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Hepatoprotective effect of methanolic *Tanacetum parthenium* extract on CCl4-induced liver damage in rats

Yavar Mahmoodzadeh, Mohammad Mazani, Lotfollah Rezagholizadeh*

Department of Biochemistry, School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran

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ABSTRACT

The purpose of this study was to investigate the effects of Tanacetum Parthenium Extract (TPE) on Lipid peroxidation, antioxidant enzymes, biochemical factors, and liver enzymes in the rats damaged by Carbon Tetrachloride (CCl4).

54 male Wistar rats were divided into 9 groups each consisting of 6 rats. Two of the groups were control groups (normal and damage control groups), 4 of them were exposure groups which were respectively administered with 40, 80, and 120 mg/kg of TPE and silymarin for 14 days before being damaged by CCl4, and the other 3 groups were post-treatment groups which received 80 and 120 mg/kg of TPE and silymarin 2, 6, 24, and 48 h after being injected with CCl4. At the end of the study, biochemical factors, serum liver enzymes, malondialdehyde level, antioxidant enzymes, and liver morphology were assayed.

Pre- and post-treatment with TPE could significantly decrease ALT, AST, ALP, TG, LDL, TC, and glucose levels and increase HDL, and albumin levels and catalase, SOD, and GPx activities compared to the CCl4-damaged control group.

The results of this study are indicative of the antioxidant activity of TPE, its potential hepatoprotective effects, and its probable therapeutic properties for laboratory animals damaged by CCl4.

1. Introduction

Liver is one of the main organs involved in the metabolism of drugs and toxic chemicals and is the first target organ for almost all chemicals [1,2]. Most of the xenobiotics enter the body through gastrointestinal tract and after absorption enter the liver through portal vein. The liver has a high concentration of toxin-metabolizing enzymes which can convert xenobiotics to compounds with low toxicity and excrete them. However, sometimes toxic substances are converted to active metabolites during metabolism which can exacerbate liver damage and cause changes in the macroscopic structure of, or damage to, specific molecules such as bile acid transporters, families of nuclear receptors, intracellular lipids, proteins, and nucleic acids [3]. Improper performance of these molecules activates some secondary paths which finally lead to planned events such as apoptosis, necrosis, autophagy, mitochondrial defects, and immune responses [4]. Moreover, the entire cellular function is also disrupted by cytolytic activities and the destruction of membranes and transmembrane transport mechanisms.

CCl_4 is the most recognized chemical substance used in developing models of liver and kidney damage [5,6]. Therefore, CCl_4-induced liver damage is one of the best ways of inducing damage by xenobiotics and also one of the common methods of screening hepatoprotective or liver treatment drugs. The metabolism of CCl_4 begins with the formation of trichloromethyl and proxy chloromethyl free radicals via the activity of oxygenase system of cytochrome P450 in endoplasmic reticulum. The trichloromethyl radical reacts with various important biological substances such as fatty acids, proteins, lipids, nucleic acids, and amino acids [7,8].

Therefore, the antioxidant activity of the body and the inhibition of the production of free radicals are important in preventing CCl_4-induced hepatopathies. In CCl_4-induced liver damages, the balance between Reactive Oxygen Species (ROS) production and antioxidant defense system is disturbed due to oxidative stress which disrupts cellular functions through some events and causes liver damage and necrosis. Despite the considerable advancements in medicine and modern pharmacology, drugs used for the treatment of liver damages have many side effects and exacerbate the disease. Therefore, it is necessary to find new drugs to replace those with many side effects [9,10]. Non-Alcoholic Fatty Liver Disease (NAFLD) includes a wide spectrum of liver damages from simple fatty liver to steatohepatitis, steatonecrosis, and non-alcoholic steatohepatitis. The aims of treatment in NAFLD are weight loss for obesity, reduction of lipid-lowering agents for

* Corresponding author.
E-mail address: Reza34055@gmail.com (L. Rezagholizadeh).

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dyslipidemia, modifying antioxidants and probiotics for oxidative stress, cytoprotective agents for apoptosis, and anti-tumor necrosis factor (TNF-α) for proinflammatory cytokines. Thus, it seems necessary to find a compound that has hepatoprotective and antihyperlipidemic effects without causing other side effects [11–13].

TPE with the common name of 'Feverfew' is a plant belonging to the family of Asteraceae which is used for the treatment of various diseases such as arthritis and migraine in traditional medicine. This plant contains various antioxidant compounds such as sesquiterpene lactone and various flavonoids [14]). Therefore, this study was conducted with the aim of assaying the effects of TPE on lipid peroxidation, antioxidant enzymes, biochemical factors, and liver enzymes in the rats damaged by CCl_4.

2. Materials and methods

2.1. Chemical substances

Commercial kits of superoxide dismutase and glutathione peroxidase were bought from Randox Company (Crumlin, UK) and those of AST, ALP, ALT, TG, TC, Urea, HDL, and glucose were purchased from Pars Azmoon Company (Tehran, Iran). Chemical substances including hydrogen peroxide, methanol, thiobarbituric acid, bovine serum albumin, coomassie blue, CCl_4, ferric sulfate, ferric chloride, sodium acetate, and butanol were purchased from Merck Company (Germany). TPTZ and ketamine were bought from Fluka Company and Alphasan Company (Netherlands), respectively.

2.2. Plant collection and extraction

The plant samples were collected at the humidity level of 45–60% early in May from Khodafarin, Arasbaran Zone, East Azarbayjan Province, Iran which is situated at the altitude of 2100 m above sea level. After confirmation of the genus and species, which was done using valid identification keys by the herbarium experts of the Research Center for Agriculture and Natural Resources of East Azarbayjan Province with the herbarium code of 2411, the samples were prepared for extraction. The aerial parts of the plant were dried separately in shadow at the ambient temperature of 20–25 °C and then powdered using a mortar. After that, they were soaked in methanol 70% for 7 days. The obtained extract was percolated and then condensed by rotary evaporator. After the evaporation of alcohol, the extract was deposited in a freeze dryer at main drying and final drying phases for 2 weeks to become powder and then kept in a freezer.

2.3. The design of the study

2.3.1. Selection of animals

In this study, male Albino Wistar rats with body weights of 180 ± 20 g were purchased from Faculty of Veterinary Medicine, Tehran University. They were kept in standard conditions, that is, the temperature of about 22 °C, 12-h light/dark cycle, and in a bed of straw. This study was approved by the Ethics Committee of Ardabil University of Medical Sciences and the identification code received for it from the Clinical Trials Registry of Islamic Republic of Iran is 'IR.ARUMS.REC.1394.67'.

2.3.2. Induction of liver damage

In order to induce liver damage in rats, CCl_4 was solved in olive oil with the ratio of 1:1 and from the obtained mixture 1.5 mg/kg was injected to them intraperitoneally [15].

2.3.3. Grouping of the rats

54 rats were randomly assigned to 9 groups each consisting of 6 rats.

Group 1 (NC): This group was the normal group in which the rats received distilled water via gavage for 14 days and were injected with 1.5 mg/kg of just olive oil (the solvent of CCl_4) on the 14th day.

Group 2 (CC): This group was the exposure group in which the rats received distilled water via gavage for 14 days and were injected with 1.5 mg/kg of CCl_4 and olive oil mixture on the 14th day.

Group 3 (bTP_{40}): This group was the pretreatment group with the dose of 40 in which the rats were administered with 40 mg/kg TPE for 14 days and received 1.5 mg/kg of CCl_4 and olive oil mixture on the 14th day.

Group 4 (bTP_{80}): This group was the pretreatment group with the dose of 80 in which the rats were administered with 80 mg/kg TPE for 14 days and received 1.5 mg/kg of CCl_4 and olive oil mixture on the 14th day.

Group 5 (bTP_{120}): This group was the pretreatment group with the dose of 120 in which the rats were administered with 120 mg/kg TPE for 14 days and received 1.5 mg/kg of CCl_4 and olive oil mixture on the 14th day.

Group 6 (bSC_{100}): This group was the positive control group with the standard drug silymarin. The rats in this group were administered with 100 mg/kg silymarin for 14 days and received 1.5 mg/kg of CCl_4 and olive oil mixture on the 14th day.

Group 7 (aTP_{80}): This group was the post-treatment group with the dose of 80 in which the rats were administered with 1.5 mg/kg of CCl_4 and olive oil mixture on the 14th day and received 80 mg/kg TPE via gavage 2, 6, 24, and 48 h after the injection of CCl_4.

Group 8 (aTP_{120}): This group was the post-treatment group with the dose of 120 in which the rats were administered with 1.5 mg/kg of CCl_4 and olive oil mixture on the 14th day and received 120 mg/kg TPE via gavage 2, 6, 24, and 48 h after the injection of CCl_4.

Group 9 (aSC_{100}): This group was the post-treatment group with silymarin in which the rats were administered with 1.5 mg/kg of CCl_4 and olive oil mixture on the 14th day and received 100 mg/kg silymarin via gavage 2, 6, 24, and 48 h after the injection of CCl_4.

50 h after the last injection, the rats were anesthetized with the injection of 200 µl (160 µl ketamine 10% + 40 µl xylazine) of anesthesia agent. Then their abdominal areas were opened and the blood samples were directly taken from their hearts. The samples were centrifuged at 3000 rpm for 10 min and after making aliquots in vials of 1.5 ml were stored in a freezer at −80 °C to perform biochemical experiments.

Immediately after blood collection, a piece of each rat's liver (right-distal lobe) was removed and kept in formalin 10% for histopathological examinations. A part of their livers was also washed with saline and kept in liquid nitrogen.

2.4. Measurement of serum factors

Serum levels of ALT, AST and ALP were measured using standard assay kits according to the manufacturer's instructions. The concentrations of glucose, cholesterol, and triglyceride were measured with GOD-POP, CHOD-POP, and GPO-POP methods, respectively. Direct measurement method was used to determine HDL-C and LDL-C concentrations while albumin was measured through bromocresol green method according to the instructions provided by Pars Azmoon kit (Tehran, Iran). The experiments were conducted according to the instructions provided in each laboratory kit using an Auto-Analyzer (Biochemistry Analyzer BT 1500, Italy).

2.5. Preparation of tissue lysate

200 mg of liver was chopped and poured in a tube and 2 ml of homogenization buffer (tris buffer) was added to it. Then, it was homogenized at 10,000 rpm for 2 min in a homogenizer set. The obtained suspension was centrifuged at 12,000 rpm for 20 min so that the unhomogenized cells deposit. The pure homogenous solution was used in the measurement of malondialdehyde, superoxide dismutase, and

glutathione peroxidase.

2.6. Measurement of malondialdehyde (MDA) in liver

MDA was measured according to Mihara & Uchiyama's method with slight modifications. In summary, 400 µl of the supernatant liquid of the homogenized tissue was solved in 400 µl of trichloroacetic acid. After centrifugation, 400 µl of supernatant liquid was mixed with 2400 µl of phosphoric acid (1%). After vortexing, 1 ml thiobarbituric acid (0.67%) was added to the test tube and after being vortexed once again was placed in a boiling bain-marie for 60 min. After getting cold, 1600 µl *n*-butanol was added to the samples and they were vortexed once again for 1–2 min. Then, they were centrifuged at 3000 rpm for 10 min. After separating the organic phase (supernatant solution), light absorption was measured in the wavelength of 532 nm against *n*-butanol as the blank. After transferring the obtained results to 1,1,3,3-tetraethoxypropane standard curve, MDA concentration of the samples were determined [16].

2.7. Measurement of superoxide dismutase (SOD) activity

The measurement of SOD was done according to the instructions of Randox kit. The basis for the reaction of SOD is the production of free radicals by xanthine and xanthine oxidase (XOD). These free radicals react with substrate 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (I.N.T) and produce dye molecules. Superoxide dismutase inhibits this reaction. The level of this inhibition depends upon the concentration of superoxide dismutase.

2.8. Measurement of glutathione peroxidase activity (GPx)

Measurement of GPx was done according to the instructions of Randox kit with Valentine and Paglia's method. First, the reduced glutathione was oxidized by glutathione peroxidase. Then, the oxidized glutathione was converted to reduced glutathione in the presence of glutathione reductase and NADPH. The decrease of light absorption as the result of conversion of NADPH to $NADPH^+$ is correlated with GPx concentration.

2.9. Measurement of tissue catalase activity

After removing the liver and homogenization in phosphate buffer (PH = 7), the pure homogeneous solution was used to measure catalase activity with hydrogen peroxide as the substrate. Catalase was measured through Aebi method. In summary, 50 µl centrifuged supernatant liquid was diluted 500 times with phosphate buffer. Then, 2 µl of this solution was poured in a cuvette. 1 ml of 30 mM hydrogen peroxide was added to it and the absorption variations were monitored at the wavelength of 240 nm against the blank in 1 min. The chemical reaction was triggered with the addition of hydrogen peroxide and then the enzyme activity was calculated [17].

2.10. Assay of total antioxidant capacity (TAC)

It was measured by ferric reducing ability of tissue (FRAP) method. This method is based on the ability of tissue in reducing Fe^{3+} to Fe^{2+} in the presence of TPTZ. The reaction of Fe^{2+} and TPTZ gives a complex with blue color and maximum absorbance in 593 nm [18].

2.11. Morphological and histopathological studies

After anesthetization and getting blood samples from the heart of the rats, their abdominal areas were opened and a piece of their liver was removed for macroscopic, histopathological, and biochemical analyses. The livers were identified and observed for any gross appearance and color change. The liver tissue was sliced and pieces were fixed in 10% formalin, dehydrated in graduated ethanol (50–100%), cleared in xylene, and embedded in paraffin. Sections 4–5 µm thick were prepared and then stained with hematoxylin and eosin (H–E) dye and examined for histopathological changes under the microscope (Olympus IX71).

2.12. Protein assay

The protein was assayed via Bradford method which is based on the binding of coomassie blue dye to proteins [19].

2.13. Statistical analysis

All of the results were reported in the form of 'mean ± standard deviation' and the analysis was done through one-way ANOVA. The significance level was taken as ($P < 0.05$). To conduct supplementary analysis, post hoc test was employed. All of the statistical analyses were performed using SPSS 16.0 software.

3. Results

3.1. The effect of TPE on biochemical factors

The effects of TPE on various biochemical factors in the groups under study are given in Tables 1 and 2. The CCl_4-damaged rats (CC) showed a significant increase in ALT, AST, ALP, cholesterol, triglyceride, LDL, and glucose levels while their albumin and HDL levels decreased significantly compared with the control group (NC) ($P < 0.001$). Pretreatment with 40, 80, and 120 mg/kg of TPE for 14 days as well as post-treatment with 80 and 120 mg/kg of TPE 2, 6, 24, and 48 h after the injection of CCl_4 led to the decrease of ALT, AST, ALP, TC, TG, LDL, and glucose levels and increase of albumin and HDL levels. The doses of 40 mg/kg in pretreatment and 80 mg/kg in post-treatment did not have a significant effect on some biochemical factors.

Table 1
Effect of TPE on liver enzymes, albumin, and glucose.

Groups	AST	ALT	ALP	albumin	Glucose
NC	156 ± 7.29	60.66 ± 3.88	621.83 ± 20.01	2.92 ± 0.11	110.33 ± 10.46
CC	1975 ± 75.9*	1290 ± 59.16*	1535 ± 72.07*	1.8 ± 0.13*	152.14 ± 12.29*
bTP40	1915 ± 48.88	1215 ± 89.38**	1495 ± 58.9	1.89 ± 0.08	145 ± 8.07
bTP80	1773 ± 68.01†	1120 ± 47.74†	1446.66 ± 49.66**	2.02 ± 0.21**	134.16 ± 15.61**
bTP120	1613 ± 56.09†	1011.66 ± 89.31†	1396.66 ± 60.88†	2.15 ± 0.23†	129.16 ± 14.17†
bSC100	1511 ± 39.7†	848.33 ± 73.05†	1338.33 ± 49.56†	2.25 ± 0.27†	126 ± 15.4†
aTP80	1913 ± 62.18	1261 ± 47.9	1515 ± 54.03	1.94 ± 0.08	135.82 ± 13.28**
aTP120	1858 ± 63.69†	1201 ± 47.9**	1446 ± 25.03**	2 ± 0.15**	135.16 ± 12.81**
aSC100	1730 ± 82.2†	1113 ± 46.33†	1418 ± 51.15†	2.11 ± 0.16**	132 ± 13.82**

Results presented in the table were expressed as the mean values ± standard deviation (SD) for 6 rats in each group. * shows the significance of differences relative to the normal control group ($P < 0.001$). ** and † show significance of differences relative to the damage control group ($P < 0.05$ and $P < 0.001$ respectively).

Table 2
Comparison of lipid profile in study groups.

Groups	Cholesterol	TAG	LDL	HDL
NC	54.33 ± 4.41	58.5 ± 4.63	4.8 ± 3.08	37.83 ± 1.72
CC	80.28 ± 4.34*	90.57 ± 5.25*	44.02 ± 3.81*	18.14 ± 1.4*
bTP40	78.33 ± 6.83	86.66 ± 6.68	40.36 ± 7.05	20.63 ± 1.4**
bTP80	73.5 ± 3.56**	82 ± 2.68**	34.61 ± 3.66†	22.48 ± 0.75†
bTP120	71.5 ± 3.14†	78.16 ± 4.07†	31.63 ± 3.28†	24.23 ± 1.37†
bSC100	67.5 ± 3.88†	74.5 ± 4.63†	27.46 ± 4.53†	25.13 ± 1.57†
aTP80	76.8 ± 3.6	86 ± 5.05	39.9 ± 2.9	19.7 ± 1.17**
aTP120	72.33 ± 4.27**	80.33 ± 3.7†	35.2 ± 3.9†	21.01 ± 1.44†
aSC100	70.5 ± 4.41†	76.5 ± 3.39†	33.15 ± 4.3†	22.05 ± 0.07†

Results presented in the table were expressed as the mean values ± standard deviation (SD) for 6 rats in each group. * shows the significance of differences relative to the normal control group ($P < 0.001$). ** and † show significance of differences relative to the damage control group ($P < 0.05$ and $P < 0.001$ respectively).

3.2. The effect of TPE on lipid peroxidation levels

Comparison of tissue MDA in CCl_4-damaged rats (CC) with the healthy rats (NC) indicated a significant increase in MDA level after the induction of oxidative stress ($P < 0.001$). However, pretreatment with the doses of 80 and 120 mg/kg and post-treatment with the dose of 120 mg/kg TPE decreased the level of lipid peroxidation which resulted in the decrease of MDA level compared to the group without treatment (Fig. 1). Silymarin also decreased lipid peroxidation level in both pre- and post-treatment groups.

3.3. Comparing the levels of oxidative stress factors in the groups under study

Comparing the levels of antioxidant catalase, superoxide dismutase, and glutathione peroxidase in the tissues of CCl_4-damaged rats (CC) with healthy rats (NC) demonstrated a significant decrease in the levels of these enzymes after the induction of damage and inflammation ($P < 0.001$). However, pretreatment with the doses of 40, 80, and 120 mg/kg and post-treatment with the dose of 120 mg/kg TPE could prevent the decrease in the activities of catalase, superoxide dismutase, and tissue glutathione peroxidase compared to the group without treatment (Figs. 2–4). Moreover, silymarin significantly increased antioxidant enzymes in the damaged rats in both pre- and post-treatment groups. Fig. 5 shows the effect of TPE on plasma total antioxidant capacity in CCl_4-damaged rats. The level of effectiveness increases with the increase in the dose of TPE in both pre- and post-treatment groups. Therefore, it seems that the effect of TPE on plasma total antioxidant capacity is dependent on the dose.

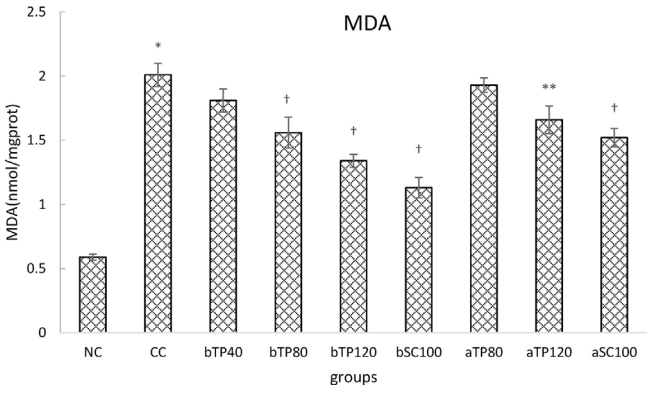

Fig. 1. The effect of pretreatment with TPE on MDA level in CCl_4-damaged rats. The bar signs on top of the columns indicate mean ± standard deviation (n = 6). * shows the significance of differences relative to the normal control group ($P < 0.001$). ** shows the significance of differences relative to the exposure group ($P < 0.05$). † shows significance of differences relative to the damage control group ($P < 0.001$).

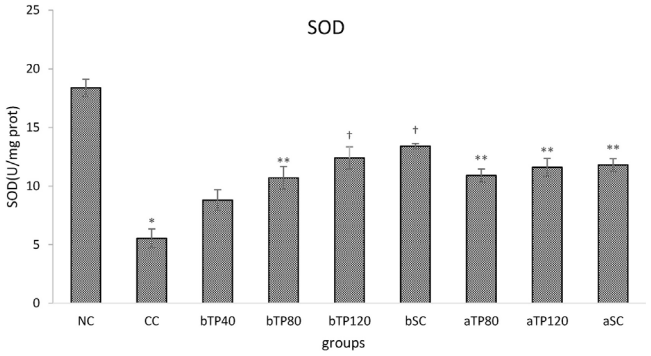

Fig. 2. The effect of TPE on the level of superoxide dismutase (SOD) in CCl_4-damaged rats. The bar signs on top of the columns indicate mean ± standard deviation (n = 6). * shows the significance of differences relative to the normal control group ($P < 0.001$). † shows significance of differences relative to the damage control group ($P < 0.001$).

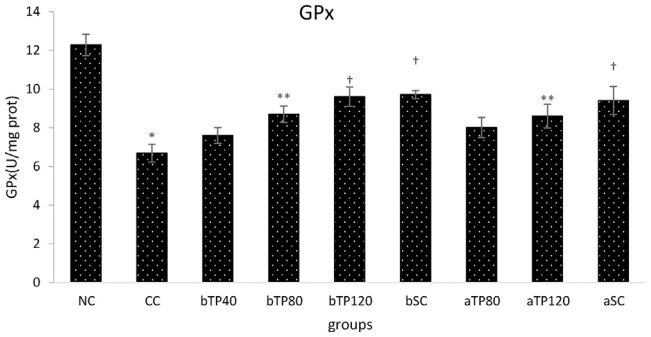

Fig. 3. The effect of TPE on the level of glutathione peroxidase (GPx) enzyme in CCl_4-damaged rats. The bar signs on top of the columns indicate mean ± standard deviation (n = 6). * shows the significance of differences relative to the normal control group ($P < 0.001$). † shows significance of differences relative to the damage control group ($P < 0.001$).

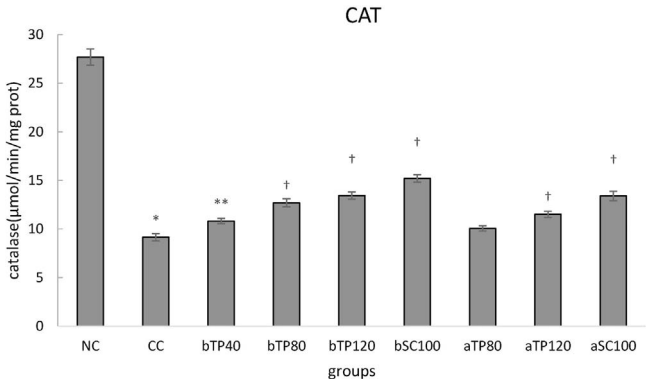

Fig. 4. The effect of TPE on the level of catalase (CAT) enzyme in CCl_4-damaged rats. The bar signs on top of the columns indicate mean ± standard deviation (n = 6). * shows the significance of differences relative to the normal control group ($P < 0.001$). ** shows the significance of differences relative to the exposure group ($P < 0.05$). † shows significance of differences relative to the damage control group ($P < 0.001$).

3.4. The effect of TPE on histopathology of liver tissue

Morphological examination of rat liver tissue at the end of the study showed the visible pale, gross, and irregular surface suggesting the severe hepatocellular damage in CCl_4-treated rats as compared to normal control group. Pretreatment with 120 mg/kg TPE as well as pretreatment with 100 mg/kg silymarin somewhat protected the liver from CCl_4-induced injuries (Fig. 6).

The hepatoprotective effect of TPE on CCl_4-induced liver damage was further confirmed by histopathological examinations. The liver

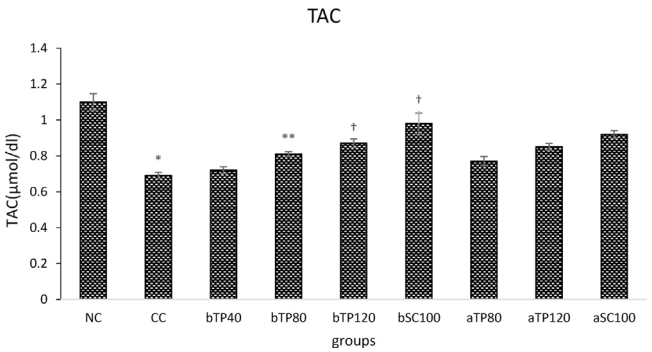

Fig. 5. The effect of TPE on the level of plasma total antioxidant capacity (TAC) in CCl_4-damaged rats. The bar signs on top of the columns indicate mean ± standard deviation (n = 6). * shows the significance of differences relative to the normal control group ($P < 0.001$). ** shows the significance of differences relative to the exposure group ($P < 0.05$). † shows significance of differences relative to the damage control group ($P < 0.001$).

samples administered with only CCl_4 indicated damages such as vacuolization of cells, more percentages of necrosis, dilation of disse space, infiltration of inflammatory cells, bridging necrosis, and degeneration of biliary. However, in the groups administered with TPE, less percentages of necrosis were observed both in pre- and post-treatment groups. Moreover, tissue healing after necrosis and degeneration of biliary in livers showed that TPE can be compared with standard drug silymarin in terms of its effectiveness on the tissue (Fig. 7).

4. Discussion

The findings of this study demonstrated that injection of CCl_4 to rats induces severe liver damage which is detected due to the increase in the serum levels of AST, ALT, and ALP. Damage to liver cells changes their functional transition, causes membrane permeability, and leads to the leakage of enzymes into extracellular space [20,21]. Pre- and post-treatment with TPE largely modulated the severity of CCl_4-induced liver damage. Enzyme levels' return to near-normal levels in treated rats shows that TPE can stabilize liver cell membranes and prevent the leakage of enzymes. Preventing the production of free radicals and neutralizing them as well as the protection potential of this plant against hepatotoxins can be other probable reasons for the healing effect of TPE [22]. Another indicator of damage to liver cells is lipid peroxidation [23]). In fact, lipid peroxidation shows the imbalance between the amount of free radicals and antioxidants in the body. That is, if the amount of antioxidants in the body is less than the amount of oxidants, these extra oxidants can bind with body's critical compounds including the double bonds of membrane and cause damage [24].

The results of MDA measurement in this study revealed that pre-treatment with TPE in all doses except for the dose of 40 mg/kg and post-treatment just with the dose of 120 mg/kg decreased lipid peroxidation. Some previous studies have indicated that compounds such as isoquercetin, kaempferol, sabinene, tannins, and flavonoids can prevent lipid peroxidation and damage to cells [25,26]. Regarding that TPE also contains these compounds as well as other antioxidants such as flavanols, apigenin, and luteolin, the decrease of lipid peroxidation in the groups treated with TPE might be due to the presence of scavenger compounds in it [27]. These findings were consistent with the findings

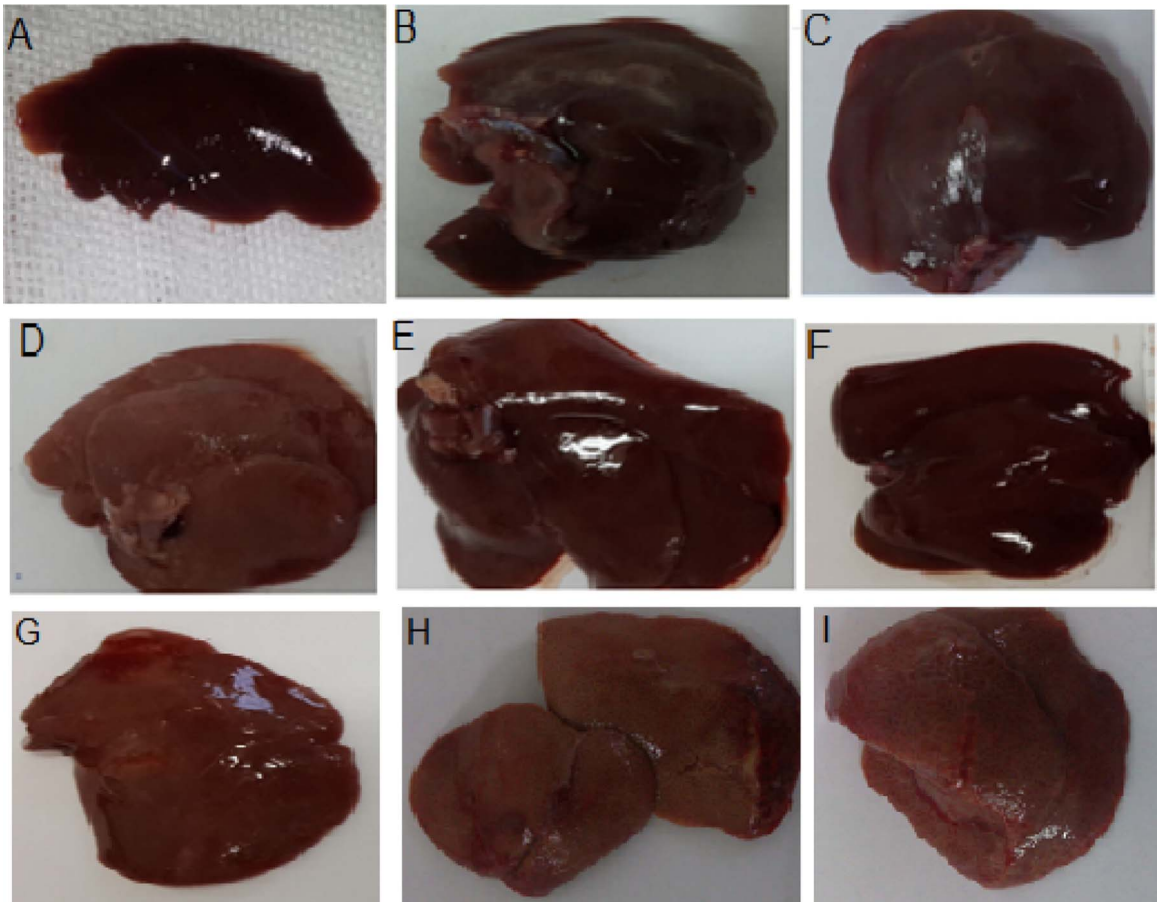

Fig. 6. Macroscopic images of liver tissue in the groups under study. A: normal group, B: exposure group, C: pretreatment with 40 mg/kg TPE, D: pretreatment with 80 mg/kg TPE, E: pretreatment with 120 mg/kg TPE, F: pretreatment with 100 mg/kg silymarin, G: post-treatment with 80 mg/kg TPE, H: post-treatment with 120 mg/kg TPE, and I: post-treatment with 100 mg/kg silymarin.

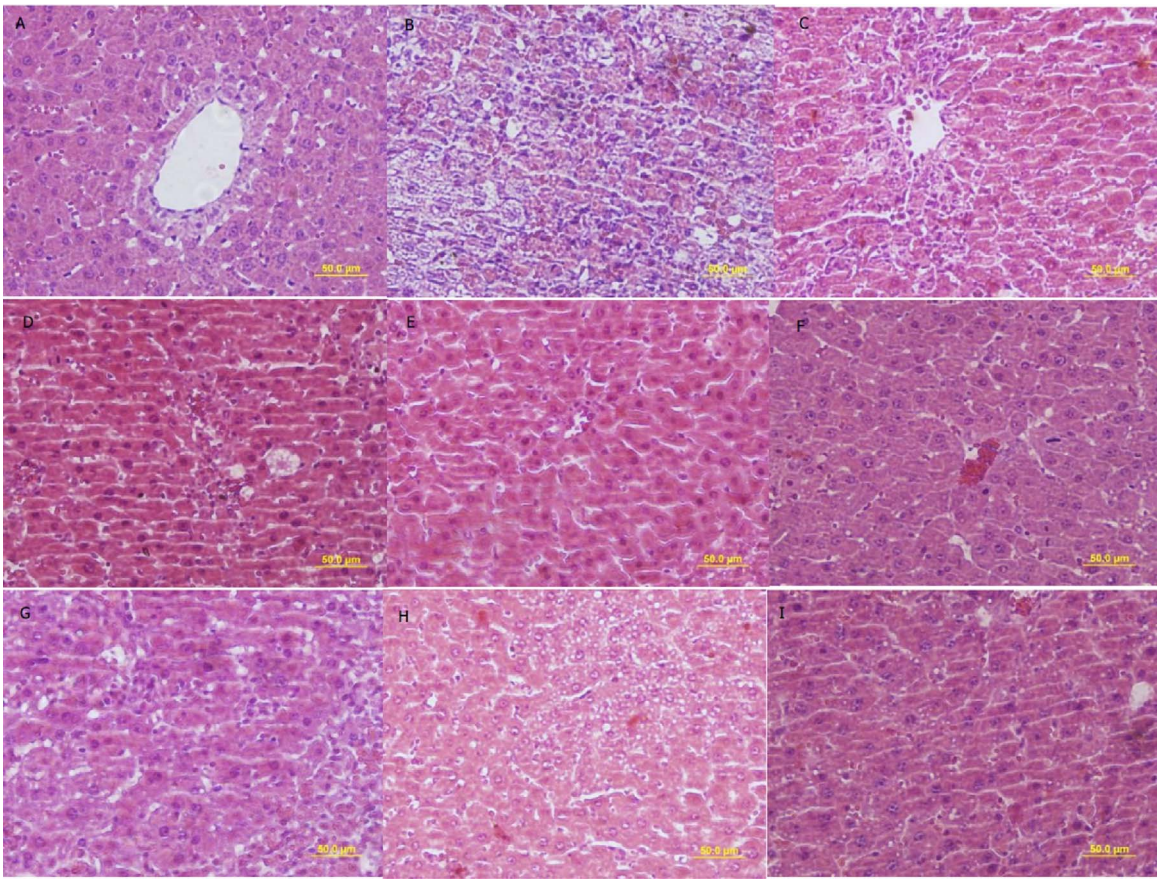

Fig. 7. H & E stained microscopic images of liver tissue in the groups under study. A: normal group, B: exposure group, C: pretreatment with 40 mg/kg TPE, D: pretreatment with 80 mg/kg TPE, E: pretreatment with 120 mg/kg TPE, F: pretreatment with 100 mg/kg silymarin, G: post-treatment with 80 mg/kg TPE, H: post-treatment with 120 mg/kg TPE, and I: post-treatment with 100 mg/kg silymarin.

of Hussain et. al.'s study in which they indicated that *Alcea Rosea* extract has antioxidant properties against free radicals due to having isoquercetin and kaempferol [25].

Liver plays a fundamental role in the metabolism of lipids, carbohydrates, and proteins. Injection of CCl_4 caused a significant increase in the triglyceride, total cholesterol, and LDL levels and decrease in HDL level. The decrease in protein synthesis and disruption in the metabolism of phospholipids might be involved in abnormal lipoprotein levels. Administration of TPE in both pre- and post-treatment groups modulated lipid profiles. Increase in the cholesterol levels might be due to the increased esterification of fatty acids, inhibition of fatty acid β-oxidation, and decreased excretion of cellular lipids [28]. CCl_4 stimulates the transfer of acetate into liver cells (probably by increasing access to acetate) and leads to an increase in cholesterol synthesis. It also increases the synthesis of fatty acids and triglyceride from acetate and enhances lipid esterification [7]. The accumulation of triglyceride in liver might occur due to the inhibition of lysosomal lipase activity and VLDL secretion [29]. Some research studies have also reported that during toxicity by CCl_4, fats stored in peripheral adipose tissue are transported to liver and kidney and accumulated there [30,31,7]. Moreover, the findings of Kamalakkannan et al.'s study indicated that CCl_4 also inhibits the synthesis of apo-lipoprotein which in consequence reduces the synthesis of lipoproteins [32]. Pretreatment with 80 and 120 mg/kg and post-treatment with 120 mg/kg TPE decreased cholesterol, triglyceride, and LDL levels and increased HDL level. From among the antioxidant compounds, phenolic compounds are the strongest in inhibiting lipid peroxidation [32]. Compounds containing hydroxyl group, especially those having it at ortho position, can easily give electron to free radicals. Regarding that TPE is rich with hydroxyl-containing sesquiterpene compounds [33], it can probably inhibit the damaging effects of ROS and prevent LDL and cell membrane oxidation. This action, on the one hand, causes the decrease of acetate transport to liver cells which results in a decrease in the synthesis of cholesterol, free fatty acids, and triglyceride. On the other hand, by increasing HDL production and decreasing LDL production, it facilitates the breakdown of acetate by liver. These findings of our study were consistent with the findings of other studies [32,29,34].

Liver is the main site of protein synthesis, especially albumin. In this study, albumin was used to assess liver synthetic function [35]. The increase in albumin level after the injection of TPE indicates that this extract can prevent the decrease of albumin probably through stabilizing endoplasmic reticulum and resynthesizing protein or through neutralizing ROS by scavenger compounds.

Antioxidant enzymes are sensitive to severe damage to cells [36]. It can be said that the decrease in SOD, GPx, and CAT levels indicates severe damage to liver by CCl_4. Damage to liver following the injection of CCl_4 is the result of lipid peroxidation induced by the free radicals derived from CCl_4. Therefore, antioxidant activity and the inhibition of free radical production are important in preventing CCl_4-induced hepatopathies. The body has effective defense mechanisms to prevent and neutralize free radicals. This action is finely accomplished through regulating endogenous antioxidants such as glutathione peroxidase, superoxide dismutase, and catalase. These enzymes form a shared support system against ROS. In CCl_4-induced liver damages, the balance between ROS production and antioxidant defense system of these enzymes is disturbed as a result of oxidative stress which disrupts cellular functions through some events and causes liver damage and necrosis. In normal conditions, ROS is detoxified via antioxidant system which contains both enzymatic and non-enzymatic antioxidants. SOD is a special antioxidant for the conversion of anyone superoxide to

hydrogen peroxide which is detoxified via catalase and glutathione peroxidase (GSH-px). Therefore, GSH-px uses GSH as the substrate and excretes free radicals with the cooperation of SOD [37]. Return of the subjects to normal after being damaged by CCl_4 in the groups administered with TPE might be due to the ability of this extract to stimulate these antioxidant enzymes to counteract the ROS produced by CCl_4. Non-enzymatic antioxidant systems like GSH determine tissue sensitivity to oxidative damages. Depletion of liver GSH causes the increase of liver sensitivity to chemical substances like CCl_4 and paracetamol [38].

Some previous studies have shown that TPE has antidiabetic and antihyperglycemic effects [39]. In assaying the effect of TPE on blood glucose, it was observed that CCl_4 injection significantly increases blood glucose level in damaged rats. In addition, administration of TPE in pretreatment groups prevented hyperglycemic effects of CCl_4. Regarding that liver is the main site of carbohydrate metabolism and its metabolic pathways including glycolysis, gluconeogenesis, and glycogenolysis occur in this part of the body, the reason for the increase in blood glucose levels in damaged rats might be due to severe damage to the liver or to the pancreas as the result of interference in the metabolic pathways of carbohydrates. Some previous findings have indicated that the flavonoid substances in the plants have antioxidant and antidiabetic effects [40]. Considering the fact that TPE is rich with flavonoids, it seems that the observed effects are related to the return of antioxidant enzymes to normal levels or the prevention of severe damage to liver or pancreas [41]. TPE might exert its effects by preventing the destruction of liver parenchyma via neutralizing ROS.

The hepatoprotective effect of TPE on CCl_4-induced liver damage was further confirmed via histopathological examinations. The liver samples administered with only CCl_4 indicated damages such as vacuolization of cells, more percentages of necrosis, and degeneration of biliary [21]. However, in the groups administered with TPE, less degeneration was detected and tissue healing was observed after necrosis and degeneration of biliary in the liver. It seems that the extract of this plant can prevent liver damage or lead to the reconstruction of damaged liver parenchyma.

5. Conclusion

The findings of this study showed that TPE has antioxidant effects and can protect liver against damages induced by free radicals which are produced as the result of CCl_4 metabolism. The results also demonstrated that the extract of this plant can decrease the abnormally increased levels of cholesterol and triglyceride which indicates its antihyperlipidemic properties.

Conflict of interest

The authors declare that there are no conflicts of interest.

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