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Trans sodium crocetinate protects against hepatotoxicity induced by bisphenol A in rats

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ABSTRACT

Objective(s): Bisphenol A (BPA) is a monomer used in producing a wide range of materials and products, and it is recognized as an endocrine disruptor. Exposure to BPA can cause toxicity in multiple organs, especially the liver. Trans sodium crocetinate (TSC) is a synthetic salt derived from crocetin extracted from Crocus sativus. TSC exhibits antioxidant, anti-apoptotic, and properties that inhibit autophagy. This study evaluates the effects of TSC on liver toxicity induced by BPA.

Materials and Methods: A total of 42 rats were allocated into seven groups, including those exposed to BPA at a dose of 75 mg/kg, BPA and trans sodium crocetinate (TSC) at doses of 10, 20, and 40 mg/kg, and groups receiving olive oil, distilled water, or TSC (40 mg/kg) alone. The total antioxidant capacity (TAC), lipid peroxidation, and glutathione, as well as serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), and total bilirubin were assessed using colorimetric methods. Reactive oxygen species (ROS) and liver protein expression were quantified using fluorimetric and western blot techniques.

Results: TSC, at the dose of 40 mg/kg, reduced the levels of ROS and lipid peroxidation induced by BPA, while remarkably increasing the glutathione content and total antioxidant capacity (TAC) in liver tissue. Moreover, TSC markedly alleviated the BPA-induced increases in caspase-3 protein levels and in the activities of ALT, AST, ALP, and LDH, as well as in serum bilirubin T.

Conclusion: Altogether, TSC can be regarded as a supplement to protect against BPA-induced hepatotoxicity due to its potent antioxidant and anti-apoptotic effects.

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Introduction

Bisphenol A (BPA) is a synthetic compound that acts as an environmental pseudoestrogen and is commonly used in manufacturing a variety of products, such as dental fillings, disposable containers, and the internal coatings of cans (1, 2). Several studies have shown that exposure to BPA may cause toxicity to various organs, including the nervous system (3), cardiovascular system (4, 5), liver (6), kidney (7), and male reproductive system (8). Animal studies have shown that glucuronidation is the primary metabolic pathway in the liver for eliminating BPA, and that the BPA-glucuronide complex is the primary metabolite of BPA in animals and humans (9). *In vivo* research has demonstrated that BPA exposure can lead to a range of liver disorders, from metabolic syndrome to steatosis and liver tumors (10, 11, 12). Research has indicated that different doses of BPA elevate oxidative stress in the livers of rats by promoting the production of reactive oxygen species (ROS), which decreases the activity of anti-oxidant enzymes and enhances lipid peroxidation (13). Additionally, BPA has been shown to increase liver enzyme levels, specifically aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (14), and it also triggers apoptosis in liver cells (13), ultimately leading to liver tissue damage.

Saffron (*Crocus sativus*) is a medicinal and culinary spice that grows in warm, dry regions of Asia and Europe. It is native to the eastern Mediterranean and widely cultivated in Iran (15, 16). Saffron requires specific conditions, including suitable soil, suitable climate, and sufficient light (17). It is a highly valued spice essential to Iran's economy, with uses in pharmaceuticals, cosmetics, and dye production. It is the world's most expensive spice, with an annual output of approximately 300 tons, primarily in Iran (76%). Due to concerns over artificial production and fraud, maintaining quality certification according to international ISO or Food and Drug Administration (FDA) standards is crucial for its trade (18). Saffron is rich in crocins, a group of carotenoid-glycosyl esters, and in dry saffron, crocin content typically ranges from 8% to 38% by mass, with some studies reporting up to 50%; crocin levels vary with origin, climate, farming practices, drying, storage, and irrigation (19). To date, MS data have identified 16 crocins differing in sugar moieties, glucoside (g), gentiobioside (G), neapolitanoside

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(n), or triglucoside (t) and in cis or all-trans crocetin forms, with the major derivatives being trans-crocetin bis(β-D-gentiobiosyl) ester, trans-crocetin (β-D-gentiobiosyl) (β-D-glucosyl) ester, and cis-crocetin (β-D-gentiobiosyl) (β-D-glucosyl) ester, together comprising over 95% of total crocins; a few other crocetin derivatives contribute to the observed chromatograms (20). Crocetin ($C_{20}H_{24}O_4$; MW: 328.4 g/mol), a dicarboxylic acid carotenoid found in saffron, offers various pharmacological benefits, including anti-oxidant and neuroprotective properties (21, 22). Crocetin has been shown to reduce fatigue and depression, improve retinopathy, and alleviate sleep disorders. Additionally, it protects against the degeneration of nigrostriatal dopaminergic neurons and helps maintain dopamine levels. It also enhances oxygen permeability in body fluids, which may lead to improved alveolar filtration and oxygen delivery to the brain, thereby reducing nervous system damage. Moreover, crocetin demonstrates a range of useful effects such as anti-tumor, anti-diabetic, anti-hyperlipidemic, neuroprotective, hepatoprotective, renoprotective, cardioprotective, and fertility-enhancing properties (23, 24); (25, 26). The mechanisms proposed for these effects involve crocetin's anti-oxidant, anti-apoptotic, anti-inflammatory, and autophagy-modulating effects (25, 27, 28).

Trans sodium crocetinate (TSC) is a synthetic salt of crocetin, classified as a bipolar trans-carotenoid (25). TSC has been shown to increase the diffusion of O_2 from erythrocytes to plasma and to decrease plasma resistance, thereby improving O_2 availability to tissues (29, 30). Moreover, TSC has been suggested to prevent liver disorders (31, 32). In addition to oxygen supply, TSC has anti-oxidant (33), anti-apoptotic (25, 33), and autophagy-inhibitory properties (25).

Given the significance of hepatotoxicity associated with the oxidative and apoptosis-inducing effects of BPA, and the anti-oxidant and anti-apoptotic properties of TSC, this study examines the protective effects of TSC against BPA-induced hepatotoxicity.

Materials and Methods

Chemicals

Bisphenol A (BPA), trichloroacetic acid (TCA), 5,5-dithiobis(2-nitrobenzoic acid) (DTNB), acrylamide, thiobarbituric acid (TBA), glycine, sodium citrate, tris(hydroxymethyl)aminomethane, tris hydrochloride (Tris HCl), and all salts utilized for buffer preparation were of analytical grade and sourced from Merck (Darmstadt, Germany). Trans sodium crocetinate (TSC) was purchased from Tinab Chem Middle East Company in Iran, while 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) was acquired from Sigma, United States.

Animals

Male Wistar rats (n = 42; weighing between 220 and 300 g) at 10 to 12 weeks of age were acquired from the Center for Reproduction and Maintenance of Laboratory Animals at Mashhad University of Medical Sciences, Mashhad, Iran. One week before the experiments began, the rats were grouped and housed in clear Plexiglas cages with hardwood bedding. To help them acclimate to their new environment, conditions were maintained at 24 °C, a 12-hr light/dark cycle, and 40% relative humidity. The animals had ad libitum access to standard food and water. All experiments followed humane practices, and the experimental protocols were approved by the local ethics committee at Mashhad University of Medical Sciences (ethics code: IR.MUMS.AEC.1401.046).

Experimental setup

The animals were administered olive oil orally and received distilled water through intraperitoneal (IP) injection as vehicle controls. BPA was administered at 75 mg/kg via oral gavage, while trans-sodium crocetinate (TSC) was injected IP at 10, 20, and 40 mg/kg in conjunction with BPA and at 40 mg/kg alone, based on previous research (33). The BPA dosage was determined based on findings from (34). All substances were freshly prepared each day and administered over 28 consecutive days. Twenty-four hours after the final dose, the rats were anesthetized with ketamine and xylazine (70/10 mg/kg) (35), and liver samples were collected. Portions of the harvested tissue were utilized to assess lipid peroxidation, GSH levels, TAC, and ROS content, while another portion was preserved at -80 °C for Western blot analysis.

Reactive oxygen species (ROS) assay

The formation of ROS in the liver was evaluated via a method previously described by (36, 37). A homogenate of liver tissue was combined with one ml of Tris-HCl buffer and 2′, 7′-dichlorofluorescein diacetate (with a final concentration of 10 μM). The mixture was incubated at 37 °C in the dark for 30 min, after which the fluorescence intensity of each sample was measured using a BioTek Synergy H4 Hybrid Multi-Mode Microplate Reader (excitation wavelength 485 nm, emission wavelength 525 nm). The fluorescent intensity for each sample was ultimately determined as a percentage of the control group (37).

Lipid peroxidation assay

The assay is based on the reaction between malondialdehyde (MDA), a marker of lipid peroxidation, and thiobarbituric acid (TBA), yielding a colored complex (38). Initially, thiobarbituric acid and phosphoric acid were added to the liver tissue homogenate. The samples were then put in a boiling water bath at 100 °C. After cooling, n-butanol was added to the mixture, which was then centrifuged. The absorbance of the resulting color in the upper phase was measured at 532 nm using a spectrophotometer.

Glutathione content

Glutathione content was determined using a method based on the reaction of sulfhydryl groups with the reagent 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) (39). The colored complex formed in this reaction has a peak absorption at 412 nm. In summary, trichloroacetic acid (TCA) was first added to the homogenate samples, followed by centrifugation. DTNB was then added to the supernatant, and the absorbance of the resulting yellow complex was measured using a spectrophotometer. The content of glutathione (GSH) was calculated according to the GSH standard curve and determined as nmol/g of tissue.

Ferric reducing anti-oxidant power (FRAP) of liver tissue

The total anti-oxidant capacity was measured according to the Ferric Reducing Anti-oxidant Power (FRAP) method. This technique relies on the formation of a blue

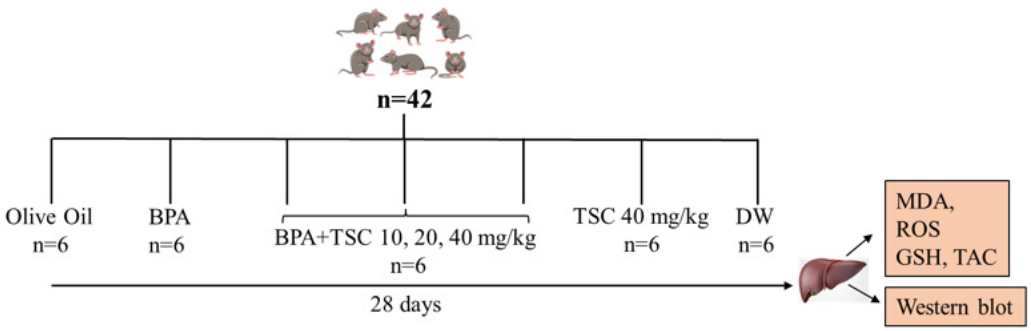

Figure 1. Schematic of the experimental design
Trans sodium crocetinate (TSC), Bisphenol A (BPA), reactive oxygen species (ROS), malondialdehyde (MDA), and glutathione (GSH).

Fe^{2+}- tripyridyltriazine complex, which occurs when colorless, oxidized Fe^{3+} interacts with electron-donating anti-oxidants (40). In brief, the liver tissue homogenate was prepared in an ice-cold Tris-HCl buffer. The FRAP reagent was then added to a mixture of the sample homogenate and deionized water. After maintaining the reaction mixture at 37 °C for 5 min, the absorbance was recorded at 595 nm using a spectrophotometric method.

Western blotting assay

For performing Western blot technique, protein extraction from tissues stored at -80 °C was conducted using a lysing buffer composed of 50 mM Tris base, 2 mM EDTA, 2 mM EGTA, 10 mM NaF, 1 mM sodium vanadate dihydrate, 10 mM β-Glycerophosphate, 10 mM 2-Mercaptoethanol, 0.2% sodium deoxycholate, along with a 1X protease inhibitor cocktail and 0.1 mM phenylmethylsulfonyl fluoride (PMSF). Protein concentration was measured by the Bradford assay at 595 nm, with bovine serum albumin (BSA) as the standard. Equal amounts of each protein sample were then loaded onto a 10% SDS polyacrylamide gel. Subsequently, proteins were transferred to a polyvinylidene fluoride (PVDF) membrane (Bio-Rad, USA) by electrotransfer. The PVDF membrane was incubated overnight in Tris-buffered saline with 0.1% Tween (TBST) at 4 °C, then blocked for 2 hours in TBST containing 5% skim milk. Following three washes (5 min each), primary antibodies were added, including rabbit anti-Bax (1:1000 dilution, Cell Signaling #2772), anti-Caspase-3 (1:1000 dilution, Cell Signaling #9665), anti-Bcl-2 (1:1000 dilution, Cell Signaling #2870), and the internal control mouse anti-β-actin (1:1000 dilution, Cell Signaling #3700) to detect the target proteins. After incubation with primary antibodies overnight, the membrane was washed three additional times (5 min each). Next, secondary antibodies, horseradish peroxidase-conjugated anti-mouse (1:3000 dilution, Cell Signaling #7076) and anti-rabbit (1:3000 dilution, Cell Signaling #7074), were applied to the membrane and maintained for 2 hours at room temperature. After this, the secondary antibodies were removed, and the membrane was rinsed three additional times (5 min each). The blots were visualized using a chemiluminescent detection method with the Gel Doc Alliance 4.7 system (UK), and the density of each blot was determined to evaluate protein expression. The concentration of each protein was measured by dividing the density of the specific blot by the density of its internal control (β-actin).

Plasma biochemistry

Plasma levels of ALT, AST, LDH, ALP, and bilirubin were assessed at the Jahad Daneshgahi laboratory of Mashhad University of Medical Sciences.

Statistical analysis

In this study, data analysis was performed using GraphPad Prism version 9 software. All measurements for glutathione (GSH), malondialdehyde (MDA), reactive oxygen species (ROS), and total anti-oxidant capacity (TAC) were derived from a minimum of seven independent experiments, while the Western blotting assays were based on at least four independent experiments. Results are expressed as mean ± standard deviation (SD). A one-way ANOVA, followed by Tukey's *post hoc* test, was utilized to compare multiple experimental groups. Statistical significance was determined at a *P*-value threshold of less than 0.05.

Results

Oxidative stress indices

Hepatic ROS and MDA assays

TSC (20 and 40 mg/kg, IP) alleviated liver tissue ROS ($P<0.001$) and lipid peroxide formation ($P<0.001$) in comparison with BPA-receiving animals (Figure 2a, b). Notably, the TSC effect on oxidative stress biomarkers, ROS, and lipid peroxidation, was dose-dependent. The MDA level and ROS in the groups treated with TSC 40 alone remained unchanged in comparison with the control groups.

Hepatic GSH and TAC levels

significant reduction in GSH and TAC levels was observed in the group treated with BPA alone compared with the control groups ($P<0.001$). TSC (20 and 40 mg/kg)

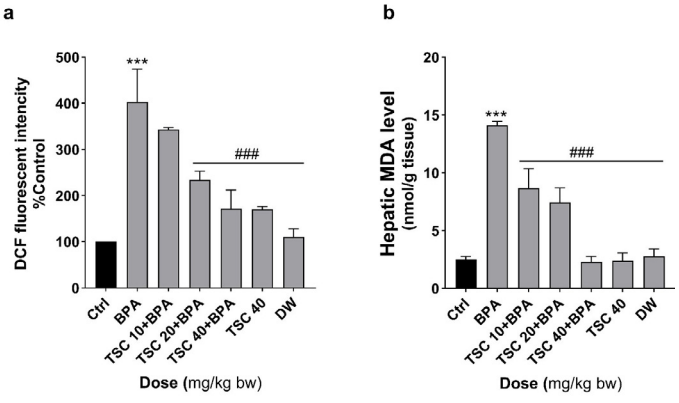

Figure 2. Effect of trans sodium crocetinate (TSC) on Bisphenol A (BPA) –induced a) reactive oxygen species (ROS) production and b) malondialdehyde (MDA) concentration in rats' liver tissue
Data were analyzed using one-way ANOVA followed by Tukey-Kramer post hoc tests, and values are expressed as mean ± SD; ***$P<0.001$ compared with the control group; ###$P<0.001$ compared with the BPA group.

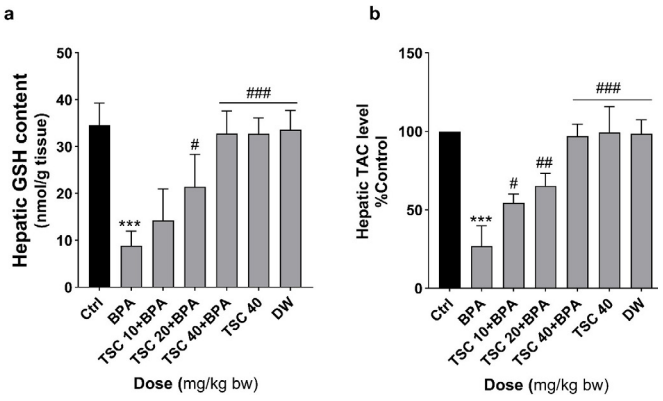

Figure 3. Effect of trans sodium crocetinate (TSC) and Bisphenol A (BPA) on a) glutathione (GSH) and b) total anti-oxidant capacity (TAC) content of rat's liver tissue

Data were analyzed using one-way ANOVA followed by Tukey-Kramer post hoc tests, and values are expressed as mean ± SD; ***P<0.001 compared with the control group; ###P<0.001 compared with the BPA group.

significantly protected liver GSH content compared with BPA-treated rats (P<0.05 for 20 mg/kg; P<0.001 for 40 mg/kg) (Figure 3a). Additionally, all doses of TSC (10, 20, and 40 mg/kg) in combination with BPA notably preserved liver TAC compared to the BPA-only groups (P<0.05 for 10 mg/kg; P<0.01 for 20 mg/kg; P<0.001 for 40 mg/kg) (Figure 3b). No significant changes in GSH and TAC levels were observed in the TSC-alone group (40 mg/kg) compared with the control group.

Western blot analysis

The effects of TSC and BPA on the protein levels of pro-apoptotic proteins Bax and Caspase-3, as well as the anti-apoptotic protein Bcl-2.

Western blot analysis showed that protein levels of Caspase-3 (Figure 4) were significantly elevated in the livers of rats receiving BPA in comparison with the control group (P<0.001). In rats treated with both BPA and TSC (at doses of 20 and 40 mg/kg), the protein level of Caspase-3 significantly decreased in comparison with the BPA-only group; no differences were observed compared to the control group (P<0.01) (Figure 4a, b). On the other hand, the protein levels of the Bax and Bcl-2 (Figure 4a) in the BPA-exposed groups did not exhibit significant changes from those in the control group (Figure 4a, c).

Serum biochemistry analysis

The impact of BPA and varying doses of TSC on serum biomarkers of liver injury is illustrated in Figure 5. Animals exposed to BPA showed a significant increase in the activities of ALT, AST, ALP, LDH, and plasma total bilirubin (Bili T) in comparison with the control groups (P<0.001 for AST; P<0.05 for ALT; P<0.01 for ALP, LDH, and Bili T). Co-administration of TSC (20 and 40 mg/kg) with BPA meaningfully mitigated the biochemical alterations in AST (P<0.001) and ALT activities (P<0.01 for 20 mg/kg and P<0.001 for 40 mg/kg) when compared to the BPA-only group (Figure 5a, b). However, no significant differences in serum biomarker levels were observed in the TSC alone group in comparison with the control groups.

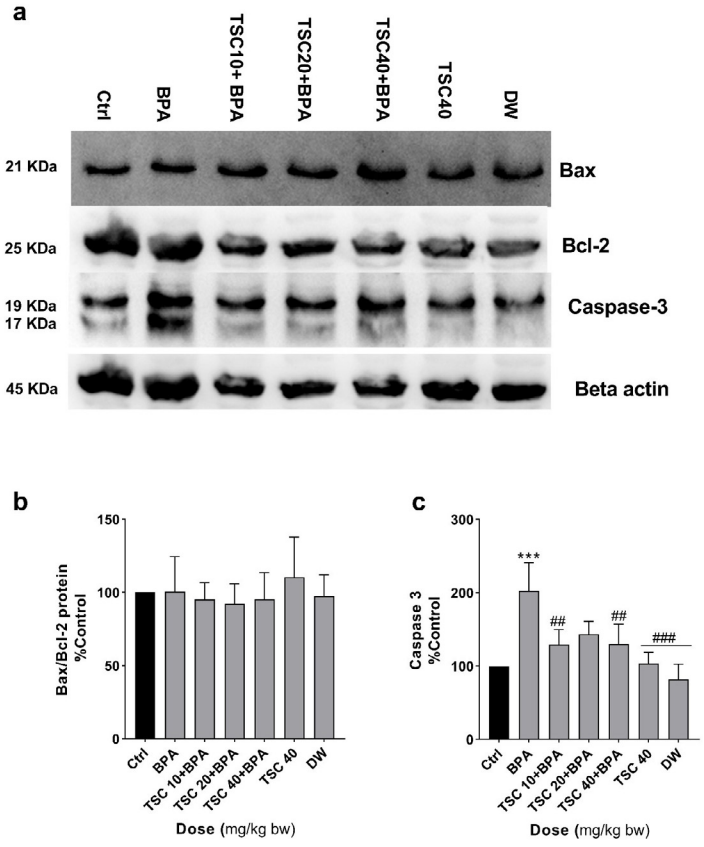

Figure 4. Western blot assay for apoptotic marker proteins detection in liver tissue of rats exposed to trans sodium crocetinate (TSC) and Bisphenol A (BPA) a) 25 kDa, 21 KDa, 17-19 KDa, and 45 KDa bands show results for Bcl-2, Bax, Caspase-3, and β-actin proteins, respectively; b) graph shows analysis results for pro-apoptotic Caspase-3 protein, and c) graph shows analysis results for Bax/Bcl-2 proteins ratio. Data were analyzed by one-way ANOVA following the Tukey-Kramer post-test, and values are expressed as means ± SD; *P<0.05, and **P<0.01 compared to control, # P<0.05, and ##P<0.01 compared to the BPA group.

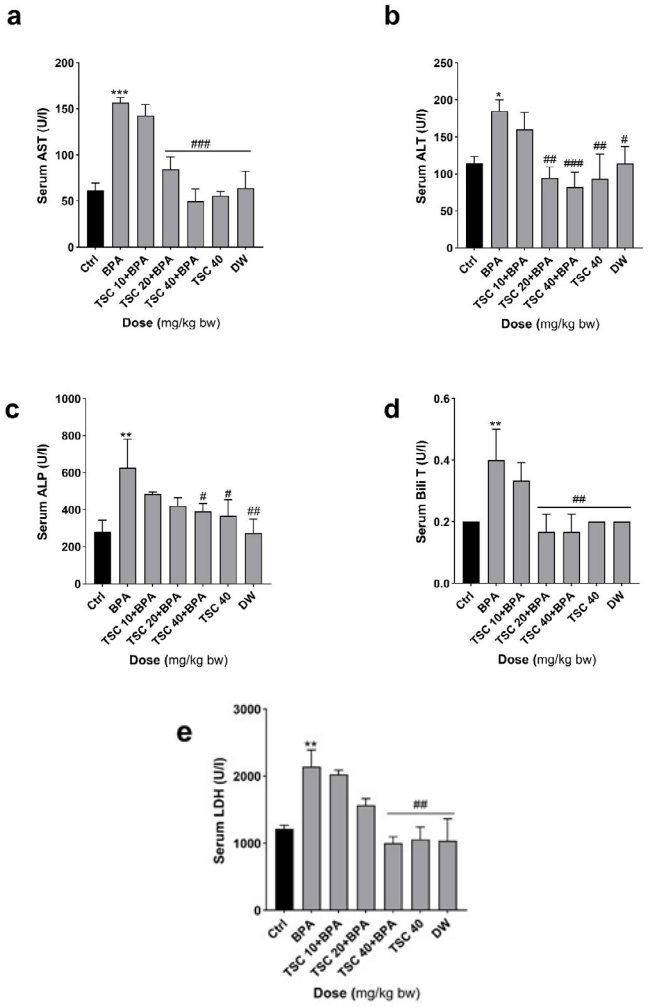

Figure 5. Serum biochemical measurements in rats exposed to trans sodium crocetinate (TSC) and Bisphenol A (BPA)
a) graph shows results for aspartate aminotransferase (AST), b) graph shows results for alanine aminotransferase (ALT), c) graph shows results for alkaline phosphatase (ALP), d) graph shows results for lactate dehydrogenase (LDH), and e) graph shows results for bilirubin T (T bili). Data were analyzed by one-way ANOVA following the Tukey-Kramer post-test, and values are expressed as means ± SD; **P*<0.05, and ***P*<0.01 compared to control, # *P*<0.05, and ##*P*<0.01 compared to the BPA group.

Discussion

The present study revealed that TSC can correct the increased levels of ROS and lipid peroxidation, as well as the decreased glutathione content and total anti-oxidant capacity, thereby mitigating oxidative stress induced by BPA. It also reduced BPA-induced apoptosis and serum biomarkers of liver damage (ALT, AST, ALP, LDH, and TBili), and TSC mitigated all BPA-induced toxic effects.

The oxidative stress examination indicated that BPA dramatically elevated ROS formation and lipid peroxidation and depleted anti-oxidant protective resources such as GSH and TAC in the liver tissue of rats. Our previous study revealed that TSC co-administration with BPA can preserve GSH and TAC content in the brain and prevent the formation of ROS overload, leading to increased lipid peroxidation (41). Although ROS has important roles in physiological (42) and immune system functions (43) and is involved as a secondary messenger in signaling pathways controlling cellular homeostasis (42), the production of increased levels of ROS leads to the creation of oxidative stress, which in turn impacts physiological activities and tissue structural integrity in the biological system (44). There is increasing evidence of oxidative stress-mediated liver damage caused by exposure to BPA. Despite the presence of an anti-oxidant defense system in liver tissue, BPA induces significant hepatocellular damage (45). A study similar to the present study, but with a different dose, showed that the oral administration of BPA (50 mg/kg) for 28 days induces severe hepatocellular damage through a disturbance in the oxidant/anti-oxidant balance. They also reported that BPA increased oxidant levels, including free radicals and lipid peroxide levels, and decreased anti-oxidant levels, including glutathione and anti-oxidant enzyme activity (45, 46). Different studies demonstrated that anti-oxidant compounds can play essential roles in reducing BPA-induced hepatotoxicity by inhibiting oxidative stress (47-49).

In the present study, we showed that TSC reverses the disturbance in the oxidant/anti-oxidant balance caused by BPA. TSC is a small molecule and trans-carotenoid salt that belongs to the carotenoid family (50) and is obtained from crocetin (51). TSC enhances oxygen delivery to tissues and reduces plasma viscosity (30). It also improves crocetin bioavailability (52) and, like crocetin (30) and other saffron compounds (53, 54), has potent anti-oxidant effects. TSC is known as a potent radical scavenger that can reduce intracellular ROS (55), particularly hydroxyl radicals (51), while boosting the activity of anti-oxidant system enzymes (56). Additional studies consistent with our results indicated that pretreatment of cells with TSC effectively reduced ROS formation and increased cell viability (25, 33). Accordingly, TSC seems to prevent BPA-induced liver injury by preserving the oxidant/anti-oxidant balance.

Our findings show that treatment with TSC, along with BPA, prevents the increase in pro-apoptotic protein, Caspase-3, levels caused by BPA. Apoptosis, or programmed cell death, is a crucial biological process that maintains homeostasis and eliminates damaged or unwanted cells. The major pathways of apoptosis are intrinsic and extrinsic. The intrinsic pathway, triggered by internal stress signals, subsequently leads to the activation of caspase-3, culminating in cell death. The extrinsic pathway is initiated by external signals binding to death receptors (e.g., Fas ligand binding to Fas receptor). This pathway activates caspase-8, which can directly activate caspase-3, leading to apoptosis (57, 58). Previous studies have shown that BPA increases gene expression of mitochondrial apoptotic pathways, such as caspase-3, -9, and -10, as well as the activity of caspase enzymes (59, 60). A team of researchers reported that BPA induces mitochondrial apoptosis pathways in hepatocytes by directly affecting mitochondrial structure and increasing mitochondrial membrane permeability, resulting in the release of proteins that activate apoptosis (61). Additionally, BPA elevates oxidative stress, which enhances the oxidation of lipid contents of the mitochondrial membrane and disrupts its structure (46). An increase in oxidative stress also promotes the induction of apoptosis (13). Accumulated documents show that crocetin has significant anti-oxidant and antiapoptotic effects (62).

The present study demonstrates that TSC, a trans salt derived from crocetin, alleviates oxidative stress and subsequent apoptosis by reducing the generation of free radicals and promoting their elimination, while maintaining

anti-oxidant levels in rat liver tissue. Our findings align with several studies that have indicated that TSC inhibits apoptosis in HEK-293 cells by reducing ROS levels and ensuing Bax/Bcl-2 ratio and caspase-3 (25, 33). Although this study found that the Bex/Bcl-2 ratio did not show a significant change in response to BPA, the increased protein level of caspase-3 caused by BPA was effectively reduced by TSC, which may suggest the extrinsic pathway inducing apoptosis. The extrinsic pathway of apoptosis is initiated by the binding of ligands, such as Fas ligand or tumor necrosis factor (TNF), to their respective death receptors on the target cell's surface. This interaction triggers receptor activation, leading to the formation of the death-inducing signaling complex (DISC) that recruits initiator caspases, such as caspase-8. After activation, these caspases cleave and activate downstream effector caspases (e.g., caspase-3), thereby executing apoptosis via processes such as DNA fragmentation and membrane blebbing (63). While the intrinsic apoptotic pathway is activated by cellular stresses, leading to the release of cytochrome c from mitochondria. This cytochrome c forms the apoptosome with Apaf-1 and procaspase-9, activating caspase-9 and subsequently caspase-3, which executes apoptosis. Key proteins involved include pro-apoptotic Bax/Bak and anti-apoptotic Bcl-2. The extrinsic pathway can operate independently of the intrinsic pathway, making it particularly relevant in contexts like cancer therapy, where the intrinsic pathway may be defective. The effectiveness of the extrinsic pathway relies on factors like the presence of appropriate ligands, the sensitivity of the target cells, and the integrity of downstream apoptotic signaling mechanisms (64). However, further studies are needed for a more comprehensive understanding of the mechanisms of TSC on apoptotic pathways.

In the current study, exposure to BPA elevated serum factors (AST, ALT, ALP, and T Bili), indicating the extent of liver damage, while TSC alleviated these biomarkers in rats' serum. Typically, enhanced serum levels of ALT and AST reflect hepatocellular damage, while enhanced levels of ALP indicate the extent of bile duct impairment (65). When liver hepatocytes are damaged, these enzymes leak into the bloodstream. An increase in serum enzyme concentrations indicates hepatocyte damage and liver dysfunction (66). According to enhanced evidence, BPA impacts the activity of liver enzymes, including AST, ALT, and ALP, and may cause liver injury (59, 67). It has been reported that exposure to BPA, either as a single dose of 1.2 mg/kg per day (59) or repeated doses of 10 and 50 mg/kg for 28 days, dramatically increases AST and ALT activity and bilirubin levels (45). Although some studies have also shown that exposure to BPA does not significantly affect the levels of AST and ALT enzymes (68, 69), more studies have mentioned, in line with the present study, that BPA can increase the serum levels of these enzymes with different doses in animals (70-72). TSC likely prevents BPA-induced liver damage by significantly reducing oxidative stress and preserving anti-oxidant capacity. As mentioned above, oxidative stress can cause severe damage to liver cell structure (45), elevated serum enzyme levels (59), and ultimately liver dysfunction.

The current study highlights several limitations that should be addressed in future research. First, it is crucial to confirm the specificity of serum biomarkers indicative of hepatic injury through histopathological examination. Although elevated liver markers suggest damage, histopathological examination remains crucial for a comprehensive diagnosis, as it can reveal the underlying causes, types of damage, and the overall state of liver tissue. Additionally, further investigation into the molecular mechanisms and pathways, especially the extrinsic apoptosis and autophagy pathways by which TSC exerts its protective effects, is necessary. The applicability of the findings to other species or humans is also uncertain, emphasizing the need for caution in generalizing results. Future research can assess the effects of prolonged exposure to both BPA and TSC and explore the specific cellular mechanisms involved in TSC's action. Moreover, exploring TSC's potential in clinical settings, particularly in combination with other hepatoprotective agents, may enhance its efficacy in combating BPA-induced liver damage.

Overall, BPA acts as an oxidant, increasing ROS and lipid peroxidation, disrupting the anti-oxidant system, inducing apoptosis, increasing liver enzyme activity, and ultimately causing liver damage and dysfunction. TSC use, which has outstanding anti-oxidant and physicochemical properties, can prevent liver tissue damage and dysfunction caused by BPA-induced oxidative damage.

Acknowledgment

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Ethical Approval

Animal maintenance conditions and all experimental procedures were conducted in accordance with the ethics committee guidelines of Mashhad University of Medical Sciences (ethics code: IR.MUMS.AEC.1401.046).

Availability of Data and Materials

The data that support the findings of this study are available upon request from the corresponding authors.

Authors' Contributions

H H and BM R have made significant contributions to study design, evaluation, drafting, and submission management. M K has made major contributions to study design, data collection and evaluation, drafting, and statistical analysis. All authors have performed editing and approved the final version of this paper for submission.

Conflicts of Interest

The authors declare no conflicts of interest.

Declaration

During the preparation of this work, the authors used AI-assisted technologies to rephrase to reduce plagiarism and improve language and grammar. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the publication's content.

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