Salvia hispanica L. (chia seeds) alleviates paracetamol-induced acute liver injury in mice by modulating oxidative stress and inflammation

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Received: 29 October 2023
Revised: 6 January 2024
Accepted: 6 January 2024
Published: 24 February 2024

Egyptian Pharmaceutical Journal 2024, 0:0–0

Background
Paracetamol [N-acetyl-p-aminophenol (APAP)] is one of the frequently prescribed antipyretics and analgesics; yet going over the recommended dose still poses a major clinical challenge and leads to serious drug-encouraged liver damage.
Objective
Our study aims to discover the hepatoprotective effect of *Salvia hispanica* L. [chia seeds (CS)] against APAP-induced acute liver injury in male mice.
Materials and methods
Paracetamol (300 mg/kg bw, once a day for two successive days) was orally administered to establish a liver injury model. Forty male albino mice were randomly divided into four groups (10/group); control, APAP group, CS-4% +APAP group: was pretreated with CS (4%) for 21 days before receiving APAP, CS-20%+APAP group: was pretreated with CS (20%) for 21 days before receiving APAP. At the end of the experiment, the levels of liver injury indices, hepatic nitro-oxidative stress, and inflammatory-associated biomarkers along with histopathological examinations were determined. Additionally, inflammatory responses of some primer sequences (nuclear factor kappa B, p38 mitogen-activated protein kinases, monocyte chemoattractant protein-1, and toll-like receptor 4) were determined by quantitative real-time PCR in liver tissues.
Results
CS markedly stabilized the APAP-motivated alterations in liver function markers, cytochrome P450 2E1 level, hepatic nitro-oxidative stress, and pathological changes. The anti-inflammatory activity of CS improved tumor necrosis factor-alpha and myeloperoxidase production. Furthermore, mRNA expression of nuclear factor kappa B, monocyte chemoattractant protein-1, p38 mitogen-activated protein kinases, and toll-like receptor 4 were significantly downregulated. Such effects were found to be responsible for its hepatoprotective effect in a dose-dependent way.
Conclusion
Our results showed evidence that the hepatoprotective effect of CS against APAP-induced liver injury was mediated through the reduction of oxidative stress damage, enhancement of antioxidant status, and inhibition of different inflammatory markers.

Keywords:
paracetamol, liver, chia seeds, oxidative stress, inflammation, mice

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1687-4315

Abbreviation: APAP, N-acetyl-p-aminophenol; CS, chia seeds; CYP2E1, cytochrome P450 2E1; MCP-1, monocyte chemoattractant protein-1; MPO, myeloperoxidase; NF-kB, nuclear factor kappa B; p38MAPK, p38 mitogen-activated protein kinases; TLR4, toll-like receptor 4; TNF-α, tumor necrosis factor-alpha.

Introduction

Acute liver failure (ALF) is a potentially critical problem of severe hepatic sickness resulting from viral hepatitis or drug use [1]. Paracetamol [acetaminophen or N-acetyl-p-aminophenol (APAP)] is considered one of the most important causes for drug-encouraged ALF. Also, it has been widely studied for its liver injury [2]. When administered at curative doses, the majority of APAP is transferred into nontoxic compounds which are secreted in the urine. Only a very small portion is excreted unchanged in the urine. The residual APAP, about 5–9% is metabolized by the cytochrome P450 enzymes (CYPs), mostly cytochrome P450 2E1 (CYP2E1), into the highly reactive intermediate metabolite N-acetyl-p-benzoquinone imine (NAPQI) [3]. Generally,

NAPQI is quickly detoxified by conjugating with glutathione (GSH). On the other hand, when metabolizing enzymes are saturated after APAP overdose, excessive NAPQI depletes GSH, resulting in the covalent binding of sulfhydryl groups in cellular proteins, especially mitochondrial proteins, which leads to the pathogenesis of ALF [4,5].

Although N-acetyl cysteine is considered the only permitted antinode for APAP poisoning, its side effects and high dose requirement, along with the extended duration of treatment that may counteract liver regeneration, have led to a search for alternative agents that are safer and more efficient in treatment of APAP toxicity [6].

Bioactive substances of natural origin have been demonstrated to protect the development of diseases related to reactive oxygen species (ROS) [7]. Accordingly, developing new efficacious complementary natural products with antioxidative and anti-inflammatory properties becomes an advanced strategy in APAP-induced acute liver injury prevention [8].

Chia (*Salvia hispanica* L.) is a herbaceous plant native to southern Mexico and northern Guatemala. It belongs to the mint family (Lamiaceae) [9]. In recent years, chia seeds (CS) have received more consideration and were included in human diets due to their massive nutritional and medicinal potential [10,11]. CS is a plentiful source of protein, dietary fiber, vitamins, minerals (such as calcium), carbohydrates, unsaturated fatty acids (mainly omega-3 and omega-6 PUFAs), and pigments [12]. Additionally, CS contains many bioactive substances with high antioxidative activity, such as phytosterols, α-tocopherol, and polyphenolic compounds. These compounds include but are not limited to rosmarinic acid, caffeic acid, gallic acid, ferulic acid, cinamic acid, quercetin, kaempferol, myricetin, flavonol glycosides, and chlorogenic acid [13]. These mentioned compounds has documented antioxidant effects which guards against the oxidative stress that represent a cornstone in initiating drug-stimulated hepatotoxicity [14].

The published data about the possible protective effects of CS on liver insult is slim.

Following the previous findings and as a part of our goal to find effective natural materials for protection against APAP intoxication, we need to investigate the hepatoprotective effect of dietary CS intake against APAP-motivated acute liver injury in mice.

Materials and methods

Animals

The present experimental study was carried out on the male albino mice.

Animals were obtained from The National Cancer Institute of Cairo University. Mice were kept in wire mesh cages under a regulated environment (25±2°C temperature, 55±5% relative humidity, and 12 : 12 h light/dark cycle), and they were given food and water supply *ad libitum*. Unnecessary disturbance of animals was avoided. Animals were treated kindly; squeezing, pressure, and tough maneuvers were avoided. Commercially available pellet diet and CS were grinded, and either 4 g of milled CS was mixed with 96 g of milled pellets to prepare 4%, w/w or 20 g of milled CS was mixed 80 g of milled pellets to prepare 20% w/w. Next, 100 ml of distilled water was added to blend the mixture, then dried in an oven at 50°C for 24 h and kept in a dusky dry stoppered glass container for weekly use [15]. Diet was offered to mice in specific food cups to avoid any loss in diet. All animal procedures were carried out in compliance with the rules established by Institutional Animal Care and according to the guidelines for the handling and use of laboratory animals. All procedures were carried out according to the research ethics committee for experimental studies at the National Organization for Drug Control and Research NODCAR/I/27/2021 in November 2021.

Chemicals

Paracetamol was purchased from Sigma-Aldrich (Saint Louis, Missouri, USA); CS were purchased from Imtenan Health Shop (Cairo, Egypt). Other chemicals and analytical grade reagents were obtained from El-Gomhoria Company, Cairo, Egypt.

Induction of experimental liver injury

Mice were fasted for 12 h before paracetamol administration and given free access to water to generate a similar condition for paracetamol metabolism [16]. Following the fasting period, liver injury was induced in healthy male albino mice by oral administration of APAP (300 mg/kg, once a day, for two successive days) dissolved in 0.9% sodium chloride at 30°C in water bath [17].

Experimental design

Following 1 week of acclimatization, mice were divided into four groups (10 animals each) as per the following:

Group 1 (control): mice were fed a commercial pellet diet and administered a daily oral dose of 0.9% sodium chloride (1 ml/kg b.w.) for 23 days.

Group 2 (APAP group): mice were fed a commercial pellet diet and administered a daily oral dose of 0.9% NaCl (1 ml/kg b.w.) for 21 days then administered paracetamol orally at a dose of 300 mg/kg b.w./day for another two successive days and served as a positive control [17].

Group 3 (CS-4%+APAP): mice were fed CS containing food pellets daily at a dose of 4% w/w [18] for 21 days then administered paracetamol orally at a dose of 300 mg/kg b.w./day for another two successive days.

Group 4 (CS-20%+APAP): mice were fed on CS containing food pellets daily at a dose of 20% w/w [19] for 21 days, then administered paracetamol orally at a dose of 300 mg/kg b.w./day for another two successive days.

At the end of the experimental period, after 24 h of paracetamol administration, blood samples were collected from retro-orbital venous plexus and left at 37°C for 20 min. The coagulated blood was centrifuged at 3000 rpm for 20 min to separate serum and stored at −20°C for further biochemical analyses such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, total cholesterol (TC) and albumin]. Afterwards, mice of all groups were euthanized by decapitation, and the liver of different groups were excised and washed with cold isotonic saline. Part of the liver tissues were weighed and homogenized in ice-cold phosphate-buffered saline (10% homogenate) for evaluation of nitro-oxidative stress biomarkers as nitric oxide (NO), malondialdehyde (MDA), reduced GSH contents, and superoxide dismutase (SOD) activity, inflammatory markers as myeloperoxidase (MPO) activity, tumor necrosis factor-alpha (TNF-α) level and CYP2E1 level. One lobe of liver was fixed in 10% (v/v) formalin for histopathological examinations, while the last part was stored at −80°C for further biochemical analysis.

Biochemical analysis

Assessment of serum hepatotoxicity indices

The activities of ALT, AST, and ALP, as well as the levels of TC, total protein, and albumin were determined through using commercial diagnostic kits obtained from Biodiagnostic Inc. (Giza, Egypt) as instructed by the manufacturer.

Evaluation of hepatic nitro-oxidative stress biomarkers

MDA level was assessed by recruiting the method of Uchiyama and Mihara [20]. GSH content was prepared according to the method illustrated by Beutler *et al.* [21]. SOD activity was determined according to the procedure of Nandi and Chatterjee [22]. NO level was determined according to Miranda *et al.* [23].

Evaluation of hepatic myeloperoxidase activity and tumor necrosis factor-alpha, cytochrome P450 2E1 levels

MPO activity was evaluated according to the method of Bradley *et al.* [24]. The levels of TNF-α and CYP2E1 were quantitatively detected in liver tissue by using mouse enzyme-linked immunoassays kits (TNF-α; Abbexa, UK and CYP2E1, MyBiosource Inc., San Diego, California, USA), respectively, as instructed by the manufacturer.

Quantitative real-time PCR for the mRNA expression levels of nuclear factor kappa B, p38 mitogen-activated protein kinases, monocyte chemoattractant protein-1, and toll-like receptor 4 in hepatic tissues

Using TRIzol reagent (Invitrogen, Sigma-Aldrich) and following the manufacturer's protocols, total RNA was extracted from hepatic tissue. The conversion cDNA reverse transcription kit (Clontech Laboratories, Takara Bio Inc., Shiga, Tokyo, Japan) was utilized for cDNA synthesis as per the manufacturer's instructions. Real-time quantitative reverse transcription PCR(RT-PCR) was executed using SYBR Green PCR Master Mix (QIAGEN, Venlo, The Netherlands) in reaction volume of 25 µl with the subsequent thermal cycling conditions: 50°C (reverse transcription) for 5 min, 95°C for 10 s, then 40 cycles of 95°C for 15 s, and 60°C for 30 s. Primer pair sequences for each gene are displayed in Table 1. For calculating the results from real-time assays, Applied Biosystems Software (Foster City, California, USA) was applied. Using the comparative threshold cycle method (Ct), the relative quantification of mRNA expression was determined. Glyceraldehyde 3-phospate dehydrogenase mRNA expression was used as invariant endogenous control (reference gene) for normalizing all data [25].

Histopathological evaluation

Prefixed liver tissues (48 h in 10% buffered formalin) were cleaned and dehydrated in escalating grades of alcohol, clarified in xylene, processed into paraffin blocks, sectioned (5 µm thick), and stained with hematoxylin and eosin. Histopathological lesions were examined through the light microscope according to the method described by Bancroft and Layton [26].

Statistical analysis

Statistical analysis was calculated using one-way analysis of variance, followed by the Tukey–Kramer

Table 1 Primers sequence for QRT-PCR analysis

Gene primer sequence
NF-κB forward 5'-CCTCTGGCGAATGGCTTTAC-3'reverse 5'-GCTATGGATACTGCGGTCTGG-3'
p38MAPK forward 5'-CGAAATGACCGGCTACGTGG-3'reverse 5'-CACTTCATCGTAGGTCAGGC-3'
MCP-1 forward 5'-TGCTGTCTCAGCCAGATGCAGTTA-3'reverse 5'-AGAAGTGCTTGAGGTGGTTGTGGA-3'
TLR4 forward 5'-CACTGTTCTTCTCCTGCCTGAC-3'reverse 5'-TGGTTGAAGAAGGAATGTCATC-3'
GAPDH forward 5'-ACCACAGTCCATGCCATCAC-3'reverse 5'-GTCCTCAGTGTAGCCCAGGA-3'

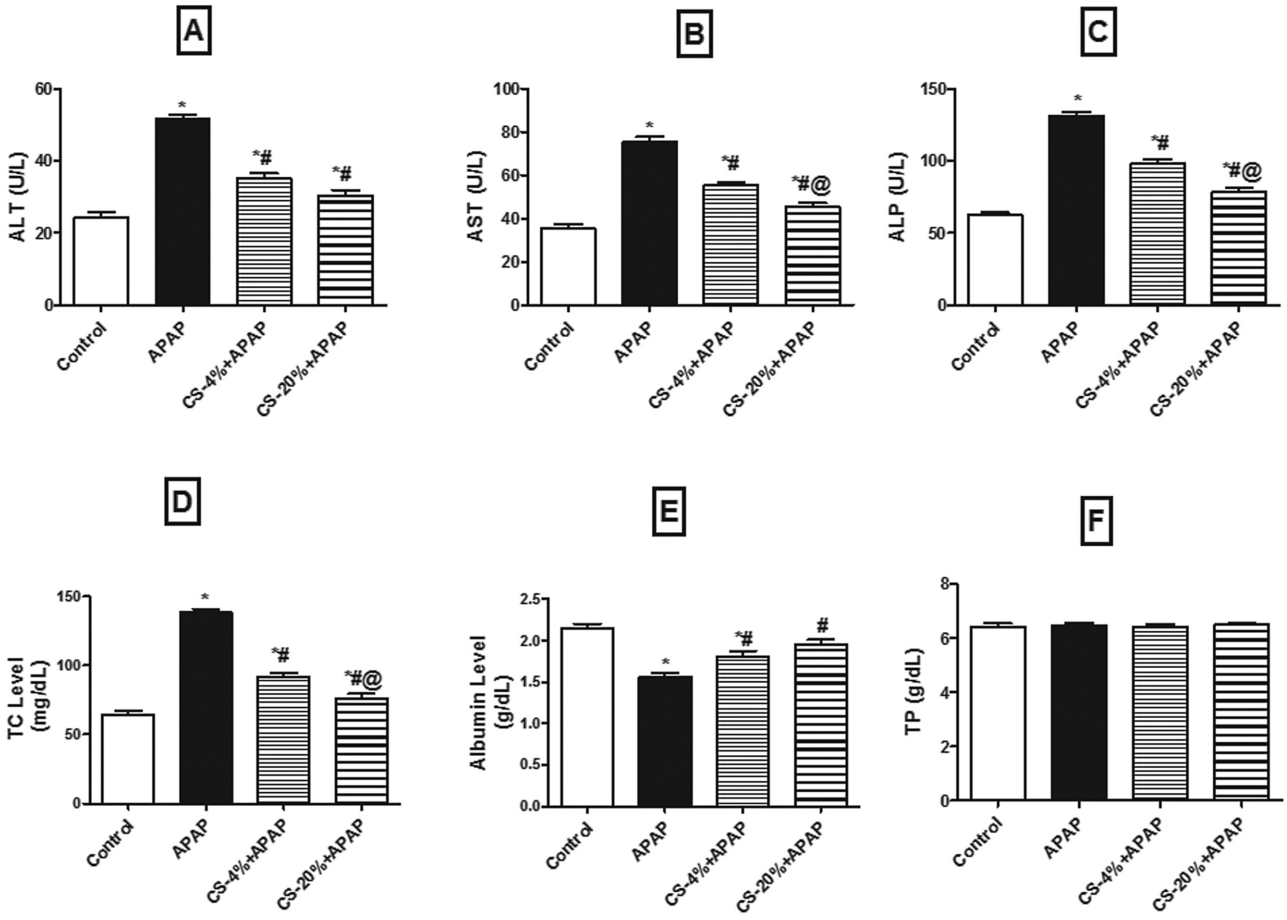
multiple comparisons test. Values of *P* less than 0.05 were considered significant. GraphPad PRISM program was used for statistical analysis (version 5, San Diego, California, USA). All experimental results were represented as mean±SEM.

Results

The protective effects of chia seeds intake on hepatic markers

Figure 1 depicts the hepatic plasma markers: APAP-induced liver injury in mice manifested by a pronounced elevation in serum ALT, AST, ALP, and TC by 114, 113, 110, and 115%, respectively, along with a reduction in albumin level by 27% as compared to the control group. In contrast, pretreatment with CS at a dose of 4 or 20% significantly attenuated APAP-induced alterations in liver function markers as revealed through the reduction of ALT by 32 and 41%, AST by 27 and 40%, ALP by 25 and 40%, TC by 35 and 45%, respectively, in addition to a significant elevation in albumin level by 16 and 25%, respectively, as compared to APAP-treated group.

Figure 1



Effect of CS at doses 4% and 20% on serum levels of (a) ALT, (b) AST, (c) ALP, (d) TC, (e) albumin, and (f) TP in mice. Results are represented as mean±SEM; *n*=6; statistical analysis was carried out by one-way ANOVA test followed by Tukey post-hoc test. *Significantly different from the control group at *P* value less than 0.05. #Significantly different from APAP-treated group at *P* value less than 0.05. @Significantly different from (CS-4%+APAP)-treated group at *P* value less than 0.05. ALP, alkaline phosphatase; ALT, alanine aminotransferase; ANOVA, analysis of variance; APAP, N-acetyl-p-aminophenol; AST, aspartate aminotransferase; CS, chia seeds; TC, total cholesterol; TP, total protein.

The antioxidant effects of chia seeds intake in N-acetyl-p-aminophenol-toxicated mice

As illustrated in Fig. 2, mice challenged with APAP triggers a pronounced elevation in oxidative stress evidenced by the decline in the level of endogenous antioxidants (SOD and GSH by 19 and 29%, respectively), concomitant with an elevation in hepatic MDA and NO levels by 129 and 86%, respectively, compared to the control group. On the contrary, pretreatment with 4 or 20% CS efficiently elevated SOD activity by 86 and 126%, respectively, and GSH content by 9 and 29%, respectively, associated with a marked decrease in MDA level by 40 and 52%, respectively, and NO level by 21 and 35%, respectively, as compared to APAP-treated group.

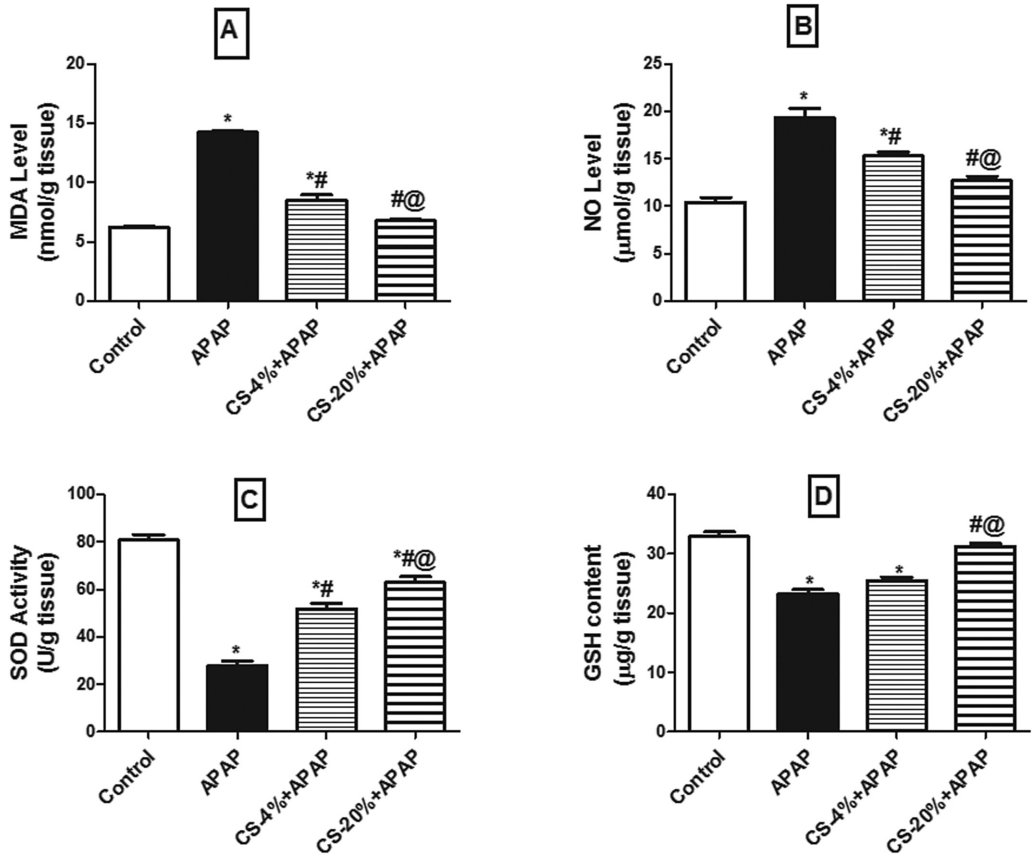
Effect of chia seeds intake on hepatic myeloperoxidase activity, tumor necrosis factor-alpha, and cytochrome P450 2E1 levels in N-acetyl-p-aminophenol-toxicated mice

As illustrated in Fig. 3, the APAP-treated group exhibited a profound elevation in hepatic MPO activity and TNF-α, CYP2E1 levels by 3.8, 4.6, and 2.3 folds, respectively, as compared to the control group. Meanwhile, pretreatment with 4 and 20% CS resulted in a considerable reduction in MPO activity by 41 and 60%, respectively, TNF-α level by 50 and 67%, respectively, and in CYP2E1 level by 41 and 55%, respectively, as compared to APAP-treated group.

Effects of chia seeds intake on mRNA expression levels of hepatic nuclear factor kappa B, p38 mitogen-activated protein kinases, monocyte chemoattractant protein-1, and toll-like receptor 4 in mice

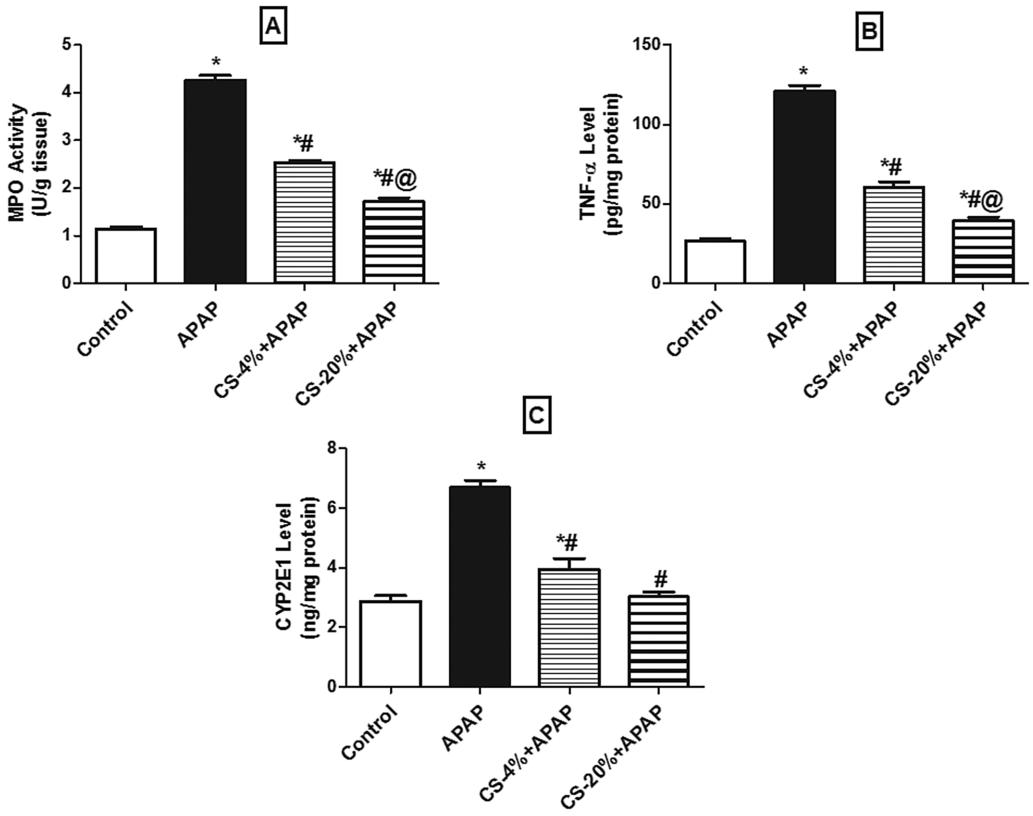
As shown in Fig. 4, APAP-intoxication caused a marked increase in the inflammation-associated factors as evidenced by the significant ($P<0.05$) elevation in the mRNA expression levels of nuclear factor kappa B (NF-kB), p38 mitogen-activated protein kinases (p38MAPK), monocyte chemoattractant protein-1 (MCP-1), and toll-like receptor 4 (TLR4) by 5.7, 7.7, 2.9, and 7.6 folds, respectively, as compared to control group. Meanwhile, prefeeding with CS at a dose of 4 or

Figure 2



Effect of CS at doses of 4% and 20% on the antioxidant levels of (a) MDA, (b) NO, (c) SOD activity, (d) GSH content in APAP-induced liver injury in mice. Results are represented as mean±SEM; $n=6$. Statistical analysis was carried out using one-way ANOVA test followed by Tukey post-hoc test. *Significantly different from the control group at P value less than 0.05. #Significantly different from the APAP-treated group at P value less than 0.05. @Significantly different from (CS-4%+APAP)-treated group at P value less than 0.05. APAP, N-acetyl-p-aminophenol; ANOVA, analysis of variance; CS, chia seeds; GSH, glutathione; MDA, malondialdehyde; NO, nitric oxide; SOD, superoxide dismutase.

Figure 3



Effect of CS at doses of 4% and 20% on (a) MPO activity, (b) TNF-α, and (c) CYP2E1 levels in mice. Results are represented as mean±SEM; *n*=6. Statistical analysis was carried out using one-way ANOVA test followed by the Tukey post-hoc test. *Significantly different from the control group at *P* value less than 0.05. #Significantly different from the APAP-treated group at *P* value less than 0.05. @Significantly different from (CS-4%+APAP)-treated group at *P* value less than 0.05. APAP, N-acetyl-p-aminophenol; ANOVA, analysis of variance; CS, chia seeds; CYP2E1, cytochrome P450 2E1; MPO, myeloperoxidase; TNF-α, tumor necrosis factor-alpha.

20% significantly ($P<0.05$) decreased mRNA expression levels of NF-kB by 45.4% (0.55 fold) and 66.6% (0.33 fold), p38MAPK by 37.4% (0.63 fold) and 63.7% (0.36 fold), MCP-1 by 34.5% (0.66 fold) and 54.9% (0.45 fold), TLR4 by 37% (0.63 fold) and 64% (0.36 fold), respectively, as compared to the APAP-treated group. Notably, CS pretreatment of the APAP-challenged group at a dose of 20% nearly showed comparable results to the control group.

Histopathological examination

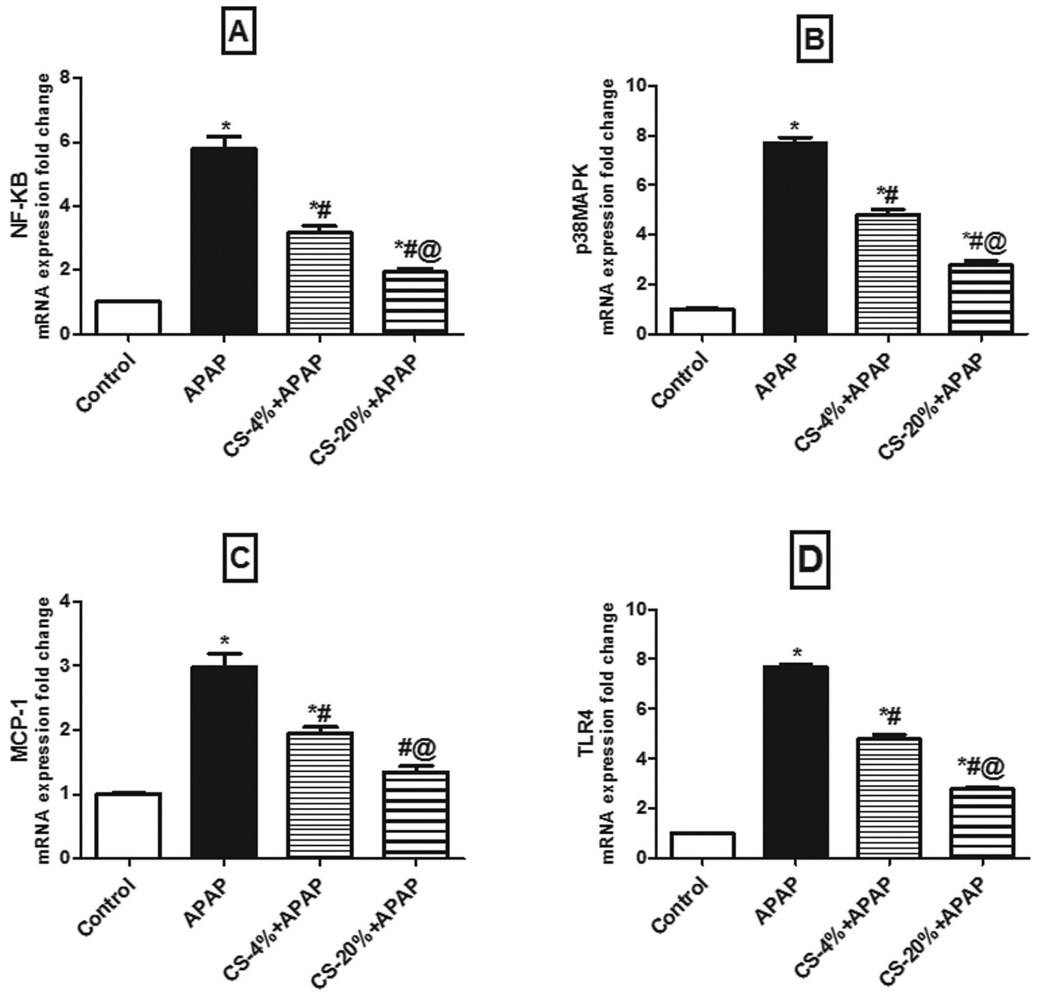
As illustrated in Fig. 5, the photomicrographs of the liver tissue sections stained with hematoxylin and eosin supported by histopathology scoring in Table 2: the control group displayed conserved lobular architecture. In addition, the APAP-intoxicated group showed marked congestion, pyknosis, necrosis, steatosis, and lymphatic infiltration compared to other groups, whereas groups pretreated with CS-4% showed a recognized decrease in the previous morphological changes. The CS-20% group showed a better amelioration in liver histology.

Discussion

Oxidative stress is implicated in the pathogenesis of several diseases. In addition, it plays a pronounced role in the damaging effect attributed to paracetamol hepatotoxicity involving CYP system.

Consequently, supplementation of exogenous antioxidants might normalize redox status throughout oxidative stress. So, this work was designed to investigate the ability of CS pretreatment to counteract APAP-induced hepatotoxicity in mice. Our results demonstrated a marked increase in the levels of ALT, AST, ALP, and TC, along with a considerable decrease in albumin levels in the APAP-intoxicated group compared to the control group. Additionally, the antioxidant status of the cell as SOD activity and GSH content were significantly decreased while the levels of CYP2E1, MDA, NO, MPO, TNF-α were significantly elevated associated with upregulation of NF-kB, p38MAPK, MCP-1, and TLR4 mRNA expression in APAP-treated group compared to the control group. Our

Figure 4

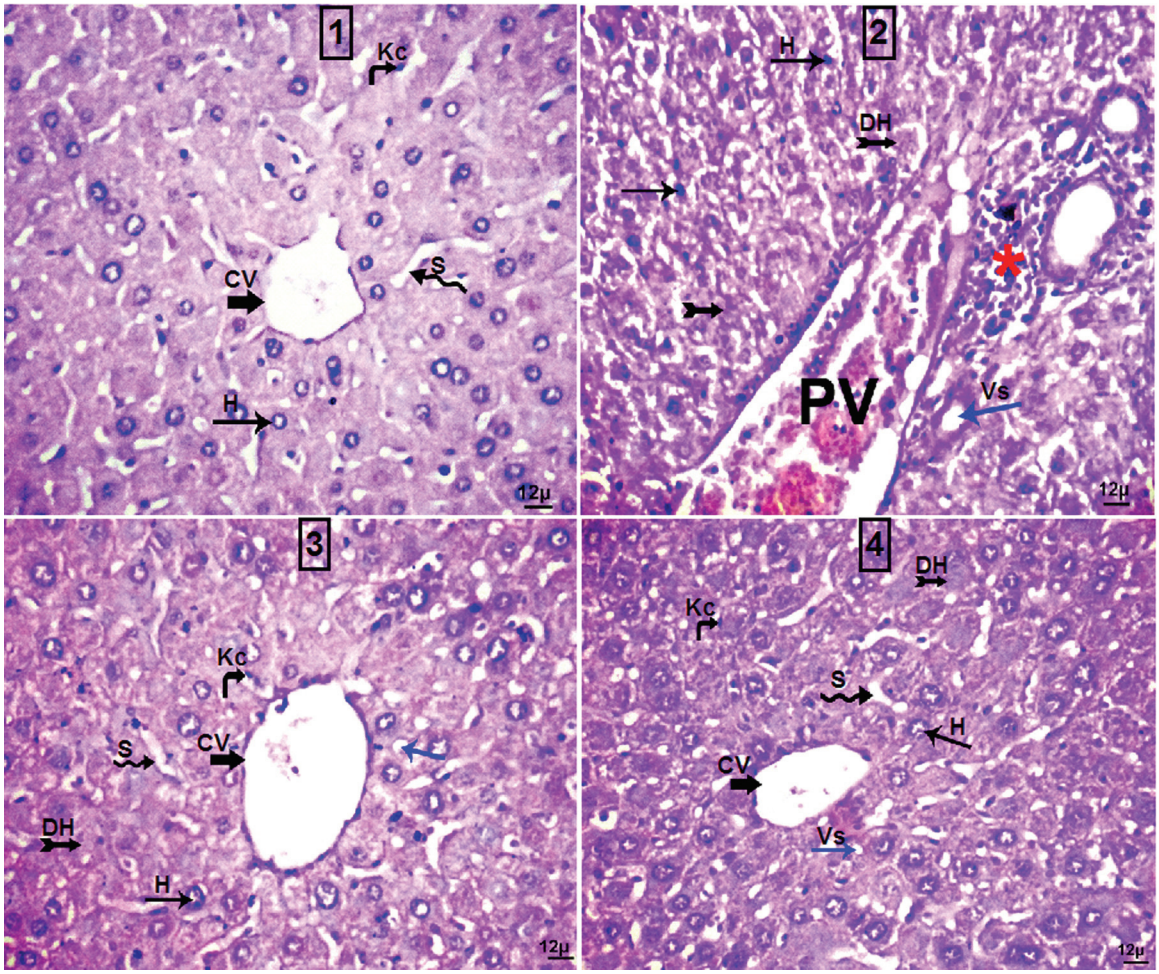


Effect of CS at doses of 4% and 20% on mRNA expression of (a) NF-kB, (b) p38 MAPK, (c) MCP-1, and (d) TLR4 in APAP-induced liver injury in mice. The levels of mRNA in the liver were analyzed by QRT-PCR assay. GAPDH was used as an invariant internal control for calculating mRNA fold changes. Results are represented as mean±SEM; n=5. Statistical analysis was carried out using one-way ANOVA test followed by the Tukey post-hoc test. *Significantly different from the control group at P value less than 0.05. #Significantly different from the APAP-treated group at P value less than 0.05. @Significantly different from (CS-4%+APAP)-treated group at P value less than 0.05. APAP, N-acetyl-p-aminophenol; ANOVA, analysis of variance; CS, chia seeds; GAPDH, glyceraldehyde 3-phospate dehydrogenase; MCP-1, monocyte chemoattractant protein-1; NF-kB, nuclear factor kappa B; TLR4, toll-like receptor 4.

results are in line with earlier studies reported that APAP overdose induced increasing in hepatic MDA level and decreasing in hepatic SOD activity and GSH content [27–30]. Our results show that APAP overdose results in a significant raise in AST, ALT, ALP, and TC levels, along with a significant decline in the level of albumin, suggesting the success of establishing a liver injury model causing cellular infiltration and loss of functional integrity, these findings match with previous studies [28,29]. Conversely CS pretreatment causes a marked ameliorative effect on these liver biomarkers levels compared to the APAP-treated group, indicating its hepatoprotective effect. These results are in accordance with Helal *et al.* [31] who reported that feeding CS powder decreased liver enzymes (ALT, AST, and ALP) and TC in hypercholesterolemic rats. These effects may be due to the plentiful content of potent natural antioxidants especially omega-3 PUFAs and polyphenolic compounds that have a preserving effect on the integrity of the hepatocytes [32,33].

An increase in CYP2E1 level was observed after APAP intoxication in several studies, which is well known as a potent inducer for ROS and has a major effect on acute hepatotoxicity [30,34], which is comparably proved by our results. Interestingly CS pretreatment exerts an important role in mitigating the early injury of APAP hepatotoxicity through the decline of CYP2E1 level, which is the embodiment of CS prohibiting APAP hepatotoxicity from the source [35]. This effect of CS may be attributed to its bioactive component of polyphenolic compounds especially ferulic acid that has a direct effect on inhibiting CYP2E1 [36].

Figure 5



Representative histological micrographs of mice liver tissue sections stained with hematoxylin and eosin (×100). (1) The control group showed no histological alterations. (2) The APAP-treated group showed lymphatic infiltration, vacuole steatosis, pyknoysed hepatocyte, and degenerated hepatocyte. (3) CS-4% +APAP group showed moderate cell degeneration, better morphology of sinusoid and hepatocyte. (4) CS-20% +APAP group showed no sign of steatosis or lymphatic infiltration, normal portal component, and hepatocyte (scale bar=12 µm) [where bold arrow: central vein (CV); thin arrow: hepatocyte (H); wavy arrow: sinusoid (S); curved arrow: Kupffer cell (Kc); blue arrow: vacuole steatosis (Vs); red asterisk*: lymphatic infiltration; and double head arrow: degenerated hepatocyte (DH)]. APAP, N-acetyl-p-aminophenol; CS, chia seeds.

As was mentioned earlier, excessive intake of APAP and the abnormal transformation to NAPQI by the action of CYP2E1 causes GSH depletion in the cytoplasm and mitochondria, which has immense pressure on the formation of ROS [37]. ROS accumulation triggers lipid peroxidation, reduces detoxifying enzymes, and damages cellular redox homeostasis as increased MDA as well as NO levels, and decreased SOD activity, causing further liver tissue injury [29,38,39]; this can lead to the leakage of liver enzymes into the serum of APAP-intoxicated animals [40]. These results are markedly reversed by CS treatment, highlighting the antioxidative effects of CS that may be attributed to the free radical scavenging activity and augmentation of the antioxidant status of its bioactive component omega-3 PUFAs [35,41].

ROS can initiate an inflammatory response by activating NF-kB, which is transported to the nucleus and induces the expression of target inflammatory genes, especially proinflammatory

Table 2 Histopathology scoring

	Congestion	Lymphatic infiltration	Necrosis	Degeneration (pyknosis, karyolysis)	Steatosis
Control	–	–	–	–	–
Paracetamol	++++	++++	++++	++++	++
4% CS	+++	++	++	++	–
20% CS	++	+	+	+	–

CS, chia seeds; –, normal; +, mild; ++, moderate; +++, severe; ++++, extremely severe.

cytokines such as TNF-α and inflammatory mediators such as iNOS [16,34]. NAPQ1 overload leads to the mobilization of monocytes and neutrophils to the liver's damaged area generates more proinflammatory chemokines as MCP-1 and cytokines as TNF-α. In turn, TNF-α can exacerbate the inflammatory response by inducing further NF-kB nuclear translocation and activation, elevation neutrophil accumulation, and induction of iNOS expression, resulting in the pathogenesis of liver injury caused by APAP [42,43]. Our results demonstrated that CS pretreatment decreased levels of MPO, NF-kB, TNF-α, and MCP-1 in APAP-intoxicated mice, confirming its hepatoprotective effect through suppression of NF-kB pathway [44,45]. This could be attributed to its high content of documented anti-inflammatory components such as omega-3 PUFAs [12,46].

NF-kB activation is also controlled by various cellular kinases, particularly the MAPKs family, which is involved in the control of gene expression linked to inflammation, cell propagation, and cell death in APAP-induced hepatotoxicity [47]. Oxidative stress and ROS accumulation activates p38MAPK which in turn stimulates apoptosis and promotes cytokines synthesis [48]. Accumulating evidence suggests that p38MAPK plays an integral role in controlling the expression of proinflammatory cytokines and inflammatory mediators by regulating the activation and canonical signaling of NF-kB in APAP-intoxicated livers [49]. Subsequently, the activation of MAPKs is another crucial molecular mechanism to assess the inflammatory responses. Our results demonstrated that up-regulation of p38MAPK mRNA expression induced by APAP was inhibited by CS pretreatment, suggesting that the inhibition of p38 MAPK by CS may contribute to CS-mediated suppression of the NF-kB pathway.

In addition, the APAP challenge leads to increased mRNA expression of TLR4, which in turn stimulates p38MAPK and NF-kB activation and the consequent release of inflammatory mediators and proinflammatory factors, resulting in aggravating liver damage. Our findings are in concordance with Liu *et al.* [50]. In contrast, CS pretreatment down-regulates the expression of NF-kB, p38MAPK, and TLR4, illustrating that the hepatoprotective effect of CS may result from the potential of CS to mediate the signaling pathway of TLR4/p38MAPK/NF-kB. The positive effect of CS pretreatment may be related to the hepatic TLR4 and p38MAPK lowering capacity of omega-3 PUFAs and polyphenolic compounds, especially ferulic acid and chlorogenic acid as abundant constituents in CS, all of which exert anti-inflammatory and hepatoprotective effects by blockage TLR4-mediated MAPK and NF-kB pathways [36,46,51].

Consistent with these results, our histopathological investigation also declared that treatment of APAP-intoxicated mice with CS significantly mitigates the liver histological abnormalities observed in APAP-treated mice, suggesting the hepatoprotective effect of CS. Similar findings were shown by Apoorva *et al.* [44], who demonstrated that treatment with CS preserves the liver architecture against the INH-RIF-induced histopathological and biochemical changes in the rats.

Conclusion

In this study, we suggest that dietary intake of CS displays significant hepatoprotective efficacy against APAP-induced liver injury in mice in a dose-dependent manner by suppressing oxidative stress, and inhibiting the production of inflammatory and proinflammatory mediators such as MPO and TNF-α. Also, CS hepatoprotective effect could be explained through decreased CYP2E1 level and suppressed TLR4/p38MAPK/NF-kB pathways.

Acknowledgments

Authors' contribution: S.M.A. developed the idea of the research. S.M.A. and M.A.M. designed the research. S.M.A. and M.A.M. carried out the experiment. S.M.A. and M.A.M. carried out the biochemical tests. S.M.A. construed the statistics. S. M.A. was a main sponsor in writing the manuscript. S. M.A. and M.A.M. revised the final manuscript.

Financial support and sponsorship

Nil.

Conflict of interest

There are no conflicts of interest.

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