rats intoxicated with CCl4 showed a significant decrease in total liver protein and NP-SH content as compared to the normal control group. Treatment with silymarin and PCE (250 or 500 mg/kg), along with CCl4, showed a significant increase ($p < 0.001$) in the total protein and NP-SH levels.

The effect of PCE on hepatic lipid peroxidation in CCl4-intoxicated rats is shown in Figure 3. The malondialdehyde (MDA) level, a marker of lipid peroxidation, was significantly ($p < 0.001$) elevated in liver tissue in the CCl4-intoxicated control group. Meanwhile, both the standard drug silymarin and the test drug PCE (250 and 500 mg/kg) treatments prevented the CCl4-induced elevation of MDA.

Photomicrographs of the liver sections of animals are shown in Figure 4, to study the hepatoprotective effect of PCE. A (group 1, normal control animals) shows the normal histological structure of the hepatic lobule. B (group 2, CCl4-only treated animals) shows hepatic steatosis (small arrow) and apoptosis of hepatocytes (large arrow) along with inflammatory cell infiltration. C (group 3, silymarin-treated animals) shows congestion of the central vein (arrow). D (group 4, PCE-250-mg/Kg-treated animals) shows dilation of the hepatic sinusoids and sinusoidal leukocytosis, while E (group 5, PCE-500-mg/kg-treated animals) shows sinusoidal leukocytosis (arrow).
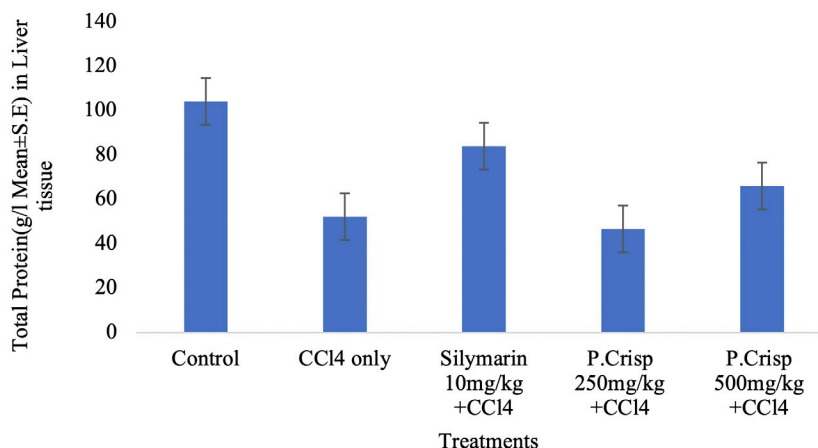
FIGURE 1 - Effect of *P. crispa* extract (PCE) on hepatic Total Protein of control and experimental rats. All values represent mean ± SEM. **p < 0.01; ***p < 0.001; ANOVA, followed by Dunnett's multiple comparison test. a As compared with Control group. b As compared with CCl4 only group.
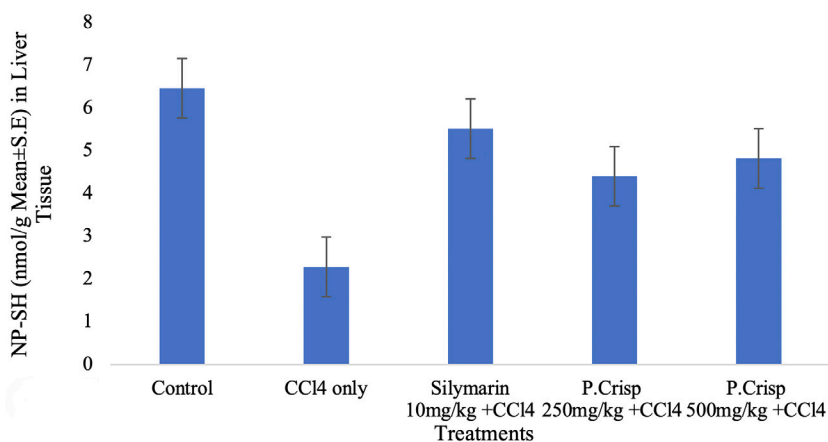

FIGURE 2 - Effect of *P. crispa* extract (PCE) on hepatic NP-SH of control and experimental rats. All values represent mean ± SEM. ***p<0.001; ANOVA, followed by Dunnett's multiple comparison test. a As compared with Control group. b As compared with CCl4 only group.
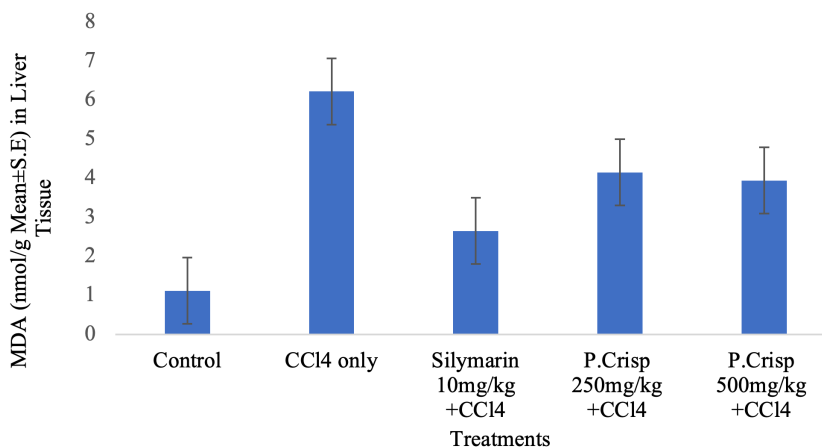

FIGURE 3 - Effect of *P. crispa* extract (PCE) on hepatic MDA of control and experimental rats. All values represent mean ± SEM. ***p<0.001; ANOVA, followed by Dunnett's multiple comparison test. a As compared with Control group. b As compared with CCl4 only group.
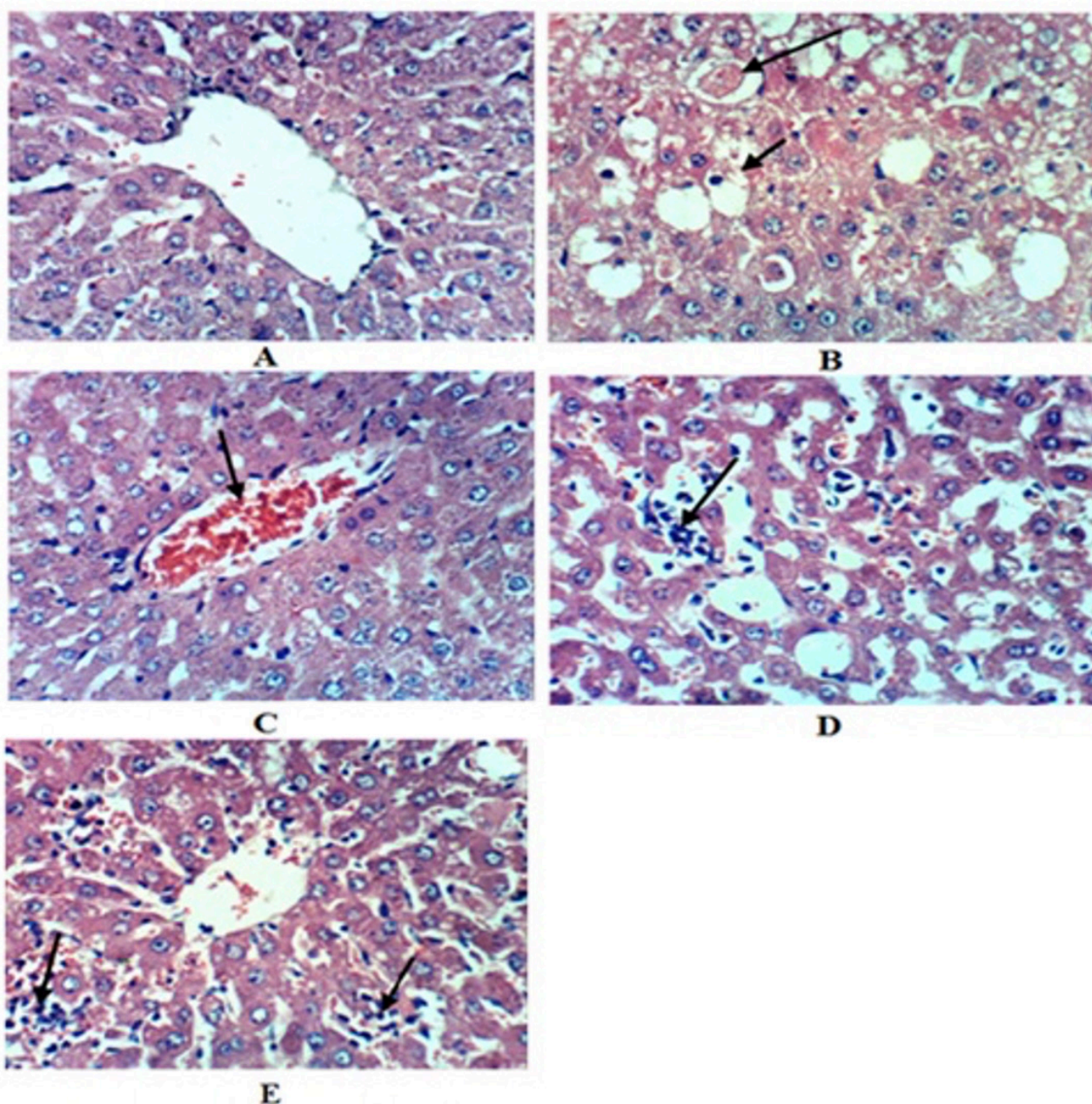
FIGURE 4 - Photomicrographs of rat liver sections (H & E X 400) (A) Group 1 (Control), showing the normal histological structure of the hepatic lobule. (B) Group 2 (Only CCl4), showing hepatic steatosis (small arrow) and apoptosis of hepatocytes (large arrow). (C) Group 3 (Silymarin + CCl4), showing congestion of central vein (arrow), (D) Group 4 (PCE-250 mg/Kg + CCl4), showing dilation of hepatic sinusoids and sinusoidal leukocytosis. (E) Group 5 (PCE-500 mg/Kg + CCl4) showing sinusoidal leukocytosis

DISCUSSION

The present study revealed that PCE possesses significant analgesic, antipyretic, anti-inflammatory, and hepatoprotective activities in experimental animals at a dose of 500 mg/kg. PCE at 250 mg/kg failed to exhibit any significant effect. Two different analgesic testing models were employed to identify the peripheral and central analgesic effects of the test extract. The

abdominal constriction response induced by acetic acid is a sensitive procedure to determine analgesia at the peripheral level. In the acetic-acid-induced writhing test model, a dose-dependent effect of PCE was observed. The substance inhibiting the writhing will have an analgesic effect, preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition (Srinivasan *et al.*, 2003). The result of the present study, therefore, hints that PCE may possess peripheral analgesic activity at a dose of 500 mg/ kg, whereas the small dose was observed to be non-effective. The hot plate test model also showed a significant effect of PCE at a higher dose. The hot plate system engages higher brain functions and is measured as a supra-spinally controlled response (Chapman *et al.*, 1985). Thus, the significant effect of PCE on the hot plate method to inhibit responses such as paw licking and jumping in mice indicates that it might be acting at the supra-spinal level.

PCE was also investigated for antipyretic effects using yeast-induced pyrexia in rats. It has already been established that yeast provokes pathogenic fever in animals by enhancing the production of prostaglandins, which elevates the set point of the thermoregulatory center in the hypothalamus (Rititid *et al.*, 2007). The increase in body temperature is due mainly to infection, inflammation, or other disease states. The desirable body temperature is a delicate balance between heat production and loss and is synchronized by the hypothalamus through the set-point control. PCE at 500 mg/kg showed appreciable antipyretic activity in rats. The antipyretic effect of PCE was observed as early as 30 min, and the effect was maintained for 180 min after oral administration. The lowering of body temperature by PCE was comparable to standard drug indomethacin. It is now clear that most antipyretics work by inhibiting the enzyme cyclooxygenase and reducing the levels of PGE2 within the hypothalamus. Most of the NSAIDs, like indomethacin, show the antipyretic activity by blocking the central cyclooxygenase production of PGE2 (Chomchuen *et al.*, 2010). It is therefore suggested that the antipyretic effect of PCE occurs similarly to that of indomethacin.

In the present study, the anti-inflammatory effect of PCE was evaluated using the carrageenan-induced paw edema model in Wistar albino rats. A significant anti-inflammatory effect of PCE was observed at a higher dose only. The observed experimental effect was also not dose-dependent. This type of activity is quite common with medicinal plant evaluation, and it is perhaps due to the multi-component nature of the medicinal plants and their extracts (Raval, Ravishankar, Ashok, 2013). Carrageenan-induced paw edema occurs in two phases. The first phase begins with the release of histamine, serotonin, and kinins after the injection of carrageenan in the first few hours (Bhukya *et al.*, 2009). In the second phase, there is a release of prostaglandins and related substances in the next 2-3 h. The second phase is susceptible to both the clinically useful steroidal and non-steroidal anti-inflammatory agents (Amdekar *et al.*, 2012). The results of the present study indicated that PCE significantly suppressed the edematous response for up to 3 h. Similar results were observed in work done by Alghaithy *et al.* (2011) on the chloroform extract of Pulicaria guestii against carrageenan-induced paw edema and ear edema induced by croton oil application in rats. Such activity may be explained on the basis that genus Pulicaria contains sesquiterpene lactones (Li-Weber *et al.*, 2002) and flavonoids that have generally been reputed to have anti-inflammatory activity that may be related to their ability to inhibit cyclooxygenase and lipooxygenase enzymes (Mazza, Kay, 2008).

In the present study, the carbon-tetrachloride-induced hepatic injury model is used for the hepatoprotective evaluation of PCE. It is a well-known experimental method for evaluating hepatoprotective drugs (Hermenean *et al.*, 2012). Levels of liver enzymes (ALT, AST, GGT, and ALP) and bilirubin in serum have long been considered a sensitive index of liver damage (Khan *et al.*, 2012). Marked elevation of ALT, AST, GGT, ALP, and bilirubin in the serum of CCl4 control rats indicates damage to the hepatic tissue. ALT and AST are the most responsive markers of hepatocellular injury (Giannini *et al.*, 1999). GGT is important in transporting amino acids and is required for the synthesis of GSH in cells, while ALP is a

membrane-bound enzyme involved in active transport across the capillary wall (Yusufoglu, Alam, Zaghloul, 2015). Oral treatments with PCE and silymarin were able to retain the serum liver function biomarkers of CCl4-intoxicated rats toward the normal levels. Bilirubin is a key degradation product of hemoglobin and is normally excreted into the bile. If hepatic parenchymal injury is severe, less bilirubin is excreted, and hyperbilirubinemia is observed (Sticova, Jirsa, 2013). The study demonstrated that PCE and silymarin had reduced the level of serum bilirubin, which was elevated by CCl4 administration.

Carbon tetrachloride is shown to induce liver damage as a result of metabolic conversion of the free radicals through lipid peroxidation (LPO); interruption of the activities of the antioxidant enzymes and produced free radicals induce oxidative stress and cause hepatic damage (Manna, Sinha, Sil, 2006) and resulted in increased concentrations of hepatic MDA. According to Luqman and Rizvi (2006), LPO is known to injure the cells through the inactivation of membrane enzymes, decreasing the fluidity of the membrane and resolving into cytotoxic aldehydes such as MDA. MDA, the last product of energy metabolism, serves as an indicator of LPO, which is known to occur in hepatic toxicity due to the generation of ROS (Dalton *et al.*, 2009). A significant reduction in MDA level was observed in rats treated with PCE and silymarin. PCE might be shielding the liver cells by impairing CCl4-mediated LPO and resulting in the prevention of the production of ROS. The level of total protein would be decreased in hepatotoxic conditions due to defective protein biosynthesis in the liver. Thus, the reduction in total protein level is a further indication of liver damage in CCl4-exposed animals (Alam *et al.*, 2013). In our study, the level of total protein has been restored toward the normal value. Restoring the levels of total protein by PCE denotes a reduction of oxidative stress and, thus, hepatoprotective effect. Another indicator of hepatotoxicity is the consumption of non-protein sulfhydryl (NP-SH) content of the liver tissues, which could induce further damage and dysfunction of the liver (Li *et al.*, 2015). The liver intoxication provoked by CCl4 causes significant depletion of the non-protein

sulfhydryl (NP-SH) content of the hepatic tissues, which is an important indicator of oxidative damage of the liver (Al-Yahya *et al.*, 2013). PCE, as well as silymarin, significantly increased the level of NP-SH in liver tissues, confirming their hepatoprotective effects. This effect is considered by Al-Yahya *et al.* (2013) to be related to the phenolic compounds. Therefore, we believe that the possible mechanism of hepatoprotection offered by PCE is due to its photo components as phenolics, flavonoids, triterpenoids, alkaloids, and saponins. The major constituents of P. crispa, which are responsible for its biological activities, are phenolics, phenolic acid, flavonoids, coumarins, alkaloids, glycosides, tannins, terpenoids, and sterol types of active secondary metabolites (Maghraby *et al.*, 2010; Adebiyi *et al.*, 2016; Arbab *et al.*, 2017). The presence of these active secondary metabolites in the extracts has already been reported to be effective against various fatal ailments including hepatotoxicity and inflammations (Saha *et al.*, 2019). These metabolites was also showed to be active against pain and fever conditions (Jan, Khan, 2016). The hepatoprotective outcome of PCE was further confirmed by the histopathological examinations. PCE at different dose levels offered hepato protection. PCE at a higher dose (500 mg/kg) exhibited more protective effects than did the lower dose (250 mg/kg). The presence of phenolics, phenolic acid, flavonoids, and coumarins in PCE might be responsible for hepatoprotective effects (Yusufoglu, 2014; Saha *et al.*, 2019).

In conclusion, PCE significantly inhibited pain sensation through both peripheral and central mechanisms and displayed a significant anti-inflammatory effect in acute inflammation. Therefore, this study supports the traditional use of the plants of the genus Pulicaria for the management of back pain and inflammation. Additionally, PCE shows antipyretic and hepatoprotective activities in experimental animals. However, further studies are necessary to fully elucidate the mechanism of action of the *P. crispa* plant.

CONFLICT OF INTEREST

All authors declare no competing interests.

REFERENCES

Abdallah H, Mohamed G, Ibrahim S, Asfour H, Khayat M. Undulaterpene A: A new triterpene fatty acid ester from Pulicaria undulata. Pharmacogn Mag. 2019;15(65):671-4.

Adebiyi A, Bassey EE, Ayo R, Bello I, Habila J, Ishaku GA. Anti-mycobacterial, antimicrobial and phytochemical evaluation of *pulicaria crispa* and *Scoparia dulcis* plant extracts. J Adv Med Pharm Sci. 2016;7(4):1-11.

Alam MN, Bristi NJ, Rafiquzzaman M. Review on in vivo and in vitro methods evaluation of antioxidant activity. Saudi Pharm J. 2013;21(2):143–52.

Alghaithy A, El-Beshbishy H, Abdel Naim A, Nagy A, Abdel-Sattar E. Anti-inflammatory effects of the chloroform extract of Pulicaria guestii ameliorated the neutrophil infiltration and nitric oxide generation in rats. Toxicol Ind Health. 2011;27(10):899-910.

Alqasoumi S. Carbon tetrachloride-induced hepatotoxicity: Protective effect of 'Rocket' *Eruca sativa* L. in rats. Am J Chin Med. 2010;38(1):75-8.

Al-Yahya M, El Sayed AM, Mossa JS, Koziowski JF, Antoun MD, Ferin M, et al. Potential cancer chemopreventive and cytotoxic agents from *Pulicaria crispa*. J Nat Prod. 1988;51(3):621-4.

Al-Yahya M, Mothana R, Al-Said M, Al-Dosari M, Al-Musayeib N, Al-Sohaibani M, et al. Attenuation of CCl4-Induced Oxidative Stress and Hepatonephrotoxicity by Saudi Sidr Honey in Rats. Evid Based Complement Alternat Med. 2013;2013:1-10.

Amdekar S, Roy P, Singh V, Kumar A, Singh R, Sharma P. Anti-inflammatory activity of *Lactobacillus* on carrageenan-induced paw edema in male wistar rats. Int J Inflam. 2012;2012:752015.

Arbab AH, Parvez MK, Al-Dosari MS, Al-Rehaily AJ. *In vitro* evaluation of novel antiviral activities of 60 medicinal plants extracts against hepatitis B virus. Exp Ther Med. 2017;14(1):626-634.

Bhukya B, Anreddy RNR, William CM, Gottumukkala KM. Analgesic and anti-inflammatory activities of leaf extract of Kydia calycina Roxb. Bangladesh J Pharmacol. 2009;4(2):101-4.

Buege JA, Aust SD. Microsomal lipid peroxidation methods. Enzymol. 1978;52:302-10.

Chapman CR, Casey KL, Dubner R, Foley KM, Gracely RH, Reading AE. Pain measurement: an overview. Pain. 1985;22(1):1-31.

Chomchuen S, Singharachai C, Ruangrungsi N, Towiwat P. Antipyretic effect of the ethanolic extract of Ficus racemosa root in rats. J Health Res. 2010;24(1):23-28.

Cryer B, Duboisø A. The advent of highly selective inhibitors of cyclooxygenase-a review. Prostaglandins Other Lipid Mediat. 1998;56(5):341-61.

Dalton HR, Bendall R, Ijaz S, Banks M. Hepatitis E: an emerging infection in developed countries. Lancet Infect Dis. 2008;8(11):698-709.

Dina TA, Rahman MA, Ahmed NU, Uddin MN. Analgesic and anti-inflammatory properties of *Argyreia argentea* methanol extract in animal model. J Taibah Univ Sci. 2010;3:1-7.

Elshiekh YS, Mona AMA. Gas chromatography–mass spectrometry analysis of *Pulicaria crispa* (whole plant) petroleum ether extracts. Am J Res Commun. 2015;3(3):24-33.

Ezoubeiri A, Gadhi CA, Fdil N, Benharref A, Jana M, Vanhaelen M. Isolation and antimicrobial activity of two phenolic compounds from Pulicaria odora L. J Ethnopharmacol. 2005;99(2):287-92.

Foudah AI, Alam A, Soliman GA, Salkini MA, Ahmed EOI, Yusufoglu HS. Pharmacognostical, Antioxidant and Antimicrobial studies of aerial part of *Pulicaria crispa* (Family: Asteraceae). Bull Env Pharmacol Life Sci. 2015;4(12):19-27.

Foudah AI, Alam A, Soliman GA, Salkini MY, Ahmed EI, Yusufoglu HS. Pharmacognostical, Antibacterial and Antioxidant Studies of Aerial Parts of *Pulicaria somalensis* (Family: Asteraceae). Asian J Biol Sci. 2016;9(1-2):19-26.

Giannini E, Botta F, Fasoli A, Ceppa P, Risso D, Lantieri PB, et al. Progressive liver functional impairment is associated with an increase in AST/ALT ratio. Dig Dis Sci. 1999;44(6):1249-53.

Griffin M, Marie R. Epidemiology of nonsteroidal anti-inflammatory drug–associated gastrointestinal injury. Am J Med. 1998;104(3):23S-9.

Gupta M, Mazumder UK, Sambath Kumar R, Gomathi P, Rajeshwar Y, Kakoti BB, et al. Anti-inflammatory, analgesic and antipyretic effects of methanol extract from Bauhinia racemosa stem bark in animal models. J Ethnopharmacol. 2005;98(3):267-73.

Hermenean A, Popescu C, Ardelean A, Stan M, Hadaruga N, Mihali CV. Hepatoprotective effects of Berberis vulgarisl. extract/β cyclodextrin on carbon tetrachloride-induced acute toxicity in mice. Int J Mol Sci. 2012;13(7):9014-34.

Hussein SR, Marzouk MM, Soltan MM, Ahmed EK, Said MM, Hamed AR. Phenolic constituents of *Pulicaria undulata* (L.) C.A. Mey. sub sp. undulata (Asteraceae):

Antioxidant protective effects and chemosystematic significances. J Food Drug Anal. 2017;25(2):333-339.

Jan S, Khan MR. Antipyretic, analgesic and anti-inflammatory effects of *Kickxia ramosissima*. J Ethnopharmacol. 2016;182:90-100.

Khan RA, Khan MR, Ahmed M, Sahreen S, Shah NA, Shah MS, et al. Hepatoprotection with a chloroform extract of Launaea procumbens against CCl4-induced injuries in rats. BMC Complemen Altern Med. 2012;12:114.

Kuete V, Wiench B, Alsaid MS, Alyahya MA, Fankam AG, Shahat AA, et al. Cytotoxicity, mode of action and antibacterial activities of selected Saudi Arabian medicinal plants. BMC Complement Altern Med. 2013;13:354.

Li S, Tan H, Wang N, Zhang Z, Lao L, Wong C, et al. The role of oxidative stress and antioxidants in liver diseases. Int J Mol Sci. 2015;16(11):26087-26124.

Li-Weber M, Giaisi M, Baumann S, Treiber MK, Krammer PH. The anti-inflammatory sesquiterpene lactone parthenolide suppresses CD95-mediated activation-induced-cell-death in T-cells. Cell Death Differ. 2002;9(11):1256-1265.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem. 1951;193(1):265-75.

Luqman S, Rizvi S. Protection of lipid peroxidation and carbonyl formation in proteins by capsaicin in human erythrocytes subjected to oxidative stress. Phytother Res. 2006;20(4):303-306.

Maghraby AS, Shalaby N, Abd-Alla HI, Ahmed SA, Khaled HM, Bahgat MM. Immunostimulatory effects of extract of *Pulicaria crispa* before and after *Schistosoma mansoni* Infection. Acta Pol Pharm. 2010;67(1):75-79.

Mandaville J. Flora of Eastern Saudi Arabia. Kegan Paul International Limited, London, England (1990).

Manna P, Sinha M, Sil PC. Aqueous extract of *Terminalia arjuna* prevents carbon tetrachloride induced hepatic and renal disorders. BMC Complement Altern Med. 2006;6:33.

Mazza GJ, Kay CD. Bioactivity, absorption, and metabolism of anthocyanins. In: Fouad Daayf and Vincenzo Lattanzio (eds) Recent Advances in Polyphenol Research., Blackwell Publishing Ltd. 2008;vol. I, 228.

Muhammad N, Saeed M, Khan H. Antipyretic, analgesic and anti-inflammatory activity of Viola betonicifolia whole plant. BMC Complement Altern Med. 2012;12(1):59.

Omisore NOA, Adewunmi CO, Iwalewa EO, Ngadjui BT, Watchueng J, Abegaz BM, et al. Antinociceptive and anti-inflammatory effects of Dorstenia barteri (Moraceae) leaf and twig extracts in mice. J Ethnopharmacol. 2004;95(1):7-12.

Raval ND, Ravishankar B, Ashok BK. Anti-inflammatory effect of *Chandrashura* (*Lepidium sativum* Linn.) an experimental study. Ayu. 2013;34(3):302-4.

Rititid W, Ruangsang P, Reanmongkol W, Wongnawa M. Studies of the anti-inflammatory and antipyretic activities of the methanolic extract of Piper sarmentosum Roxb. Leaves in rats. Songklanakarin J Sci Technol. 2007;29(6):1519-26.

Sagitdinova GV, Éshbakova KA, Khushbaktova ZA, Malikov VM, Olimov V. Flavonoid pulicarpin from Pulicaria salviifolia and its hypolipidemic activity. Chem Nat Compd. 1992;28(3):286-8.

Saha P, Talukdar AD, Nath R, Sarker SD, Nahar L, Sahu J, et al. Role of natural phenolics in hepatoprotection: A mechanistic review and analysis of regulatory network of associated Genes. Front Pharmacol. 2019;10:509.

Sahreen S, Khan MR, Khan RA, Alkreathy HM. Cardioprotective role of leaves extracts of Carissa opaca against CCl4 induced toxicity in rats. BMC Res Notes. 2014;7(1):224.

Sedlak J, Lindsay RH. Estimation of total protein-bound and non-protein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 1968;25(1):192–205.

Srinivasan K, Muruganandan S, Chandra S, Tandan SK, Raviprakash V, Kumar D. Antinociceptive and Antipyretic Activities of Pongamia pinnata Leaves. Phytother Res. 2003;17(3):259-264.

Sticova E, Jirsa M. New insights in bilirubin metabolism and their clinical implications. World J Gastroenterol. 2013;19(38):6398-407.

Vane J, Botting R. New insights into the mode of action of anti-inflammatory drugs. Inflamm Res. 1995;44(1):1-10.

Williams CA, Harborne JB, Greenham JR, Grayer RJ, Kite GC, Eagles I. Variations in lipophilic and vacuolar flavonoids among European Pulicaria species. Phytochemistry. 2003;64(1):275-83.

Winter CA, Risley EA, Nuss GW. Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. Proc Soc Exp Biol Med. 1962;111:544-7.

Yusufoglu HS, Alam A, Salkini AM, Alam P, Zaghloul AM. Anti-inflammatory and hepatoprotective study of astragalus Plumosus var. Akardagicus and astragalus lamarcki (family: Fabaceae) in wistar albino rats. Int J Biol Pharm Allied Sci. 2014;3(8):1846-62.

Yusufoglu HS, Alam A, Zaghloul AM. Isolation of Astragaloside-IV and Cyclocephaloside-I from *Astragalus*

Gamal A. Soliman, Mohd N. Ansari, Mohammed H. Alqarni, Ahmed I. Foudah, Aftab Alam, Mohammed A. Salkini, Hasan S. Yusufoglu

gummifera and Evaluation of Astragaloside-IV on CCl$_4$ Induced Liver Damage in Rats. Asian J Biol Sci. 2015;8(1):1-15.

Yusufoglu HS. Analgesic, antipyretic, anti-inflammatory, hepatoprotective and nephritic effects of the aerial parts of Pulicaria arabica (Family: Compositae) on rats. Asian Pac J Trop Med. 2014;7(1):S583-90.