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Evaluation of hematoprotective, hepatoprotective, and anti-inflammatory potentials of chia seed (*Salvia hispanica* L.) extract in rats

Sabbya Sachi [a], Mst. Prianka Jahan [b], Purba Islam [a], Kazi Rafiq [a], Md. Zahorul Islam [a,*]

[a] Department of Pharmacology, Bangladesh Agricultural University, Mymensingh, 2202, Bangladesh
[b] Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh, 2202, Bangladesh

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ABSTRACT

This study was conducted to evaluate the effects of chia seed extract on CCl_4-induced hepatotoxicity, hematological profile, and carrageenan-induced inflammation in rats. Water-ethanol-acetone extract of chia seeds at the doses of 200 and 400 mg/kg body weight/day were applied to evaluate the comparative protective roles. Hematological profile and serum biochemical parameters were measured to evaluate the hematoprotective, and hepatoprotective effects of chia seed extract. Paw thickness and motility level were assessed at 0, 1, 3, 5, and 7 h after sub-planter injection of carrageenan to evaluate the anti-inflammatory potential. Tissue histopathology was performed in both cases. Chia seed extract reduced the elevated level of serum AST and ALT significantly in a dose-dependent manner following intra-peritoneal injection of CCl_4. Histopathological study of the liver tissue exhibited acute impairment of the hepatocytes and liver parenchyma following CCl_4 exposure, which was markedly regenerated by the chia seed extract treatment. Protective effects of the extracts were also evidenced by the RBC count, Hb (%), PCV (%), ESR, and neutrophil count. Chia seed extract was found to inhibit the carrageenan-induced paw edema and increase motility level in a dose-oriented fashion. Histological examination of the paw tissue revealed severe inflammation characterized by massive infiltration of inflammatory cells in the carrageenan group, which was significantly reduced by chia seed extract treatment. The higher dose of chia seed extract showed significant increases in bodyweight gain and feed efficiency ratio but decrease in visceral fat deposition. These results suggest that chia seeds possess potentials for hematoprotective, hepatoprotective, and anti-inflammatory activities.

1. Introduction

Nowadays, the identification of novel functional foods having potential pharmacological properties with limited toxic effects has been a matter of growing interest worldwide. *Salvia hispanica* L. (chia) is an annual herbaceous plant belongs to the family Lamiaceae (Khalid et al., 2023). This oil seed is considered as one of the most distinguished nutritious foods having a wide range of medicinal properties (Motyka et al., 2023). Nutritionally, chia seed is rich in carbohydrates (26–41 %), proteins (15–25 %), fats (30–33 %), dietary fiber (18–30 %), balanced amounts of vitamins and minerals, as well as high quantity of antioxidants (Otondi et al., 2020). It is a great source of polyunsaturated fatty acids (PUFAs), consisting up to 68 % of n-3 (mainly α-linolenic acid) and 19 % of n-6 fatty acids (Dąbrowski et al., 2018; Otondi et al., 2020). Chia seed is also rich in polyphenolic compounds such as rosmarinic acid, kaemferol, myricetin, quercetin, flavonol glycosides, caffeic acid, and chlorogenic acid (Al-Attar & Al-Rethea, 2017). These bioactive compounds are associated to weight loss, inflammation, oxidative stress, and blood pressure (Capitani et al., 2012).

Liver disease is a worldwide health problem and increasing day by day due to environmental pollution and adulteration of food. Available literature showed that free radicals are formed due to liver-toxic chemicals, and drug exposure (Al-Harbi et al., 2014). The produced free radicals can be associated with the commencement of lipid peroxidation process to generate reactive oxygen species (ROS) substantially, which is responsible for hepatocyte injuries and inflammatory processes (Chen et al., 2020). Polyphenolic compounds having free radical scavenging property counteract oxidative stress induced highly reactive oxygen species (Mas et al., 2024). Recent studies showed that bioactive constituents, such as phenolic compounds and n-3 fatty acids have antioxidant properties, which is related to be involved in hepatoprotective effect and cure hepatotoxicity (Poudyal et al., 2012).

* Corresponding author.
E-mail address: drzahorul@bau.edu.bd (Md.Z. Islam).

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Therefore, antioxidant properties of the polyphenolic compounds and α-linolenic acid (18:3n-3) in chia seed can effectively protect the liver from free radicals generated by chemical toxin-induced oxidative stress.

Corticosteroids and nonsteroidal anti-inflammatory drugs are the conventionally used anti-inflammatory drugs, which are associated with many undesirable side effects. The long-term use of these drugs has been linked to gastrointestinal, cardiac, and renal complications (Bindu et al., 2020). Hence, the attention towards evaluation of various natural antioxidants as an alternative source of remedies with a transcriptional mode of action, good efficacy, and minimal adverse effects is of greater interest nowadays (Mitra et al., 1998). The n-3 PUFA has a role to inhibit 5-lipoxygenase and cyclooxygenase-1-2 pathway which suppress inflammatory process (Roohi, 2020). As chia seed is a potential source of both n-3 and n-6 fatty acids, it may have role in suppressing inflammatory process.

Several studies have been conducted worldwide on assessing the nutritional and medicinal values of chia seed. Protective effects of chia seed in metabolic disorders in the liver of rats have been evidenced in the literature (de Paula Dias Moreira et al., 2022). An investigation on phenolic profile revealed the antioxidant and antibacterial activities of chia seed (Abdel-Aty et al., 2021). Alpha-linolenic acids (ALA) in chia seed has been reported to be involved in decreasing body weight, total cholesterol, and low-density lipoprotein in human (Medina-Urrutia et al., 2020). A study showed that the dietary inclusion of chia seed in rats fed on high-fat diet had significant effects on postprandial hyperglycemia and some hematological parameters (Mihafu et al., 2020). Wound healing properties and the roles of peptide fraction in chia seed have been evaluated in a few studies (Chan-Zapata et al., 2019; Pintapagung & Asawapattanakul, 2020). Although chia seed has been investigated in diversified perspective of medicinal properties, there are insufficient data regarding the ameliorative roles of chia seed extract on hematological profile, hepatotoxicity, and inflammatory condition. Therefore, the current research was undertaken to investigate the hematoprotective, hepatoprotective, and anti-inflammatory activities chia seed extract in male Wistar rats.

2. Materials and methods

2.1. Drugs and chemicals

CCl_4, silymarin, and acetone were obtained from Sigma-Aldrich Co. (St. Louis, USA). Carrageenan and dexamethasone were collected from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan), and Nacalai Tesque, Inc. (Kyoto, Japan), respectively. Analytical grade chemicals were used in all the cases to conduct the experiment.

2.2. Collection of chia seed and preparation of chia seed extract (CSE)

Chia Seed (*Salvia hispanica* L.) was collected from a local market of Mymensingh, Bangladesh. The authentication of the seeds was confirmed by a taxonomist of the Department of Crop Botany, Faculty of Agriculture, Bangladesh Agricultural University, Mymensingh. Coarse impurities in the seeds were removed by handpicking, which was followed by washing with distilled water to clean sand and other fine debris. Following this, the seeds were dried in an incubator for 3 days at 40 °C, levigated as dry powder by using an electric grinder, and macerated in water-ethanol-acetone (1/6–1/6–2/3) mixture following the method described by Alcântara et al. (2019), where the presence of all the major phenolic components in the extract was reported to be identified by applying the HPLC-MS technique. The marc was pressed and re-extracted using the solvent mixture, followed by filtering through Whatman grade-1 filter paper. The filtrate was evaporated slowly under vacuum, using a rotary evaporator (40 °C) to obtain the concentrated extract of chia seed. The final chia seed extract (CSE) thus obtained was weighed immediately and stored at 4 °C in a refrigerator, keeping in an airtight plastic container before being used in the in vivo studies.

2.3. Collection and acclimatization of experimental animals

A total of 66 male Wistar rats (*Rattus norvegicus*) about 4 weeks of age, weighing about 100–115 ± 5 g were purchased from the International Centre for Diarrhoeal Diseases Research Bangladesh (ICDDR, B), Mohakhali, Dhaka. The rats were accustomed to the new environmental setting for a period of 7 days before the experiment started, maintaining temperature 26 ± 1 °C, relative humidity of 50–60 %, and a periodical interval of 12 h light/12 h dark. The animals were allowed for the access of water ad libitum and hand-made feed pellet, which was prepared in accordance with the nutrient requirements as recommended by the National Research Council (Council, 1995) with few modifications.

2.4. Ethical approval

The study was performed in conformance with the code of ethics and standards set for the handling and management of laboratory animals with proper addressing the animal welfare issue. The methodology of the experiment was critically assessed and endorsed by the Animal Welfare and Experimental Ethics Committee, Bangladesh Agricultural University [Approval No.: AWEEC/BAU/2021 (62); Date: 23.12.2021].

2.5. CCl_4-induced hepatotoxicity study

2.5.1. Experimental design

A total of 36 rats were arbitrarily categorized into 6 groups (6 rats in each group). Hepatotoxicity was induced by a single intra-peritoneal (IP) injection of CCl_4, maintaining the dose of 2 ml/kg body weight (BW) mixed with olive oil (1:1) (Janakat & Al-Merie, 2002). Silymarin (45 mg/kg BW, orally) was employed as a reference standard hepatoprotective agent (Parveen et al., 2011). Silymarin (SIL) and CSE were administered orally by mixing these with the calculated amount of feed during feed formulation in both cases.

Groups of the rats were as the follows:

Group-I (Control): The rats were supplied with basal diet only.

Group-II (CCl_4): Basally maintained rats were exposed to CCl_4.

Group-III (CCl_4 + SIL): CCl_4 exposed rats were treated with SIL.

Group-IV (CCl_4 + CSE 200): CCl_4 exposed rats were treated with CSE 200 mg/kg BW/day.

Group-V (CCl_4 + CSE 400): CCl_4 exposed rats were treated with CSE 400 mg/kg BW/day.

Group-VI (CSE 400): Treatment with CSE 400 mg/kg BW/day without the exposure of CCl_4.

Treatments with CSE and SIL were continued for 21 days. Collection of blood was performed by cardiac puncture method using a 5-ml syringe attached with 21-gauge needle after 24 h of CCl_4 injection. The animals were kept under fasting condition prior to sacrifice using diethyl ether anesthesia for the collection of liver tissue.

2.5.2. Feed consumption and weight records

Feed intake and bodyweight gain were monitored in every alternate day. The data were employed to calculate the feed conversion ratio (FCR) and feed efficiency ratio (FER) for assessing the growth competency and feed utilization capability of rats in different groups. At necropsy, whole liver and visceral fat were collected, washed with physiological saline, and dried with filter paper for weighing to evaluate liver index (%), visceral fat index (%) and subsequent usage for histopathological analysis.

2.5.3. Sample preparation

A portion of blood was collected into 2 ml EDTA (ethylenediaminetetraacetate) tubes for hematological analysis. Another portion of the blood was allowed to coagulate at room temperature, followed by performing centrifugation for 10 min at 5000 rpm. The serum was aspirated and transferred carefully by using 20–200 μl micropipette into 2 ml Eppendorf tubes, which was used for analysis of biomarker

enzymes and creatinine. Both whole blood and serum sample were stored at −20 °C until being used.

2.5.4. Hematological analysis

Hematological assessment of the unclotted whole blood was performed in the Department of Pharmacology, Bangladesh Agricultural University by using an Automated Hematology Analyzer (BHA 560, Benemed Industry Co., Ltd, China). The parameters determined were red blood cells (RBC) count, white blood cells (WBC) count, hemoglobin (Hb), erythrocyte sedimentation rate (ESR), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and differential leukocyte count (DLC): neutrophils (NEU), lymphocytes (LYM), monocytes (MON), eosinophils (EOS), basophils (BAS).

2.5.5. Biochemical analysis

Activities of the biomarker enzymes, serum ALT and AST, and creatinine concentration were evaluated by using an autoanalyzer (Dimension® RXL MAXTM, Siemens, USA).

2.5.6. Histopathological assessment

The liver was dissected out and washed in physiological saline for histopathological evaluation. The sliced tissues of liver were then fixed in 10 % neutral buffered formalin, which was followed by dehydrating in graded alcohol. The specimens were further processed to embed in paraffin block, followed by sectioning at a thickness of 4–6 μm, and staining with hematoxylin–eosin to examine histomorphological alterations microscopically. The degree of centrilobular degeneration, hepatocellular ballooning, necrotic changes, steatosis, congestion of central vein, and sinusoidal dilatation were recorded for comparative evaluation among the groups.

2.6. Anti-inflammatory study

2.6.1. Paw edema assay

A total of 30 rats, selected randomly, were categorized into 5 groups (6 in each group). Inflammatory paw edema was induced by a single sub-plantar injection of 100 μL carrageenan (CAR) into the left hind paws of the rats, which was freshly prepared as a 1 % solution in distilled water. Dexamethasone (DEX) (3 mg/kg BW, IP) was employed as a reference standard anti-inflammatory drug (Thuo et al., 2022). Treatment with the extracts was performed by oral administration of CSE mixed with the calculated amount of feed during feed formulation. Groups of the rats were as follows:

Group-I (Control): The rats were subjected to the provision of basal diet and exposure to injection of 100 μL normal saline solution into the right hind paw.

Group-II (CAR): Basally maintained rats were exposed to CAR.

Group-III (CAR + DEX): CAR exposed rats were treated with DEX.

Group-IV (CAR + CSE 200): CAR exposed rats were treated with CSE 200 mg/kg BW/day.

Group-V (CAR + CSE 400): CAR exposed rats were treated with CSE 400 mg/kg BW/day.

Treatments with CSE (Group-IV & V) were continued for 7 consecutive days. Thirty minutes after last administration of CSE and DEX, an acute inflammatory edema was induced by CAR. The pre-carrageenan paw thickness at "0 h" and then at 1, 3, 5, and 7th h after CAR injection were measured using a vernier slide calipers according to the method described by Mohamed et al. (2020) with few modifications. The following equations were used to determine the paw edema rate (Cui et al., 2020) and inhibition (%) of edema (El-mekkawy et al., 2020):

Edema (%) = $(V_a - V_b)/V_b \times 100$; where V_b and V_a are the volumes of paw before and after the induction of inflammation at different time points, respectively.

Inhibition of edema (%) = $[1 - (A - X) / (B - Y)] \times 100$; where "$X$" and "$Y$" denote the volumes of paw in the treated and control groups before CAR treatment, respectively. "A" and "B" denote the volumes of paw in the treated and control groups after CAR treatment, respectively.

2.6.2. Motility test

The motility level of the rats was evaluated as the protocol described previously (Costa et al., 1981) with few modifications. The rats were observed for a period of 15 min after CAR injection for scoring motility pattern. The following scoring system was applied: the rats laid down = 1; the rats hobbled and avoided touching the inflamed paw on the floor = 2; the rats walked with difficulty, but the inflamed toe touched the floor = 3; the rats abled to run and climb with some difficulty = 4; the rats walked and ran easily = 5.

2.6.3. Histopathological analysis

The rats were sacrificed after 7 h of CAR injection. Sub-plantar tissues of the animals were collected and processed as described by Ghlissi et al. (2016) to evaluate the histological changes of the paw under light microscopy.

2.7. Statistical analysis

The results were presented as mean ± SEM (standard error of the mean). One-way and two-way analysis of variance (ANOVA) were implemented to measure the inter-group variation. Least significant difference (LSD) and Duncan's Multiple Range Test (DMRT) were accomplished as post hoc tests to analyze and interpret the data statistically. The values were accepted as statistically significant at $p < 0.05$ in all cases of interpretation. Histopathological data were considered to be nonparametric; therefore, no statistical tests were performed.

3. Results

3.1. Effects of CSE on body weight and feed efficiency

The effects of consumption of CSE on bodyweight changes along with feed utilization parameters in different groups are summarized in Table 1. CSE 400 mg/kg and SIL exhibited considerable increase in bodyweight and decrease in FCR ($p < 0.01$). No significant variation was observed in FCRs between CSE 400 and SIL ($p > 0.05$). Visceral fat index (%) were measured significantly low in CSE 400 and SIL groups ($p < 0.05$). However, non-significant changes in liver index (%) was found among the groups ($p > 0.05$). The highest FER, 24.21 ± 0.42 % was observed in SIL group, which was followed by CSE 400, 21.46 ± 0.27 %, CSE 200, 18.76 ± 0.24 %, and control, 14.18 ± 0.22 %. Although SIL group witnessed a noticeable increase in bodyweight gain (BWG) all through, the CSE-treated groups displayed a significant reduction in BWGs at the end.

3.2. Hematological parameters

The comparative hematological parameters found for different groups are demonstrated in Table 2. Silymarin and CSE 400 treatment groups showed significantly higher RBC count in contrast to the control and CCl_4 groups ($p < 0.01$). There were significant increases in the mean concentrations of Hb in the silymarin and CSE 400 treatment groups as opposed to the control ($p < 0.05$). The ESR level of all the treated groups declined significantly than that of the CCl_4 group ($p < 0.001$) and aligned with the control. In the case of PCV (%), there were no considerable variation between CSE and silymarin treatment groups, but significant changes were observed with that of the control and CCl_4 groups ($p < 0.01$). There were no significant variations observed in MCH or MCHC values, and WBC count. In differential leukocyte count, the changes found for neutrophils and lymphocytes in silymarin and CSE 400 treatment groups were significant as compared with the CCl_4, where the maximum neutrophil count was observed in the CSE treated group ($p < 0.01$).

Table 1
Effects of CSE on bodyweight, liver weight, visceral fat content, and feed efficiency of rats.

Parameters		Control	CSE 200	CSE 400	SIL
Bodyweight gain (BWG) (g)	Day-7	11.96 ± 0.15^{a}	12.30 ± 0.31^{a}	14.46 ± 0.23^{b}	14.51 ± 0.18^{b}
	Day-14	11.69 ± 0.11^{a}	13.84 ± 0.42^{b}	16.78 ± 0.35^{c}	15.93 ± 0.27^{c}
	Day-21	12.52 ± 0.83^{a}	11.33 ± 0.77^{b}	11.26 ± 0.47^{b}	17.76 ± 0.62^{c}
	Total	36.16 ± 0.55^{a}	37.50 ± 0.48^{a}	42.50 ± 0.53^{b}	48.24 ± 0.83^{c}
Liver weight (g)		6.12 ± 0.37^{a}	5.89 ± 0.28^{a}	6.43 ± 0.34^{a}	7.04 ± 0.57^{b}
Liver index (%)		3.87 ± 0.03^{a}	3.66 ± 0.08^{a}	3.98 ± 0.14^{a}	4.18 ± 0.02^{a}
Visceral fat (g)		3.14 ± 0.07^{a}	3.08 ± 0.13^{a}	2.93 ± 0.31^{b}	3.17 ± 0.23^{a}
Visceral fat index (%)		1.97 ± 0.02^{a}	1.93 ± 0.07^{a}	1.81 ± 0.04^{b}	1.88 ± 0.12^{b}
FCR		7.06 ± 0.11^{a}	5.33 ± 0.07^{b}	4.66 ± 0.06^{c}	4.13 ± 0.07^{c}
FER (%)		14.18 ± 0.22^{a}	18.76 ± 0.24^{b}	21.46 ± 0.27^{c}	24.21 ± 0.42^{c}

The values are presented as mean ± SEM in each group ($n = 6$). Bodyweight gain (BWG) = final bodyweight (g) - initial bodyweight (g), Liver index (%) = [liver weight (g) / final bodyweight (g)] × 100, Visceral fat index (%) = [visceral fat weight (g) / final bodyweight (g)] × 100. Feed conversion ratio (FCR) = food intake (g) / BWG (g), Feed efficiency ratio (FER) = [BWG (g)/food intake (g)] × 100. Values having the dissimilar superscripts within the same row indicate statistically significant variation.

Table 2
Comparative effects of CSE on hematological parameters.

Parameters		Groups					
		Control	CCl_4	CCl_4 + SIL	CCl_4 + CSE 200	CCl_4 + CSE 400	CSE 400
RBC (million/mm^3)		6.73 ± 0.23^{a}	6.61 ± 0.15^{a}	8.47 ± 0.37^{b}	7.07 ± 0.33^{a}	7.18 ± 0.51^{a}	8.40 ± 0.46^{b}
Hemoglobin (g/dl)		11.00 ± 0.11^{a}	10.25 ± 0.17^{ab}	12.93 ± 0.29^{d}	11.70 ± 0.17^{abc}	11.95 ± 0.41^{bc}	12.20 ± 0.16^{cd}
ESR (mm in 1st hour)		0.33 ± 0.33^{a}	2.00 ± 0.41^{b}	0.00 ± 0.00^{a}	0.25 ± 0.25^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
WBC (thousand/mm^3)		10.35 ± 0.24^{a}	10.63 ± 0.30^{a}	10.07 ± 0.18^{a}	10.32 ± 0.38^{a}	10.11 ± 0.14^{a}	10.64 ± 0.29^{a}
PCV (%)		35.00 ± 0.58^{a}	32.00 ± 0.41^{b}	37.50 ± 0.88^{c}	35.25 ± 0.64^{a}	36.50 ± 1.32^{c}	37.25 ± 0.48^{c}
MCV (fl)		52.01 ± 1.32^{a}	47.44 ± 2.13^{b}	44.27 ± 3.22^{c}	49.82 ± 0.64^{ab}	50.79 ± 0.58^{a}	48.34 ± 2.53^{bc}
MCH (pg)		16.24 ± 0.53^{ab}	15.50 ± 0.28^{bc}	15.21 ± 0.49^{bc}	16.45 ± 0.32^{ab}	16.27 ± 1.04^{ab}	14.12 ± 0.24^{bc}
MCHC (g/dl)		31.43 ± 0.23^{a}	32.05 ± 0.52^{a}	34.47 ± 0.84^{abc}	33.16 ± 0.77^{bc}	32.71 ± 0.14^{a}	32.75 ± 0.38^{a}
DLC (%)	**NEU (%)**	33.00 ± 0.58^{ab}	32.00 ± 0.91^{a}	35.00 ± 1.53^{bc}	33.00 ± 0.82^{ab}	36.50 ± 1.04^{c}	36.50 ± 0.29^{c}
	LYM (%)	63.33 ± 0.67^{ab}	65.25 ± 1.31^{b}	61.67 ± 1.76^{ab}	63.50 ± 0.87^{ab}	60.25 ± 0.95^{a}	60.75 ± 1.03^{a}
	MON (%)	1.33 ± 0.33^{a}	0.75 ± 0.25^{a}	1.33 ± 0.33^{a}	1.00 ± 0.41^{a}	0.75 ± 0.25^{a}	0.75 ± 0.48^{a}
	EOS (%)	2.33 ± 0.33^{a}	2.00 ± 0.71^{a}	2.00 ± 0.58^{a}	2.50 ± 0.29^{a}	2.50 ± 0.50^{a}	2.00 ± 0.41^{a}
	BAS (%)	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}

The values are denoted as mean ± SEM in each group ($n = 6$). Values not sharing a common letter as superscripts in the same row are regarded to vary significantly ($p < 0.05$).

3.3. Effects of CSE on CCl_4-induced hepatotoxicity

3.3.1. Biochemical parameters

The exposure of CCl_4 significantly raised the level of ALT, AST, and serum creatinine [Fig. 1(A, B, C)]. The treatment with CSE significantly lowered the concentrations of ALT and AST in a dose-dependent way ($p < 0.001$) [Fig. 1(A, B)]. Although the lower dose of CSE couldn't make significant variation in serum creatinine level ($p > 0.05$), the higher dose showed significant diminution in comparison with the CCl_4 control ($p < 0.001$) [Fig. 1(C)]. SIL-treated group showed maximum decline in serum ALT, AST, and creatinine ($p < 0.001$) [Fig. 1(A, B, C)]. The dietary supplementation of CSE at the higher dose, 400 mg/kg didn't show any adverse effects as evidenced by the non-significant changes in serum ALT, AST, and creatinine level when compared with the control ($p > 0.05$) [Fig. 1(A, B, C)].

3.3.2. Histopathological study of liver

Normal histological architecture of the hepatic tissue was observed in the control group [Fig. 2a]. CCl_4-induced hepatotoxic group showed severe centrilobular necrosis, massive degeneration of hepatocytes and steatosis, vacuolation and ballooning of hepatocyte, congestion of central vein, sinusoidal dilatation, and diffuse infiltration of inflammatory cells [Fig. 2b]. Treatment with CSE markedly alleviated the CCL_4-induced hepatic lesions in a dose-dependent manner [Fig. 2c, d]. The higher dose (400 mg/kg) of CSE exerted almost similar extent of hepatoprotection [Fig. 2d] to that of the silymarin treatment [Fig. 2e] with massive diminution of hepatocyte degeneration and necrosis. No detrimental effect of CSE was found on liver as evidenced by the appearance of normal morphology of hepatocytes and hepatic parenchyma [Fig. 2f].

3.4. Anti-inflammatory study of chia seed extract

3.4.1. Determination paw edema volume

Administration of CAR markedly triggered the paw volume ($p < 0.001$), with the maximum edema, 91.05 ± 0.75 % at 3 h. There were significant elevations of edema at 1 h for all the groups, rose slightly at 3 h in the groups CAR, CAR + CSE 200, and CAR + CSE 400, followed by a gradual decrease at the end. The group, CAR+DEX witnessed a subsequent fall in edema percentage from 1 h of injection to all through the period of observation, reaching its minimum at 30.52 ± 0.55 %. Pretreatment with CSE significantly reduced paw edema at 1, 3, and 5 h after inflammation was induced ($p < 0.05$). The maximum inhibition of paw edema, 53.92 ± 0.69 % was observed in CAR+DEX, which was followed by group CAR + CSE 400, and CAR + CSE 200. The effects of CSE on induced paw edema (%) is demonstrated in Fig. 3. The representative photographs of the concentration-dependent effects of CSE in carrageenan-induced paw edema are shown in Fig. 4.

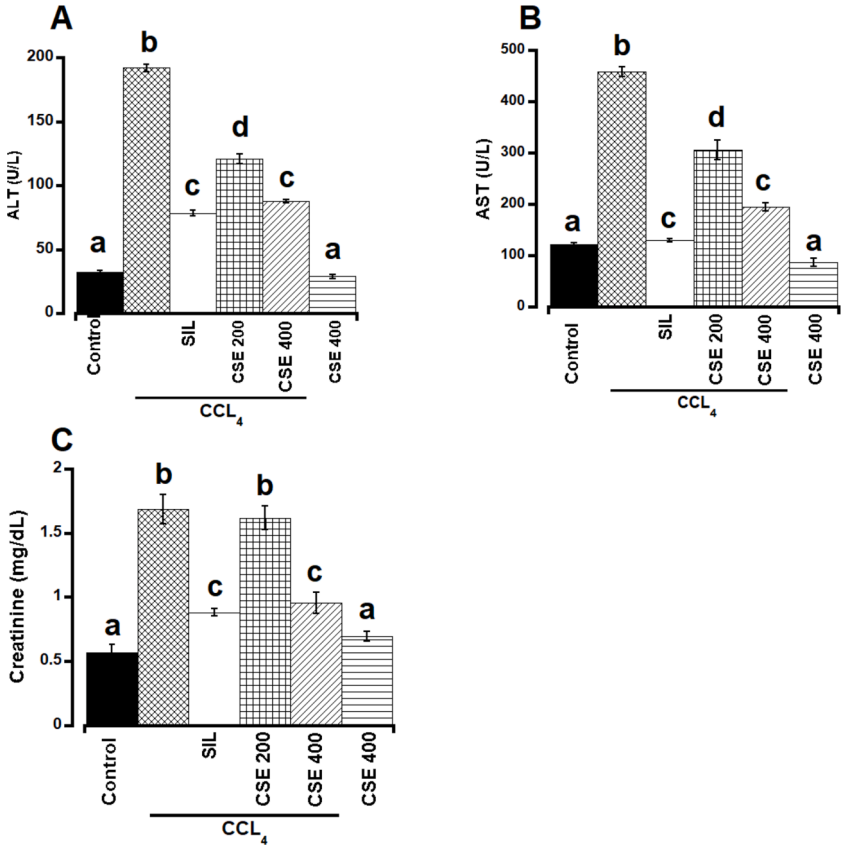

Fig. 1. Effects of CSE on CCl4-induced hepatotoxicity. Different lowercases indicate significant differences among the groups ($p < 0.05$). Values are expressed as mean ± SEM ($n = 6$).

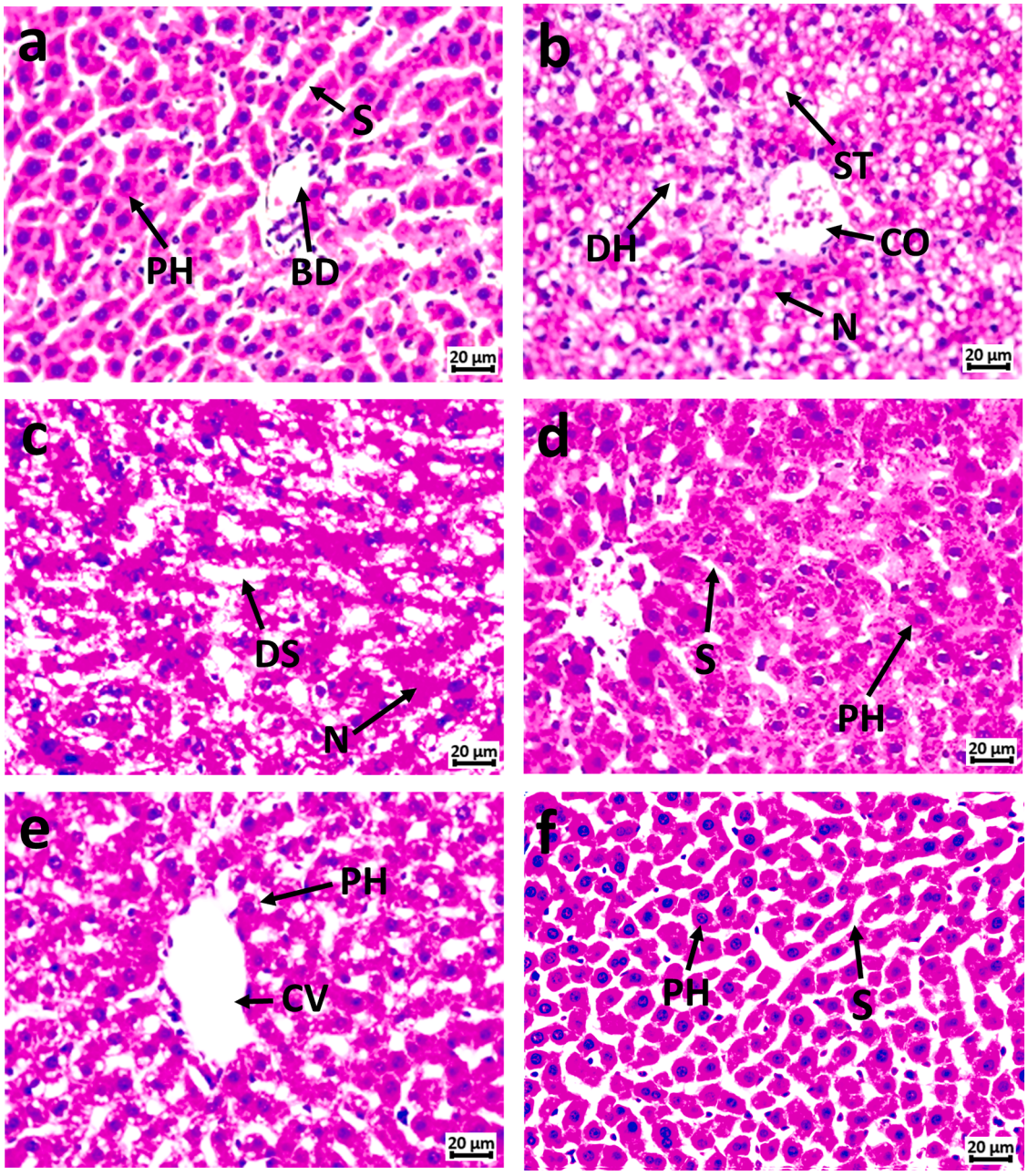

Fig. 2. Effects of CSE on CCl4-induced histopathological changes in rat liver (H&E staining). a: Control; b: CCl4; c: CCl4 + CSE 200; d: CCl4 + CSE 400; e: CCl4 + SIL; f: CSE 400 (magnification = 400×; scale bar = 20 µm). Arrows denote polygonal hepatocytes (PH), sinusoids (S), central vein (CV), bile duct (BD), degenerated hepatocytes (DH), steatosis (ST), dilated sinusoid (DS), necrosis (N), Congested central vein (CO).

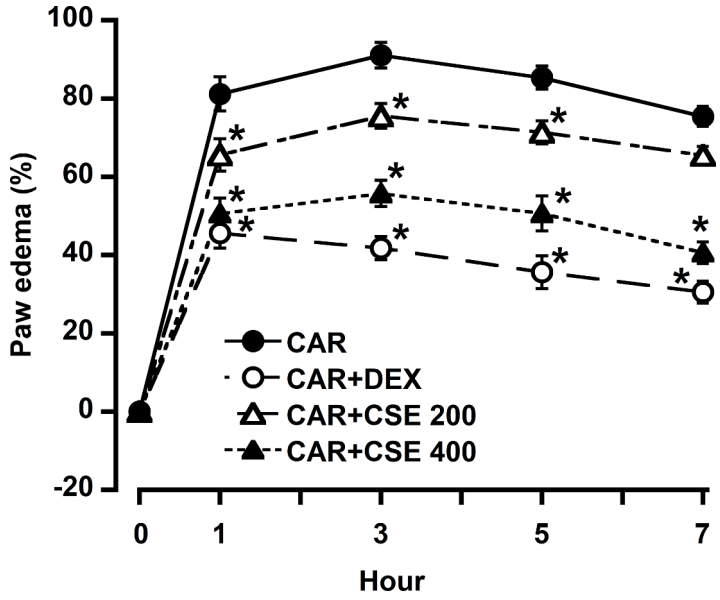

Fig. 3. Time-dependent effects of CSE on inflammatory paw edema (%). Effect of pretreatment with CSE on carrageenan-induced inflammation in rats at $t = 0$, 1, 3, 5, and 7 h. The plotted points represent mean ± SEM ($n = 6$). Asterisks denote the significant variations vs. CAR ($p < 0.05$).

3.4.2. *Assessment of motility*

The motility score was the lowest, 1.17 ± 0.17 in CAR group at 5 h. A dramatic decline in motility was also noticed in CAR + CSE 200 and CAR + CSE 400 until 3 h, at 1.50 ± 0.22 and 2.53 ± 0.41, respectively which were gradually improved at 5 and 7 h. Among the CSE treated groups, the dose of CSE 400 mg/kg scored maximum, 3.83 ± 0.32 in terms of motility at 7 h ($p < 0.001$). The score in CAR+DEX dropped slowly to 3.17 ± 0.17 at 3 h, followed by a gradual increase to 4.17 ± 0.17 at 7 h. The comparative motility level is demonstrated in Fig. 5.

3.4.3. *Histopathological study of paw tissue*

Normal histological architecture of paw tissue was noticed in the control group [Fig. 6a], while the carrageenan control group exhibited massive infiltration of inflammatory cells with disruption of tissue architecture [Fig. 6b]. The pretreatment with CSE showed the reduction of inflammatory response in a dose-dependent mode [Fig. 6c, d]. The higher dose of CSE markedly ameliorated the inflammatory condition of paw tissue as manifested by the significant decrease in the count of inflammatory cells [Fig. 6d], which was found almost as potent as in the case of dexamethasone treatment [Fig. 6e].

4. Discussion

The present study revealed that higher dose of CSE and SIL caused significant increases in the bodyweight gain and FER (%) of rats, which was supported by the findings in the previous studies (Alamri, 2019; Montes Chañi et al., 2018). The higher amount of soluble fiber in chia seed may have altered feed digestion and improved intestinal capacity to absorb nutrients which may have contributing roles in bodyweight gain and better feed utilization (Da Silva et al., 2016). On the other hand, stimulatory effects of silymarin on protein synthesis accelerated the bodyweight gain (Alozhy et al., 2019). Although the rate of bodyweight gain increased initially, a decreasing trend was observed after the completion of second week of treatment with CSE. However, SIL group exhibited a continuous increasing trend in bodyweight gain till the end of experiment. The decreasing rate of bodyweight gain in the CSE treated groups may be linked with the significant reduction of visceral fat deposition because of high content of phenolic compounds and alpha-linolenic (ALA) fatty acids in CSE (Rabail et al., 2022). It has been evidenced that dietary consumption of polyphenols is associated with lowering visceral fat deposition by improving homeostasis of fat and glucose, which is suggestive to be lessening the risks connected with obesity (Ballard et al., 2019).

The extract exerted an ameliorative effect on hematological abnormalities caused by CCl_4 toxicity. There was probable diminution of lipid peroxidation level in RBC membrane because of the effects of CSE, which reduced susceptibility of erythrocytes to hemolysis by membrane stabilization and led to increased RBC count, PCV (%), and Hb (%) (Elkirdasy et al., 2015). Research suggests that antioxidants possess hematopoietic stimulating activity, responsible for production of RBC (Wambi et al., 2008). CSE contains various polyphenolic compounds having antioxidant properties, like rosmarinic acid, kaemferol, myricetin, quercetin, flavonol glycosides, and chlorogenic acid etc. These components might have contained erythropoietin-like properties or contributed to the formation of erythropoietin, resulted in stimulation of the stem cells to produce RBC. Treatment with CSE and SIL significantly lowered the increased level of ESR in CCl_4 group. The higher level of ESR is mainly associated with the inflammatory processes involved in infections or cancerous conditions (Tishkowski & Gupta, 2023). Treatments with the extracts might have reduced the chance of microbial infection or CCl_4-induced inflammation, resulted in lowering ESR. Moreover, increased RBC counts in the treated groups are linked up with the rising viscosity level (Tishkowski & Gupta, 2023), which might have played a role in reducing ESR. Although the total leukocyte count remained unchanged, the relative neutrophil counts were found maximum in the groups treated with higher dose of CSE. In contrast, lymphocyte counts declined in the higher dosed groups as evidenced by the differential leukocyte counts, which might be the effects of cell margination (Ofem et al., 2012). As neutrophils play the vital role in the first line of defense mechanism against invading infectious agents

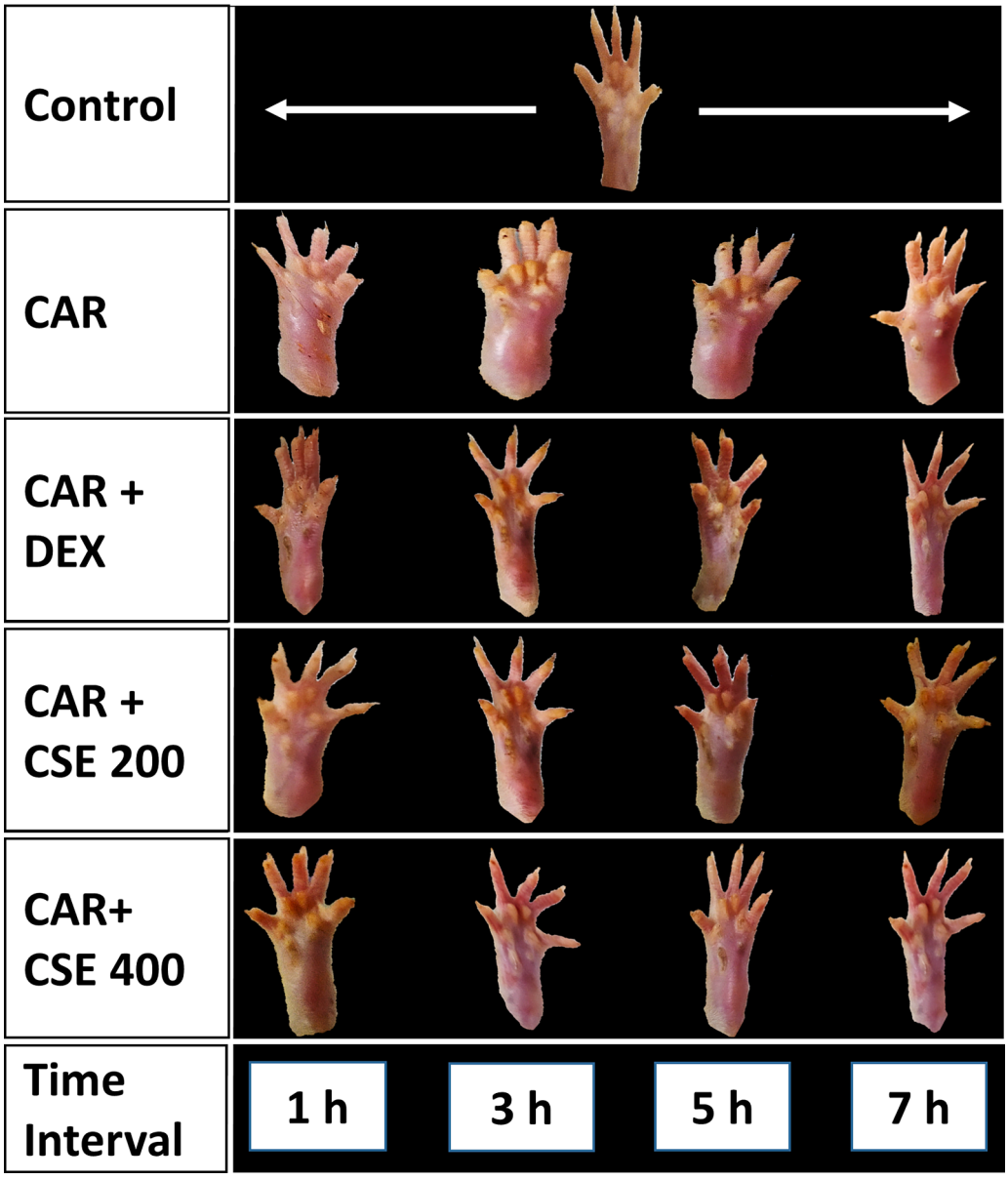

Fig. 4. Photographic representation of comparative effects of CSE at different time intervals.

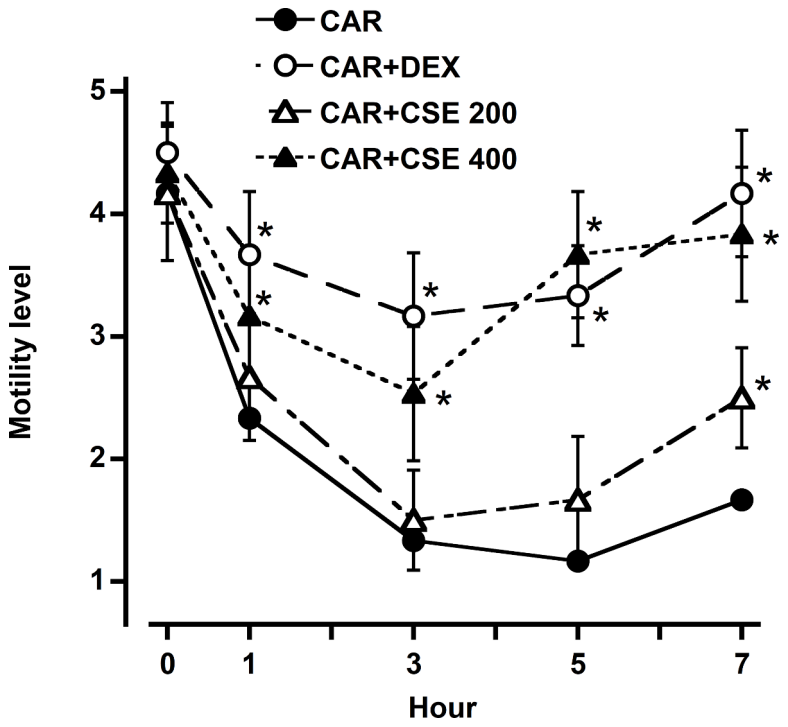

Fig. 5. Effect of CSE on motility level at $t = 0$, 1, 3, 5, and 7 h. Values are plotted as mean ± SEM ($n = 6$). Asterisks denote the significant variations vs. CAR ($p < 0.05$).

(Wishart et al., 2023), their higher counts can be correlated with the potential of CSE in boosting up the immune system.

CCl_4-induced hepatocellular damage has been demonstrated to be reversed successfully by the usage of CSE and SIL. Trichloromethyl and trichloromethyl peroxy free radicals are the highly reactive metabolites, which are generated following CCl_4 biotransformation. These free radicals are accounted for accelerating lipid peroxidation process, ultimately leading to cellular necrosis (Weber et al., 2003). The elevated level of marker enzymes, ALT, and AST in serum is indicative for the loss of structural and functional integrity of hepatocytes (Kanawati et al., 2021). Moreover, there is a positive association of serum creatinine level with the risks of hepatic steatosis as concluded by the previous study (Ma et al., 2022). The increased activities of serum ALT, AST, and creatinine level has been demonstrated in our CCl_4 challenged group, which is the consequence of hepatocyte leakage, suggestive for hepatonecrosis or hepatosteatosis as aligned with the others' findings (Ma et al., 2022). Biochemical alterations were in agreement with the histopathological evidence of CCl_4-induced hepatic injury like degeneration of hepatocytes, steatosis, ballooning of hepatocytes, sinusoidal dilatation, congestion of central vein, and necrosis as evidenced by the previous reports (Hamid et al., 2018). Our investigation showed marked diminution and restoration of biomarker enzymes and serum creatinine to the normal levels in the CSE treated groups in a dose-dependent manner, which was manifested by the progressive amelioration of histological architecture of liver (Hamid et al., 2018). These findings can be attributed to the hepatoprotective role of CSE by stabilizing membrane integrity of hepatocytes (Shah et al., 2017). The defense mechanism of antioxidants, caffeic acid, and chlorogenic acid in chia seeds against lipid peroxidation might be the underlying cause of hepatoprotection. The previous research confirmed that polyphenols in chia seed also possesses properties to alleviate chemical-induced hepatotoxicity (El-Hashash et al., 2020).

Significant attenuation of inflammatory paw edema and improvement in motility level have been demonstrated in the CSE and DEX treated groups. Carrageenan played a role as a chemical inducer for proinflammatory medicators like histamine, leukotrienes, prostaglandins, bradykinin, TNF-α etc. (Amdekar et al., 2012). Histopathological findings of paw tissue corresponded to the gross lesion, where normal histological architecture was observed in the control group. However, carrageenan-induced group revealed degenerated tissue structure along with massive infiltration of inflammatory cells. Pretreatment with CSE exhibited alleviation of the inflammatory condition in a dose-dependent fashion as evidenced by the substantial reduction of inflammatory cell count. Due to the higher content of phenolic compounds, CSE could possess anti-inflammatory and immunomodulatory potentials in consequences of diminutive expression of IL-6, IL-8, and TNF-α following blockade of NF-κB pathway as in compliance with the published outcomes (Lu et al., 2022). Moreover, ALA in chia seed can be converted into eicosapentaenoic acid and docosahexaenoic acid (Calder, 2017), which could lead to suppress 5-lipoxygenase and cyclooxygenase-1-2 to play role in alleviating inflammatory response (Grancieri et al., 2019).

5. Conclusion

The investigation demonstrates that chia seeds possess ameliorative effects on hematological parameters, induced liver damage, and inflammation in rats. Hepatic regeneration is attributed to the potency of the extracts in hepatoprotection as in harmony with the biochemical parameters. Although the extracts have potential to increase the body mass, the reduction aptitude for visceral fat deposition is prominent. Hematological parameters indicate protective effects of chia seed in oxidative stress induced abnormalities. The downregulating effects of chia seed oil on reactive oxygen species and proinflammatory cytokines are hypothesized in suppressing inflammatory response. The present

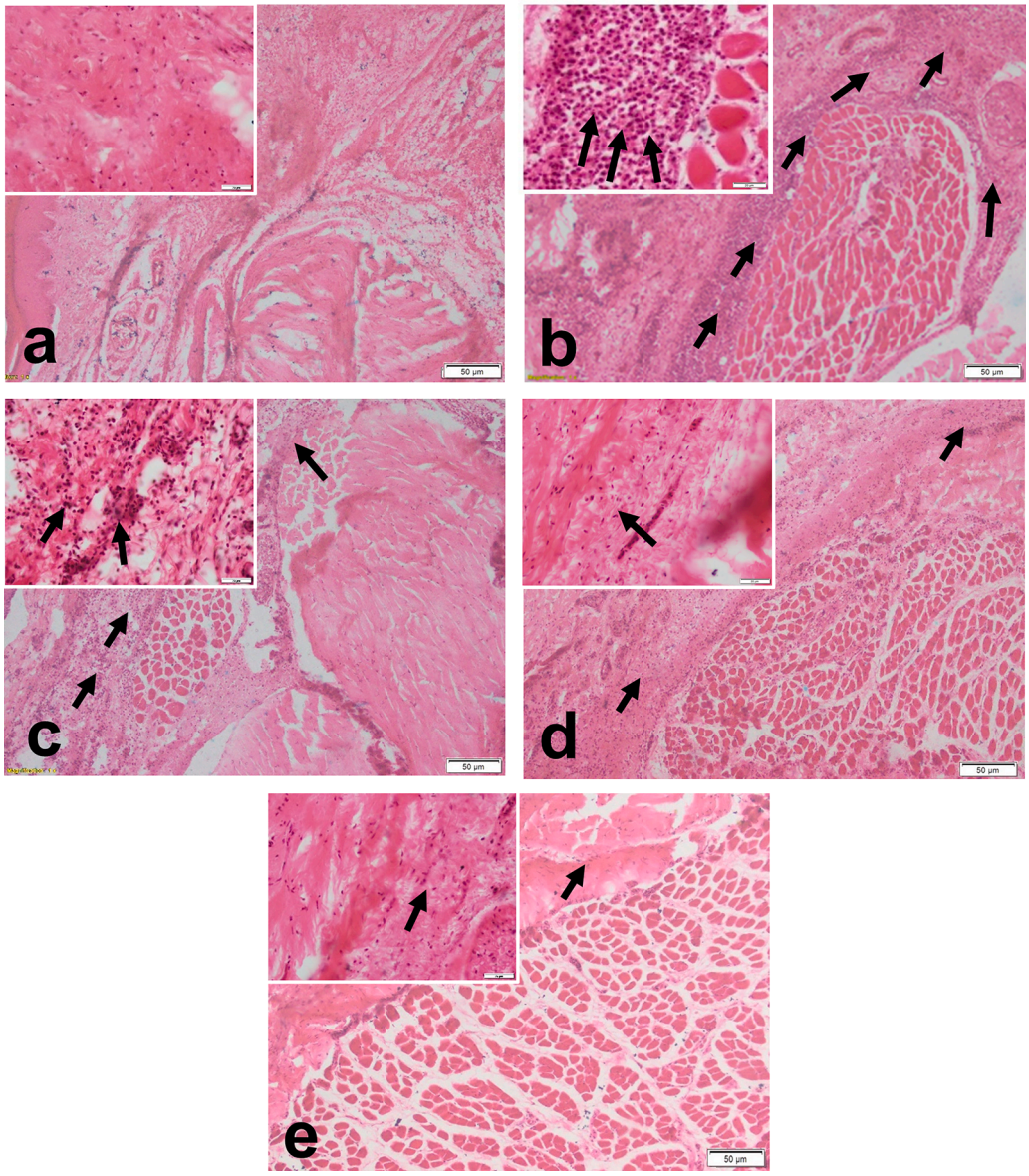

Fig. 6. Paw tissue stained with H & E (original magnification = 100× and scale bar = 50 µm; Insets = 400× and scale bar = 20 µm). (a) Negative control; (b) CAR control; (c) CAR + CSE 200; (d) CAR + CSE 400; (e) CAR + DEX (n = 6 per group), Number of arrows denotes the comparative density of infiltrated inflammatory cells.

study recommends that 400 mg/kg BW/day is the most effective concentration of chia seed extract for the significant alleviation of pathophysiological parameters.

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Ethical statement

The study was performed in conformance with the code of ethics and standards set for the handling and management of laboratory animals with proper addressing the animal welfare issue. The methodology of the experiment was critically assessed and endorsed by the Animal Welfare and Experimental Ethics Committee, Bangladesh Agricultural University [Approval No.: AWEEC/BAU/2021 (62); Date: 23.12.2021].

CRediT authorship contribution statement

Sabbya Sachi: Writing – review & editing, Writing – original draft, Visualization, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Mst. Prianka Jahan:** Writing – review & editing, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Purba Islam:** Writing – review & editing, Supervision. **Kazi Rafiq:** Writing – review & editing, Supervision. **Md. Zahorul Islam:** Writing – review & editing, Visualization, Supervision, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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