

WITHDRAWN: The Amelioration Role of Acacia Senegal on the Systemic Oxidative Stress and Necroinflammation Induced by Carbon Tetrachloride in Rats

Noha Habashy

noha.habashi@alexu.edu.eg

Alexandria University

Marwa Abu-Serie

City of Scientific Research and Technological Applications

Asmaa Hamouda

Jazan University

Research Article

Keywords: Acacia Senegal, Systemic Oxidative Stress, Systemic necroinflammation, CCl₄, Rats

Posted Date: February 4th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-155300/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

EDITORIAL NOTE:

The full text of this preprint has been withdrawn by the authors while they make corrections to the work. Therefore, the authors do not wish this work to be cited as a reference. Questions should be directed to the corresponding author.

Abstract

Acacia Senegal (AS, Gum Arabic) is a polysaccharide with several potential health benefits that is recognized by the Food and Drug Administration (FDA) agency as a secure dietary fibre. The present research evaluated the systemic oxidative and necroinflammatory stress induced by CCl₄ administration and the alleviating effect of AS aqueous extract (ASE, 7.5 g/Kg b.w.). The results demonstrated the presence of certain phenolic compounds in ASE as well as its *in vitro* potent scavenging ability to ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), NO, and lipid peroxide radicals. Also, the outcomes observed an improvement in the CCl₄-induced liver, lung, brain, and spleen toxicity by reducing ROS, lipid peroxidation, NO, and the pro-inflammatory mediators. Moreover, the TAC (total antioxidant capacity) as well as the enzymatic and non-enzymatic antioxidants were significantly upregulated in these organs after the treatment with ASE. These results were confirmed by enhancing the morphological features of each organ. Therefore, ASE can ameliorate the systemic toxicity induced in rats by CCl₄ due to its antioxidant and anti-inflammatory potential, which is related to its phytochemical composition.

Introduction

Oxidative stress exhibits an asymmetry between the exposure to the reactive oxygen species (ROS) and the ability of the physiological system to directly scavenge the reactive intermediates or adjust the outcome's damage¹. Disturbances in the normal redox state of cells can produce lethal results by building ROS that damage all cell parts, involving lipids, proteins, and DNA^{1,2}. ROS performs a crucial role in the pathogenesis of various human organs, including liver, heart, lung, spleen, and kidney^{3,4}. Various toxic chemicals, such as carbon tetrachloride (CCl₄), bromobenzene, aromatic hydrocarbons, and methanol⁵, are implicated in the development of organ damage by generating ROS^{5,6}. CCl₄ is a prevalent hepatotoxin used to provoke liver complications in various experimental studies^{7,8}. Its hepatotoxic effect is demonstrated histologically to induce fibrosis, hepatocellular death, and cirrhosis^{9,10}. CCl₄ is incorporated with its quick break by cytochrome P450 (CYP2E1) in liver cells, providing trichloromethyl radicals (CCl₃*) driving lipid peroxidation and subsequent membrane impairment^{4,9}. In addition to this, CCl₄ caused the activation of hepatic Kupffer cells that generate inflammatory mediators and ROS, yielding an ending in hepatic parenchymal cells' distress^{2,10}. CCl₄ can also induce similar damage to other organs such as kidney, lung, and spleen¹¹.

Nowadays, numerous functional foods and their ingredients were involved in the amelioration of the chemical's toxicity due to their antioxidant and anti-inflammatory roles¹². *Acacia Senegal* (AS, Gum Arabic) is one of these functional foods that have numerous medicinal effects. It is a neutral or slightly acidic dietary fibre that is present naturally as mixed magnesium, calcium, and potassium salt polysaccharidic acid^{13,14}. Gum Arabic has anti-inflammatory and antioxidant activities and protects from kidney, liver, and heart injury. It also ameliorates some biochemical, physiological, and behavioral effects in rats^{15,16} besides its ability to relieve the disadvantageous effects of chronic kidney malfunction in

humans^{14,16}. Few previous studies have assessed the ameliorative effect of AS on the drug and chemical-induced toxicity^{17,18}, but no study has evaluated its effect on CCl₄-induced systemic damage. Thus, the present work was conducted to evaluate the therapeutic effect of AS aqueous extract (ASE) on the CCl₄-induced toxicity in rat liver, lung, brain, and spleen tissues. The necroinflammatory and oxidative stress mediators have been examined due to their critical role in systemic destruction. Moreover, the phytochemistry and the *in vitro* antioxidant activity of ASE was assessed to provide a more persuasive proof of its therapeutic opportunities.

Results

Phenolic content and antioxidant activity of ASE

The phytochemical analysis showed that ASE contains some amounts of phenolic compounds (0.717 ± 0.102 mg gallic acid eq/g ASE) and has no flavonoids. Certain types of these phenolic compounds were identified by HPLC (Fig. 1A) such as gallic acid (0.1 µg/g ASE), ellagic acid (1.6 µg/g ASE), benzoic acid (0.3 µg/g ASE), and O-coumaric acid (0.1 µg/g ASE). However, the retention times (RT) of the other tested standard phenolics (catechol, caffeine, vanillin, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, cinnamic acid, and salicylic acid) didn't match any RT in the ASE chromatogram.

Regarding the antioxidant activity of ASE (Fig. 1B), it exhibited potent anti-lipid peroxidation activity (β-carotene bleaching activity) that was significantly ($p < 0.05$) higher than that of butylated hydroxytoluene (BHT) by 73.754%. In addition, it showed high scavenging ability to the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) cation radical and NO radical, but less than that of BHT by 1187.058% and 714.527%, respectively.

The ameliorating effect of ASE on CCl₄-induced systemic toxicity

The results of the present study demonstrated that ASE was able to ameliorate the CCl₄-induced toxicity in liver, lung, brain, and spleen. This was indicated by a significant improvement in the oxidative stress and necroinflammation in these tissues.

Organ/body weight ratio and liver function markers

The organ/b.w ratio results showed a non-significant difference between all the studied groups for the liver and brain tissues. However, a significant ($p < 0.05$) elevation in these ratios was observed in the lung and spleen tissues after CCl₄ administration only, by 276.431 and 86.067%, respectively (Fig. 1C).

The serum analysis for the liver function markers (Fig. 1D) showed significant ($p < 0.05$) decline in the ALT activity (75.146%) and albumin level (36.707%) in the rats that were injected with CCl₄ compared to the control rats. The remaining studied serum markers (AST and total proteins) revealed slight changes (7.323% and 3.287%, respectively) than the control. Concerning rats in the CCl₄-ASE group, the ALT

activity and albumin level were significantly ($p < 0.05$) increased by 604.471% and 21.081%, respectively, but there was no change in the AST activity (21.254%) and total protein level (6.134%) compared to those in the CCl_4 group. The V group displayed a non-significant difference in the ALT activity (13.255%), AST activity (2.834%), albumin level (3.584%), and total protein level (7.658%), relative to the control. Similarly, the serum of rats that administered ASE alone showed slight differences in the AST activity (5.883%), albumin level (12.640%), and total protein level (9.550%) relative to control. In contrast, the activity of ALT was significantly ($p < 0.05$) elevated (195.865%) in the rats of this group compared to that in C group.

Improving the CCl_4 -induced hepatic redox state disturbance by ASE

Figure 2A and B shows that CCl_4 induced hepatic oxidative stress, which was explained by a significant ($p < 0.05$) elevation of ROS (256.163%), NO (369.80%), and lipid peroxidation (904.382%) levels as well as myeloperoxidase (MPO) activity (163.715 %) relative to the C group. This is connected with significant ($p < 0.05$) decrease in the TAC (55.495%), enzymatic (superoxide dismutase "SOD" (46.893%) and glutathione peroxidase "GPX" (81.913%)) and non-enzymatic (reduced glutathione "GSH" (54.123%)) antioxidants relative to the C group (Fig. 2A, C, D). While the administration of ASE after CCl_4 injection (CCl_4 -ASE group) significantly ($p < 0.05$) reduced the levels of ROS, NO, TBARS, and the activity of MPO by 48.057%, 59.599%, 68.208%, and 26.847%, respectively, relative to the CCl_4 group (Fig. 2A, B, D). Moreover, ASE significantly ($p < 0.05$) augmented the levels of hepatic TAC (103.374%) and GSH (19.864%), and the activities of GPX (346.491%) and SOD (92.01%) compared to those in the CCl_4 group (Fig. 2C, D).

Regarding rats that were injected with olive oil alone (V group), they showed non-significant changes in all the studied oxidative stress parameters. The changes in ROS, TAC, TBARS, NO, GPX, GSH, SOD and MPO were 7.520%, 21.979%, 21.823%, 34.118%, 3.536%, 3.339%, 12.207%, and 4.219%, respectively, compared to those in the control rats. On the other hand, the rats that were administered ASE alone for 10 days (ASE group) showed a non-significant change in the levels of ROS, TAC, TBARS, and NO and the activity of SOD and MPO than the control (7.779%, 6.143%, 5.492%, 1.859%, 3.550%, and 5.953%, respectively). While the activity of GPX and the level of GSH were significantly ($p < 0.05$) elevated by 12.783% and 20.611%, respectively when compared to those in the C group (Fig. 2C).

Improving the CCl_4 -induced brain redox state disturbance by ASE

Figure 3A and B shows the toxic effect of CCl_4 on brain tissue through the induction of oxidative stress, which is indicated by the significant ($p < 0.05$) rise in the levels of intracellular ROS (185.182 %), NO (140.579%), and TBARS (480.468%) as compared to the C group. Otherwise, there was a non-significant (36.179%) difference in the MPO activity in the brain tissue of rats injected with CCl_4 and the control ones (Fig. 3D). Moreover, the cellular redox state (TAC), the activity of GPX and SOD, and the level of GSH were extremely reduced by 63.778%, 42.000%, 37.463 %, and 58.677%, respectively related to the C group

(Fig. 3A, C, D). Treatment of CCl₄-injected rats with ASE (CCl₄-ASE group) significantly incremented the cellular redox state of the brain tissue. This was shown by a significant ($p < 0.05$) decrease in the levels of accumulated intracellular ROS (28.951%), TBARS (61.184%), and NO (46.038%), respectively relative to that of CCl₄ group (Fig. 3A, B). In contrast, the data analysis found a significant increase ($p < 0.05$) in the TAC (48.006%) and GSH (59.799%) levels and the GPX (51.519%) and SOD (72.019%) activities after ASE treatment compared to those in the CCl₄ group (Fig. 3A, C, D). While a non-significant change (41.511%) was observed in the activity of MPO in this group compared to those in the CCl₄ group (Fig. 3D).

The results also detected a significant ($p < 0.05$) elevation in the ROS, TBARS, and NO levels in the brain of rats injected with olive oil only (V group) compared to those in the control group (Fig. 3B). However, the antioxidant indices (Fig. 3A, C, D) in the brain of these rats, such as the TAC (44.444%), GSH (29.822%), GPX (17.578%), and SOD (30.741%) were significantly ($p < 0.05$) diminished relative to control. But the MPO activity decreased insignificantly (17.903%) in the brain tissue of these rats, relative to the control group (Fig. 3D). On the other hand, administration of ASE alone (ASE group) insignificantly affected the levels of ROS (11.584%), TAC (0.500%), TBARS (11.519%), NO (0%), and GSH (8.239%) or the activities of GPX (3.209%), SOD (7.587%), and MPO (30.220%) compared to those in the control group.

Improving the CCl₄-induced lung redox state disturbance by ASE

Figure 4A, B shows that CCl₄ caused harm to the lung redox state with a massive rise in the levels of ROS (591.297%) and NO (483.908%), compared to those in the C group, which led to an incredible formation of TBARS (361.120%). Also, compared to that of the control group, there was a significant increase in the MPO activity by 57.318%. Conversely, the lung enzymatic (GPX and SOD) and non-enzymatic (GSH) antioxidants enormously reduced by 69.426%, 27.635%, and 82.387%, respectively, compared to those in the C group (Fig. 4C, D). Consequently, TAC of lung tissue significantly dropped in the CCl₄-injected rats by 65.780%, related to the control group (Fig. 4A). The treatment with ASE after CCl₄-injection (CCl₄-ASE group) improved the redox state in lung tissue extremely. This occurred due to the significant ($p < 0.05$) decrease in the studied oxidative stress parameters, ROS, NO, TBARS, and MPO levels, by 43.448%, 50.098%, 72.211%, 20.594%, respectively, compared to those in the CCl₄ group (Fig. 4A, B, D). Besides, ASE significantly ($p < 0.05$) enhanced the lung antioxidant system (SOD, GPX, and GSH) by 48.505%, 160.157%, and 386.431%, relative to the CCl₄ group (Fig. 5C, D). These results concomitantly led to an increase in the lung tissue TAC (64.683%) related to that in the CCl₄ group (Fig. 5A).

On the other hand, the lung tissue of rats in the vehicle (V) group showed an alteration in the redox state parameters. Hence ROS, NO, and TBARS significantly ($p < 0.05$) increased by 43.619%, 158.818%, and 24.490%, compared to the C group. Also, the antioxidant parameters, including GPX (22.390%), GSH (24.611%), and SOD (3.469%) depleted significantly ($p < 0.05$) and as a result, the TAC (27.389%) decreased compared to the control group (Fig. 4A, C, D), while the activity of MPO in the lung of the rats in this group insignificantly increased (0.456%), compared to that in the C group. Nevertheless, the

administration of ASE alone for 10 days (ASE group) displayed non-significant changes in the studied oxidative stress parameters in lung tissue compared to the C group. These parameters include ROS (12.146 %), TBARS (2.306%), NO (10.344%), MPO (11.409%), GPX (18.502 %), GSH (5.813%), SOD (10.135%), and TAC (4.137%).

Improving the CCl₄-induced spleen redox state disturbance by ASE

The results showed that CCl₄ injection can also influence spleen oxidative stress and cause a massive elevation in ROS (292.700%) and NO (182.978%) levels, which leads to an excessive generation of lipid peroxide (TBARS, 395.516%), compared to that of the C group (Fig. 5A, B). Likewise, there was a significant ($p < 0.05$) elevation in MPO activity (165.852%) compared to the control group (Fig. 5D). In contrast, there was a significant ($p < 0.05$) falling in the spleen antioxidant content, GPX (77.092%), GSH (75.735%), and SOD (50.388%), related to the control group (Fig. 5C, D). Therefore, the TAC (63.063%) of spleen tissue significantly ($p < 0.05$) diminished in the CCl₄-injected rats, compared to the C group (Fig. 5A). Otherwise, the treatment of the CCl₄-injected rats with ASE (CCl₄-ASE group) enormously decreased the oxidative stress examined parameters, including ROS, NO, TBARS, and MPO level by 37.638%, 50.024%, 68.477%, 38.071%, sequentially, compared to those of the CCl₄ group (Fig. 5A, B, D). Moreover, ASE enhanced the spleen antioxidant status by a significant ($p < 0.05$) elevation in the activity of GPX and SOD, and the level of GSH by 323.942%, 124.237%, and 126.792%, compared to those in the CCl₄ group (Fig. 5C, D). In addition, the TAC of the spleen tissue was augmented by 195.244% more than the CCl₄ group (Fig. 5A).

On the other hand, the spleen of rats that were injected with olive oil only for 3 weeks (V group) showed a slight increase in the levels of ROS (10.537%), TAC (0.677%), and TBARS (16.592%), compared to the C group (Fig. 5A, B). Similarly, there was a slight change in the activities of SOD (5.972%) and MPO (36.980%) (Fig. 5D). However, the level of NO significantly ($p < 0.05$) increased by 36.504% and in contrast, the activity of GPX and the level of GSH significantly ($p < 0.05$) reduced in the spleen of rats in this group by 15.281% and 25.714%, respectively, relative to the control rats (Fig. 5B, C). Further, the administration of ASE alone for 10 days (ASE group) showed a non-significant difference in the levels of splenic ROS (0.692%), TBARS (0.605%), NO (0.426%), and GSH (0.186%), compared to the C group. Likewise, the activities of MPO (5.071%), GPX (2.398%), and SOD (7.924%) changed slightly. Nevertheless, the level of TAC (64.865%) significantly ($p < 0.05$) increased in the spleen tissue after ASE intake, compared to that in the C group.

The alleviating effect of ASE on the CCl₄-induced systemic necroinflammation

Figure 6 demonstrates the toxic effect of CCl₄ on the studied organs by upregulating the gene expression of some pro-inflammatory and pro-fibrotic cytokines. Hence, the fold expression of the nuclear factor-kappa (NF- κ)B, inducible nitric oxide synthase (iNOS), cyclooxygenase (COX)-2, and tumor necrosis factor

(TNF)- α , was highly upregulated after CCl₄ injection, compared to the control group. These elevations were observed in liver (2691.194%, 3110.867%, 2400.977%, and 847.982%, respectively), brain (1479.924%, 1322.286%, 355.513%, and 71.140%, respectively), lung (478.041%, 455.108%, 1066.674%, and 1101.253%, respectively), and spleen (907.976%, 737.934%, 449.203%, and 52.784%, respectively). Furthermore, the fold expression of the hepatic pro-fibrotic cytokines (collagen type I alpha one chain "COL1A1", transforming growth factor- β 1 "TGF- β 1") was massively increased after CCl₄ injection by 3082.635% and 2138.017%, respectively, compared to those in the C group (Fig. 6A). The results also detected a significant ($p < 0.05$) elevation in the fold expression of the interleukin (IL)-1 β (957.250%) and IL-8 (557.650%) in the lung tissue of rats in the CCl₄ group, compared to those in the C group (Fig. 6C).

As shown in the graphs in Fig. 6, the treatment with ASE (ASE-CCl₄ group) significantly ($p < 0.05$) reduced the fold expression of the pro-inflammatory cytokines (NF- κ B, iNOS, COX-2, and TNF- α) compared to the CCl₄ group. The percentages of this depletion in the liver and brain tissues were 68.969%, 45.839%, 83.713%, and 89.234%, respectively, and 43.415%, 21.889%, 81.595%, and 37.826%, respectively. Moreover, these percentages in the lung and spleen tissues were 56.183%, 91.091%, 47.988%, and 56.609%, respectively, and 41.020%, 19.242%, 6.393%, and 35.778%, respectively. Also, ASE was capable of depleting the fold expression of IL-1 β (36.460%) and IL-8 (71.668%) in lung tissue, as well as COL1A1 (12.500%) and TGF- β 1 (59.812%) in liver tissue, compared to those in the CCl₄ group.

The present study reported that the administration of olive oil only (V group) did not greatly influence the fold expression of the pro-inflammatory and profibrotic cytokines in the liver compared to the those in the control group (Fig. 6A). Hence, the percentage change of these mediators, including NF- κ B, iNOS, COX-2, TNF- α , COL1A1, and TGF- β 1, compared to those in the control group was 7.250%, 15.535%, 0.686%, 8.699%, 7.412, and 15.794%, respectively. Similarly, in the brain tissue, there was a small change in the fold expression of the studied inflammatory mediators (9.327%, 16.204%, and 10.658%, respectively), except for the COX-2, which showed a significant upregulation in its fold expression by 79.0641%, compared to that in the C group (Fig. 6B). While in the lung, the fold expression of the pro-inflammatory cytokines, comprising of NF- κ B, iNOS, COX-2, TNF- α , IL-1 β , and IL-8 were significantly ($p < 0.05$) upregulated compared to those in the C group (Fig. 6C). The percentage change for these mediators were 290.183%, 229.548%, 286.581%, 48.410%, 234.688%, and 117.364%, respectively. Regarding the spleen tissue, there was a significant ($p < 0.05$) elevation in the fold expression of some pro-inflammatory mediators (NF- κ B "177.005%", iNOS "68.012%"), and no changes in the others (COX-2 "71.528%", TNF- α "6.648 %") after the injection with the olive oil, compared to those in the C group (Fig. 6D).

Further, the intake of ASE alone (ASE group) demonstrated a non-significant change in the fold expression of NF- κ B, iNOS, COX-2, and TNF- α in the liver (6.735%, 98.537%, 6.673%, and 20.418%, respectively) and brain (99.846%, 94.732%, 4.284%, 1.494%, respectively) as shown in Fig. 6A, B. The same results were obtained with lung (7.488%, 2.495%, 7.346%, and 4.403%, respectively) and spleen (2.799%, 95.119%, 1.866%, and 0.578%, respectively) tissues (Fig. 6C, D). Besides, the fold expression of

COL1A1 (9.421%) and TGF- β 1 (2.564%) in the liver tissue (Fig. 6A) and IL-1 β (1.072%) and IL-8 (1.386%) in the lung tissue (Fig. 6C) changed slightly compared to those in the C group.

Comparison of the ASE therapeutic efficiency in the four studied organs

Supplementary Fig. 1 represents the heatmap plot, which clusters the oxidative stress and necroinflammation tested parameters that were observed after the treatment with ASE (CCl₄-ASE) in the different studied organs. The color in the heatmap elucidates the quantity (% increase relative to CCl₄ group) of these different studied parameters, the higher the quantity, the darker the color. Figure (1 A) showed two clusters, one for the liver and spleen, and the other for the lung and brain. This figure illustrates the high capability of ASE in elevating the level of TAC and the activity of GPX and SOD in the liver and spleen, more than the lung and brain. However, it increased the GSH level in the lung more than in the other organs. While the heatmap in Supplementary Fig. 1 (B) demonstrates two clusters, the liver with the lung and the brain with the spleen. The figure shows the ability of ASE to decrease the levels of ROS, NO, TBARS, NF- κ B, iNOS, and TNF- α , and the activity of MPO in liver and lung more than the brain and the spleen. Therefore, these heatmaps revealed that the liver was the most influenced tissue, and the therapeutic values of ASE and the brain were the lowest.

Histopathological results

The microscopic examination of the processed formalin-fixed sections of the studied organs revealed the systemic toxicity of CCl₄ and the ameliorating impact of ASE on the different tested organs (Fig. 7). The control sections of the liver revealed the hepatic central vein (CV) with normal lobular architecture and radiating liver cell cords. CCl₄ injection showed severe hepatotoxicity, which indicated severe steatohepatitis with fibrous bands. In addition, the features of necroinflammation such as congested blood vessels with infiltration of mononuclear leucocytes and necrotic and binucleated hepatocytes were observed. The treatment with ASE (CCl₄-ASE) restored the normal morphology of the liver with only a mild dilation of the CV. Injection with olive oil only (V group) showed severe dilation of the CV with infiltration of mononuclear leucocytes, as well as mild sinusoidal cells activation. However, no pathological changes were detected in the liver of rats that were administered ASE only for 10 days (ASE group).

Regarding the brain, the control tissues showed normal architecture with normal glial cells and neurons. This normal morphology was changed after CCl₄ injection to severe damage with severe degeneration of neurons that was characterized by the appearance of pyknotic nuclei and vacuoles in the neuronal cells. Administration of ASE restored the architecture of the brain and relieved the CCl₄ toxicity. Injection with olive oil to rats (V group) caused mild degeneration of the neurons, which appeared with pyknotic nuclei and vacuoles in addition to the accumulation of the inflammatory cells in their vicinity. All these features reflected the induction of necroinflammation in the brain tissue of rats in the V group, while the oral administration of ASE alone (ASE group) didn't affect the normal morphology of the brain.

The lung tissue in control rats had the normal appearance of the alveolar sacs and alveolar walls. Massive disruption was observed in its morphology after injection with CCl_4 , which is characterized by inflammatory cells influx with alveolar wall thickness and alveolar epithelium damage. Great amelioration in the lung morphology was observed following the administration of ASE (CCl_4 -ASE) compared to those in the CCl_4 group. Hence, the lung tissue seemed normal with only mild thickness in the alveolar septae. In contrast, thickness in the alveolar septae with infiltration of the inflammatory cells and narrowing of the alveolar air spaces have appeared in the lung tissue sections of the animals in the V group, while no abnormal features were observed in the morphology of lung tissue in the rats of the ASE group.

Regarding the spleen tissue, it appears normal with well-defined red and white pulp regions in the control rats. Severe disturbance in these regions with extreme infiltration of inflammatory cells was detected following the injection with CCl_4 . This damage was relieved after the intake of ASE (CCl_4 -ASE), except for the slight influx of inflammatory cells. Moderate disorganized white pulp compartment with unclear regions and mild recruitment of inflammatory cells were observed in the spleen tissues of the V group. However, the spleen tissues of animals in the ASE group showed normal architecture and well distinct white and red pulp compartments like the control group.

Discussion

Arabic gum has been well-known since ancient times and consists mainly of complex sugars such as galactose, rhamnose, arabinose, and glucuronic acid with about 25% proteins. In addition, the current study detected the presence of some amounts of phenolic compounds such as gallic, ellagic, benzoic, and O-coumaric acids (Fig. 1A). The presence of these compounds explains the current outcomes about the antioxidant activities of AS *in vitro* through scavenging the ABTS, NO, and lipid peroxide radicals. Hence, the antioxidant activities of the phenolic acids and reducing sugars were reported extensively in the previous researches^{2,19,20}.

AS is used in the traditional medicines of Arabs to improve renal function in patients with renal failure²¹. The present study evaluated its effect on the CCl_4 -induced systemic toxicity in rats. This highly toxic chemical caused metabolomics variations for the mammalian liver, which have been widely investigated. It is less reported in other organs^{11,22,23}. To examine the systemic toxicity induced by CCl_4 , we investigated the alterations in oxidative stress, inflammation, and necrosis markers in rat liver, lung, brain, and spleen. The CCl_4 is a common hepatic toxin that induces oxidative stress, necroptosis, fibrosis, and inflammation in rats^{2,23}. This can be due to the liver's metabolism, which is caused by CYP2E1. This catabolic process resulted in the formation of CCl_3^* , which converted to the additional reactive trichloromethyl peroxy radical (CCl_3OO^*), following its combination with oxygen^{2,24}. Formation of these radicals will augment the ROS level in the liver and induce a disruption of its redox state and in turn, cause the occurrence of the oxidative stress, leading to hepatic damage. The present study confirmed this damage in the liver after CCl_4 exposure through a significant ($p < 0.05$) depletion in the ALT activity and albumin level relative to the C group. The previous studies reported also the ability of CCl_4 to induce

metabonomic changes in rat kidney, lung, and spleen¹¹. In line with this study, the present work provided fundamental data on CCl₄ toxicity to various rat organs. Hence, injection with CCl₄ significantly ($p < 0.05$) induced disturbance in the oxidative stress indices and necroinflammation in the rat liver, brain, lung, and spleen tissues. This may be due to the free radical metabolites of CCl₄ inducing oxidative stress and generate inflammatory mediators in the liver, leading to an inappropriate inflammatory response and alteration in the functions of other organs^{11,22,23}. The present study observed a substantially high level of ROS in the brain, lung, and spleen tissues in addition to the liver tissue. Concomitant to this, the TAC and non-enzymatic (GSH) and enzymatic (GPX and SOD) antioxidants dropped dramatically compared to those in the C group in all the studied organs after CCl₄ injection. This probably occurred due to the high cellular content of the free radicals, including the NO radicals in these tested organs that affect the cellular macromolecules, particularly the membrane lipids causing lipid peroxidation (high TBARS level)^{2,10}. Peroxidation of lipids led to further formation and accumulation of ROS in the studied rat organs and resulted in the exhaustion of the cellular antioxidants such as GSH, GPX, and SOD^{25,26}. The elevation in the activity of MPO after CCl₄ injection magnifies the oxidative stress condition due to its vital role in the production of hypochlorous acid (HOCl) in the neutrophils, which interacted with and consumed the GSH. This reaction occurred in the presence of H₂O₂ and halide ions and serves in the elevation of lipid peroxidation²⁷⁻²⁹. These disturbances caused severe depletion in the cellular redox environment (TAC) in all studied organs (Fig. 2–5). All these outcomes are in agreement with the previous studies of Muhammad et al. and Limiao et al.^{3,16}.

The administration of ASE for 10 days to rats with systemic toxicity alleviated the CCl₄-induced organ damage. This was clearly noticed from the significant ($p < 0.05$) decrease in the levels of NO and TBARS and the activity of MPO along with the significant elevation in the GSH, GPX, and SOD, compared to those in the CCl₄ group. Subsequently, the balance between the ROS level and TAC of each organ was about the achievement, indicating the reduction in the oxidative stress. These results were in accordance with the previous study of Babiker et al.³⁰. The *in vivo* antioxidant effect of ASE was in line with our obtained *in vitro* results that confirm its scavenging ability to the ABTS, NO, and lipid peroxides (Fig. 1B). The ameliorating role of ASE could be linked to its composition, comprising phenolic acids, sugars, amino acids, and minerals. Hence, the free radical scavenging and antioxidant enhancement capabilities of gallic, ellagic, benzoic, and O-coumaric acids were recorded previously³¹. The AS mainly contains (97%) sugars, including L-arabinose, D-galactose, rhamnose, and glucuronic acid along with others³² that have reducing power and potent antioxidant activities³³. AS also contains different types of amino acids such as histidine, tyrosine, and lysine, which are generally considered antioxidant compounds³⁴. In addition to this, copper and zinc are two essential minerals in the AS³⁵ known for their vital role in SOD antioxidant activity³⁶. The current results (Supplementary Fig. 1, heatmap plots) revealed that the improving efficiency of ASE to the CCl₄-induced oxidative stress in the liver was more than the other organs, although the liver is the most vulnerable human organ to all aspects of injury, especially those caused by chemical toxicity, due to its essential role in xenobiotic metabolism. This may be due to the ability of this

organ to regenerate itself by replacing the dead cells with new ones. Liver regeneration is a counterbalancing response to liver injury and it is defined for some toxicants, including CCl_4 ³⁷.

Regarding the rats in the ASE group, all the studied oxidative stress parameters were similar to the control, except for the hepatic GPX activity and GSH level, as well as the splenic TAC level, which were all significantly upregulated. These results not only referred to the safety of ASE on the liver, brain, lung, and spleen but also the antioxidant enhancement ability of this extract in the liver and the spleen. This capability may be attributed to the ASE phenolics such as gallic and coumaric acids, which were reported previously to upregulate the hepatic gene expression of GPX and elevate the GSH level by increasing the level of E2-related factor (the antioxidant response element regulating transcription factor)³⁸. The improvement in the splenic TAC may be associated with the ability of ASE to modulate other types of the complex antioxidant network in this organ than those investigated in this study.

The current study interestingly revealed the mild toxicity of olive oil injection on the brain, lung, and spleen tissues. These findings were in line with the recent study of Kouka et al., who demonstrated the ability of olive oil administration to induce oxidative stress in certain tissues³⁹. This may be influenced by the multiple intakes of this antioxidant-rich oil, which has led to the consumption of the cellular antioxidant defence biomolecules, implying a prooxidant effect, which is called antioxidative stress⁴⁰. The differential prooxidant activities of olive oil in the different organs can be owed to the differences of the concentration, availability, time of persistence, and the distribution of the olive oil antioxidant molecules and their metabolites in different rat organs^{39,41}. This assumes that these organs didn't make valuable adaptations after antioxidant ingestion, and the observed negative effect may be reversed if the olive oil was administered for a longer period⁴². Thus, the systemic toxicity in rats injected with CCl_4 is associated with CCl_4 itself, not the vehicle olive oil.

To further confirm the therapeutic potential of ASE on CCl_4 -induced systemic toxicity, we examined the level of inflammatory markers in the different tissues studied (Fig. 6). In addition, the fibrotic mediators were evaluated in the liver tissue. The present study found that the fold expression of NF- κ B, iNOS, COX-2, and TNF- α was elevated in liver, brain, lung, and spleen tissues of the CCl_4 -injected rats. Moreover, the fold expression of IL-1 β and IL-8 in the lung tissue, as well as the fibrotic mediators, including COL1A1 and TGF- β in the liver tissue, were significantly ($p < 0.05$) raised. The inflammatory process is the earliest healing and defence mechanism of tissue damage and there is a serious concern about the commitment of the ROS in supporting this process. Hence, ROS can activate NF- κ B and, in turn, it can result in upregulating the gene expression of the relevant pro-inflammatory mediators such as iNOS, COX-2, and TNF- α ⁴³. Therefore, due to the increased level of ROS in the rat liver, brain, lung, and spleen after CCl_4 injection, the NF- κ B with other pro-inflammatory cytokines were upregulated. Activation of the NF- κ B pathway interrelated to the steatohepatitis⁴⁴ that was observed in the histopathological photomicrographs after CCl_4 injection (Fig. 7). The hepatic morphology of the rats in the CCl_4 group also showed the presence of fibrous bands that may be linked to the upregulation of COL1A1 and TGF- β 1 gene expression (Fig. 6). These two cytokines are secreted from the activated hepatic stellate cells and

serve as essential activators for the hepatic fibrogenesis⁴⁵. Further, the IL-8, IL-1 β , and TNF- α are the main responsible cytokines for inflammatory cells infiltration in the lung tissue. It was reported that IL-1 β and TNF- α are the key regulators for IL-8 expression⁴⁶. TNF- α and IL-1 β stimulate NO production by iNOS, which induces nitrosative stress due to the formation of the highly reactive peroxynitrite radical after NO interaction with superoxide radical. As a result, the lung tissue showed thickening in the airway wall (Fig. 7), which reflected the mucosal inflammation, increase in mucus glands, muscle mass, and vessel area, as well as connective tissue deposition on the extracellular matrix⁴⁷. The upregulation of COX-2 gene expression also can augment the CCl₄-induced systemic damage by catalyzing the formation of prostaglandin H₂ from arachidonic acid along with the production of superoxide radicals²⁶. Furthermore, TNF- α and IL-1 β activate cellular apoptosis and necrosis pathways by altering the balance of the receptor and the ligand⁴⁸. On the other hand, the elevation of the MPO activity also contributed to increasing the systemic inflammatory response of the CCl₄ by activating the neutrophils²⁸. Therefore, the injection of CCl₄ to rats induced systemic necroinflammation by recruitment of the inflammatory cells to the different studied organs (Fig. 7), as well as cell necrosis by upregulating the TNF- α fold expression. All of these results are in accordance with our previous recent study².

The administration of ASE (CCl₄-ASE group) relieved the systemic inflammatory state induced by CCl₄ by downregulating the gene expression of the proinflammatory mediators, including the NF- κ B, iNOS, COX-2, TNF- α in all the studied organs. Also, the gene expression of IL-1 β and IL-8 in lung tissue was significantly ($p < 0.05$) decreased, compared to those in the CCl₄ group. Moreover, ASE ingestion significantly ($p < 0.05$) declined the hepatic profibrotic markers (COL1A1, TGF- β) compared to those in the CCl₄ group. These results were in harmony with the histopathological data that showed an improvement in the morphology of the different studied organs (Fig. 7). The anti-inflammatory and antifibrotic impacts of ASE that were confirmed previously^{49,50} may be related to its potent antioxidant activity and its potential effect in reducing the main inflammatory inducer, ROS. All of these influences may be implied in the presence of the antioxidant polyphenols in ASE such as ellagic, gallic, benzoic, and O-coumaric acids⁵¹. These results were in line with the previous investigations that revealed the ability of AS to block or treat the toxic signs of some drugs such as aspirin, acetaminophen, and cisplatin. Additionally, it plays a possible prophylactic role against the toxicity of some chemicals such as trichloroacetic acid and mercuric chloride⁵²⁻⁵⁴. The cluster heatmap plot (Supplementary Fig. 1) revealed that the anti-inflammatory potency of ASE was higher in the liver than in the other organs. This may be due to the regeneration ability of the liver that requires certain types of cytokines such as TNF- α , NF- κ B, and other factors. Hence, TNF- α is one of the hepatocytes priming factors that enhance their proliferation in addition to its stabilization role to the NF- κ B for hepatic regeneration³⁷. Further, the administration of ASE alone for 10 days (ASE group) demonstrated non-significant changes in the studied proinflammatory and profibrotic mediators in all the investigated organs. Furthermore, the architecture and morphology of the studied organs are still well preserved.

On the other hand, the injection with olive oil only (V group) had no adverse effect on the liver but increased the gene expression of certain inflammatory cytokines in the brain, lung, and spleen (Fig. 6). These adverse effects may be owed to the inability of these organs to make adaptations after the olive oil intake as discussed above⁴². These biochemical outcomes were in agreement with the histopathological findings that showed infiltration of inflammatory cells in the brain, lung, and spleen, along with other pathological changes. However, slight changes were observed in the liver of rats in this group (Fig. 7).

Conclusions

The current study revealed the toxic effect of CCl₄ on rat liver, brain, lung, and spleen (systemic toxicity) and the therapeutic impact of ASE. The biochemical and histopathological findings explained the mechanism of ASE in alleviating this toxicity through a decrease in systemic oxidative stress and necroinflammation induced by CCl₄. The phenolic compounds, sugars, and other valuable constituents of ASE are the main cause for this potency. Therefore, ASE can be a promising extract that can be used for the treatment of the systemic toxicity induced by CCl₄.

Materials And Methods

Chemicals

Folin-Ciocalteu reagent, phenolic standards, ABTS, BHT, CCl₄, 2',7'- dihydro-dichlorofluorescein diacetate (DCFH-DA) probe, thiobarbituric acid (TBA), tetra methoxy propane (TMP), GSH, and O-dianisidine dihydrochloride (ODD) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Gene JET RNA purification kit, cDNA synthesis kit, and SYBR green master mix 2X kit were provided from Thermo Fisher Scientific, USA. Forward and reverse primers were bought from Bioneer, Korea. ALT and AST, albumin, and protein kits were obtained from Biosystem, Spain. Other chemicals were received with a high grade.

Animals

Fifty male Albino rats were obtained from MISR University for Science and Technology with pet welfare (assurance number: A5865-01). The rats were adapted under ordinary circumstances of about 30°C temperature with a 12-hour light-dark period for two weeks. Throughout this time, the rats had free access to tap water and regular nutrition as required. All experimental protocols and methodology were carried out in accordance with the Institutional Animal Care and Use Committee (IACUC) and approved by the Alexandria University Committee of Animal Care and Use. This study was carried out in compliance with the ARRIVE guidelines.

Preparation of ASE

The AS (NCBI:txid138043) was provided from Egypt and handled to prepare the crude extract. It was crushed using an electric grinder (Telstar, Terrassa, Spain) to receive the powder; and 50 g of it was dissolved in 200 mL of distilled water and left for 24 h in a refrigerator (4°C) to be dissolved completely.

The solution was filtered to eliminate the impurities and then lyophilized to get the powdered extract (ASE), which was stored at -20°C until used.

Phytochemical analysis of ASE

The total amount of flavonoids and phenolics in ASE were assessed using the spectrophotometric and chromatographic methods. Total phenolics were measured by Folin-Ciocalteu reagent and the calibration curve of gallic acid⁵⁵. Total flavonoids were estimated using 5% sodium nitrite and 10% AlCl₃. The developed yellow color with ASE or the catechin standard was recorded at 510 nm⁵⁶.

The High-performance liquid chromatography (HPLC) using 12 different types of pure phenolic standards were used to detect the phenolics in ASE. Twenty microliters of ASE was distributed on the Zorbax Eclipse plusC18 column (100 mm × 4.6 mm Agilent Technologies, Palo Alto, CA, USA). The separation was accomplished at 284 nm using a mobile phase of methanol, acetonitrile, and 0.2% H₃PO₄ with a ternary linear elution gradient and a flow speed of 0.75 mL/min⁵⁷.

In vitro antioxidant activities of ASE

The antiradical (ABTS and NO radicals) activities and β-carotene-linoleate bleaching analysis of ASE were tested. The 50% inhibitory concentration (IC₅₀) for each test was recognized by the GraphPad InStat software version 3.

The ABTS⁺ radical cation-decolorization assay was used to study the ability of ASE to neutralize ABTS⁺ radical to ABTS⁵⁸. ASE or BHT (standard antioxidant) was mixed with ABTS⁺ radical (7 mM ABTS was incubated with 140 mM potassium persulphate for 16 h at 25°C) in dark. Then the absorbance of the blue color was recorded at 734 nm to calculate the percentage inhibition of ABTS⁺ radical.

The NO scavenging ability of ASE was detected by measuring the generated bright-reddish-purple azo dye color from Griess reaction at 490 nm. Sodium nitroprusside and Griess reagent (0.1% naphthylethylenediamine dihydrochloride, 2% phosphoric acid, and 1% sulfanilamide)⁵⁹ was used in this reaction.

The anti-lipid peroxidation effect of ASE can be determined by examining the β-carotene-linoleate bleaching. The assay was performed using the emulsion of linoleic acid, β-carotene, and Tween-80⁶⁰. The ability of ASE to scavenge the produced radicals from linoleic acid oxidation was indicated by the decrease in the rate of β-carotene bleaching. The absorbance of the β-carotene color was recorded at 490 nm immediately (a) and after 180 min (b). Then the value of the degradation rate (DR) of ASE, control (without extract), and BHT (standard) was calculated from the equation: $[DR = \ln(a/b) \times (1/180)]$. The ASE scavenging capability was determined as the percentage of inhibition from the formula: $\text{Antioxidant activity (\%)} = (DR_{\text{control}} - DR_{\text{ASE}}/DR_{\text{control}}) \times 100$.

Evaluation of the systemic anti-toxicity effect of ASE

Experimental design

Fifty male Albino rats (weighing 140–200 g, six weeks' age) were randomly divided into five groups (ten animals in each). The handling of animals in each laboratory group is presented in Fig. 8. The systemic toxicity in rats was induced by intraperitoneal (IP) injection with 50% CCl₄ in olive oil (1 ml /kg b.w.) every Sunday and Wednesday each week for three weeks^{61,62}. The animals in CCl₄-ASE group was orally administered with ASE (7.5 g/kg b.w., dissolved in water) by gavage, every day for ten days. At day thirty, rats were anaesthetized and dissected immediately to obtain the blood by cardiac puncture and the tissues (liver, brain, lung, and spleen). The heparinized blood was centrifuged for 15 min at 6000 rpm to collect plasma for liver function parameters quantification. Liver, brain, lung, and spleen tissues were cleaned with cold saline (0.9% NaCl) and weighed. Then, the small parts were fixed in 10% formalin for histopathological examination. The remaining tissues were stored at -80°C until utilized in the biochemical and molecular investigations.

Plasma investigation

Using the specific kits, the levels of liver function parameters (ALT, AST, total proteins, and albumin) in the separated plasma specimens of all the examined groups were measured colorimetrically.

Biochemical assessment of the CCl₄-induced systemic oxidative stress

The oxidative stress (cellular redox state disturbance) was determined in the studied organs by quantification of the intracellular ROS, TAC, lipid peroxidation, NO, MPO, and the enzymatic and non-enzymatic antioxidant markers. The organ homogenates were obtained by homogenizing one gram of each studied organ separately in 10 mL of cold PBS. Then the cleared homogenates were collected after centrifugation for 30 min (6000 rpm, 4°C) for the analyses².

The concentration of ROS was determined by mixing an equal volume of the diluted homogenate (dilution with PBS, 2-fold) with 5 μM DCFH-DA fluorescent probe (diluted 1000-fold with 10 μM dimethyl sulfoxide). The amount of the fluorescence was recorded at 485 (excitation) and 520 nm (emission) after keeping the reaction mixture in dark at 37°C for 5 min. Then the ROS level in each studied organ was calculated using the H₂O₂ calibration curve⁶³.

The TAC of the tested organs was determined using the ABTS radical cation method by mixing 2 mL of the radical solution with 20 μL of each organ homogenate or standard (BHT) or PBS (control)⁵⁸. The preparation of ABTS solution and the method proceeded as indicated above in the *in vitro* assay to obtain the percentage of inhibition. Then the TAC of each studied organ was calculated as BHT equivalent/g tissue using the BHT standard curve.

The level of lipid peroxidation was measured colorimetrically using TBA reactive substances (TBARS) assay using the standard curve of TMP⁶⁴. NO was examined using Griess reaction as referred in the *in*

vitro part⁶⁰.

The activity of MPO as μmol of $\text{H}_2\text{O}_2/\text{min}$ was quantified spectrophotometrically using 16.7 mg% ODD and 1.2% H_2O_2 ⁶⁵, while the activity of the enzymatic antioxidants (GPX and SOD) were examined by Rotruck method⁶⁶ and pyrogallol autooxidation method³⁶, respectively. The specific activities of these enzymes were calculated as IU/mg protein using the value of the protein content in each organ homogenate that was measured by the biuret method using the specific kit. Ellman's reagent (5, 5'-dithio bis(2- nitrobenzoic acid) was used to determine the nonenzymatic antioxidant (GSH) level using the GSH calibration curve⁶⁷.

Real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

The necroinflammation in all the studied tissues and the fibrotic markers in the liver tissue were assessed at the molecular level using the qRT-PCR technique. The NF- κ B, iNOS, COX-2, and TNF- α were evaluated here as good markers of necroinflammation in all the examined tissues, and IL-1 β and IL-8 (essential pulmonary pro-inflammatory cytokines) were assessed in lung tissue. In addition, the COL1A1 and TGF- β 1 (critical hepatic fibrosis cytokines) were tested in liver tissues.

Each studied tissue was homogenized in lysis buffer containing β -mercaptoethanol and then centrifuged (14,000 rpm for 5 min) and the total RNA was extracted from the obtained supernatant, using Gene JET RNA Purification Kit and quantified. Then the cDNA Synthesis Kit was used to synthesis the cDNA. The levels of target genes expression were determined via real-time PCR using SYBR green master mix kit, specific primers (Supplementary Table 1), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a housekeeping gene. The comparative Ct method (number of threshold cycle at cross-point between amplification plot and threshold) was used to calculate the fold expression of the studied genes².

Histopathological study

The formalin-fixed tissue specimens were embedded in paraffin wax then each sample was cut into small slices (5 μm thickness) and stained with hematoxylin and eosin following the standard histopathological examination protocol. Then, the phase-contrast microscope was used to visualize the pathological features of the examined tissues in all the studied groups, and high-resolution images (200x magnification) were captured².

Statistical analysis

The results (symmetric with skewness values from 0 to 0.941) of the present study were expressed as mean \pm SE of seven rats. The parametric analysis, one-way analysis of variance (ANOVA) using Duncan's test, was used to determine the difference between the mean values in all the studied groups. The analysis was done by SPSS software version 16 and the significance was considered at p -value < 0.05 . The GraphPad Instate software version 3 was used to calculate the IC50 values for the *in vitro* antioxidant analyses.

Declarations

Acknowledgements

This research received no specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contributions

M.M.A. and N.H.H. equally contributed to the designing and performing of the experiments and interpreting and analyzing data, as well as revising the manuscript. A.F.H. contributed in writing and revising of the manuscript and explaining the results.

Competing interests

The authors declare no competing interests.

References

1. Birnboim, H. C. DNA strand breaks in human leukocytes induced by superoxide anion, hydrogen peroxide and tumor promoters are repaired slowly compared to breaks induced by ionizing radiation. *Carcinogenesis* **7**, 1511–1517 (1986).
2. Abu-Serie, M. M. & Habashy, N. H. Vitis vinifera polyphenols from seedless black fruit act synergistically to suppress hepatotoxicity by targeting necroptosis and pro-fibrotic mediators. *Sci. Rep.* **10**, 1–15 (2020).
3. Shah, M. D., Gnanaraj, C., Haque, A. E. & Iqbal, M. Antioxidative and chemopreventive effects of *Nephrolepis biserrata* against carbon tetrachloride (CCl₄)-induced oxidative stress and hepatic dysfunction in rats. *Pharm. Biol.* **53**, 31–39 (2015).
4. Singh, N., Kamath, V., Narasimhamurthy, K. & Rajini, P. S. Protective effect of potato peel extract against carbon tetrachloride-induced liver injury in rats. *Environ. Toxicol. Pharmacol.* **26**, 241–246 (2008).
5. Hamouda, A. F. A Biochemical Study of Agarwood on Methanol Injection in Rat. *Ashdin Publ. J. Drug Alcohol Res.* **8**, 1–14 (2019).
6. Srivastava, A. & Shivanandappa, T. Hepatoprotective effect of the root extract of *Decalepis hamiltonii* against carbon tetrachloride-induced oxidative stress in rats. *Food Chem.* **118**, 411–417 (2010).
7. Suzuma, I. Suzuma, K. Takagi, H. & Honda, Y. Cyclic Stretch induced Reactive Oxygen Species (ROS) Generation in Porcine Retinal Pericytes (PRPC) through PKC- Activation | IOVS | Investigative Ophthalmology & Visual Science. Vol.45, 3202. (2004).

8. Adesanoye, O. A. & Farombi, E. O. Hepatoprotective effects of *Vernonia amygdalina* (astereaceae) in rats treated with carbon tetrachloride. *Exp. Toxicol. Pathol.* **62**, 197–206 (2010).
9. El-Boshy, M. E. et. al. Attenuation of CCl₄ Induced Oxidative Stress, Immunosuppressive, Hepatorenal Damage by Fucoïdan in Rats. *J. Clin. Toxicol.* (2017) doi:10.4172/2161-0495.1000348.
10. Hamouda, A. F., Sameeh, M. Y. & Shrourou, R. M. Effect of Avocado (*Persea Americana*), Cabbage (*Brassica Oleracea*) and Ginger (*Zingiber Officinale*) on Rat Liver and Thyroid Injuries Induced by CCl₄ (Carbon Tetrachloride). *J. Pharm. Pharmacol.* **4**, 108–118 (2016).
11. Jiang, L., Huang, J., Wang, Y. & Tang, H. Metabonomic analysis reveals the CCl₄-induced systems alterations for multiple rat organs. *J. Proteome Res.* **11**, 3848–3859 (2012).
12. Abdel-Daim, M. M., Aly, S. M., Abo-El-Sooud, K., Giorgi, M. & Ursoniu, S. Role of Natural Products in Ameliorating Drugs and Chemicals Toxicity. *Evid. Based Complement. Alternat. Med.* **2016**, 7879406.
13. Dhanasekaran, M., Ignacimuthu, S. & Agastian, P. Potential hepatoprotective activity of ononitol monohydrate isolated from *Cassia tora* L. on carbon tetrachloride induced hepatotoxicity in wistar rats. *Phytomedicine* **16**, 891–895 (2009).
14. Ali, B., Ziada, A. & Blunden, G. Biological effects of Gum Arabic: A review of some recent research. *Food Chem. Toxicol.* **47**, 1–8 (2008).
15. Ali, B. H. et al. Effect of gum arabic on oxidative stress and inflammation in adenine-induced chronic renal failure in rats. *PLoS One* **8**, e55242 (2013).
16. Ali, B. H., Ziada, A., Al Husseni, I., Beegam, S. & Nemmar, A. Motor and behavioral changes in rats with adenine-induced chronic renal failure: influence of acacia gum treatment. *Exp. Biol. Med. (Maywood)*. **236**, 107–112 (2011).
17. Ayaz, N. O., Ramadan, K. S., Farid, H. E. A. & Alnahdi, H. S. Protective role and antioxidant activity of Arabic gum against trichloroacetate-induced toxicity in liver of male rats. *Indian J. Anim. Res.* **51**, 303–309 (2017).
18. Said Elshama, S., El-Meghawry El-Kenawy, A., Osman, H.-E. H. & Youseef, H. M. Amelioration of indomethacin systemic toxicity by gum arabic administration in adult albino rats. *International Journal of Medicinal Plants and Alternative Medicine* vol. 2(3), pp. 032-046, (2014).
19. Abu-Serie, M. M., Habashy, N. H. & Attia, W. E. In vitro evaluation of the synergistic antioxidant and anti-inflammatory activities of the combined extracts from Malaysian *Ganoderma lucidum* and Egyptian *Chlorella vulgaris*. *BMC Complement. Altern. Med.* **18**, 154 (2018).
20. Habashy, N. H., Abu Serie, M. M., Attia, W. E. & Abdelgaleil, S. A. M. Chemical characterization, antioxidant and anti-inflammatory properties of Greek *Thymus vulgaris* extracts and their possible synergism with Egyptian *Chlorella vulgaris*. *J. Funct. Foods* **40**, 317–328 (2018).
21. AL-Majed, A. A., Mostafa, A. M., AL-Rikabi, A. C. & AL-Shabanah, O. A. Protective effects of oral Arabic gum administration on gentamicin-induced nephrotoxicity in rats. *Pharmacol. Res.* **46**, 445–451 (2002).
22. Laleman, W., Claria, J., Van der Merwe, S., Moreau, R. & Trebicka, J. Systemic Inflammation and Acute-on-Chronic Liver Failure: Too Much, Not Enough. *Can. J. Gastroenterol. Hepatol.* **2018**,

- 1027152 (2018).
23. Bisht, S. *et al.* A polymeric nanoparticle formulation of curcumin (NanoCurc) ameliorates CCl₄-induced hepatic injury and fibrosis through reduction of pro-inflammatory cytokines and stellate cell activation. *Lab. Invest.* **91**, 1383–1395 (2011).
 24. Xiao, J. *et al.* Lycium barbarum polysaccharides protect mice liver from carbon tetrachloride-induced oxidative stress and necroinflammation. *J. Ethnopharmacol.* **139**, 462–470 (2012).
 25. Kunwar, A. & Priyadarsini, K. I. Review Free radicals, oxidative stress and importance of antioxidants in human health. *J. Med. Allied Sci.* **1**(2):53-60 (2011).
 26. Morgan, M. J. & Liu, Z. G. Crosstalk of reactive oxygen species and NF-κB signaling. *Cell Research* **21**, 103–115 (2011).
 27. Zhang, R. *et al.* Myeloperoxidase functions as a major enzymatic catalyst for initiation of lipid peroxidation at sites of inflammation. *J. Biol. Chem.* **277**, 46116–46122 (2002).
 28. Kettle, A. J. & Winterbourn, C. C. Superoxide enhances hypochlorous acid production by stimulated human neutrophils. *Biochim. Biophys. Acta - Mol. Cell Res.* **1052**, 379–385 (1990).
 29. Winterbourn, C. C. Comparative reactivities of various biological compounds with myeloperoxidase-hydrogen peroxide-chloride, and similarity of oxidant to hypochlorite. *Biochim. Biophys. Acta - Gen. Subj.* **840**, 204–210 (1985).
 30. Amanullah, M. Protective Effect of Long Term Administration of Gum Arabic on Oxidative Stress in Hepatic Tissue of Diabetic Rats. *Biomed. J. Sci. Tech. Res.* **4**, 1-7 (2018).
 31. Szwajgier, D., Borowiec, K. & Pustelniak, K. The Neuroprotective Effects of Phenolic Acids: Molecular Mechanism of Action. *Nutrients* **9**, 477 (2017).
 32. Lopez Torrez, L., Nigen, M., Williams, P., Doco, T. & Sanchez, C. Acacia senegal vs. Acacia seyal gums - Part 1: Composition and structure of hyperbranched plant exudates. *Food Hydrocoll.* **51**, 41–53, (2015).
 33. Khatri, D. & Chhetri, S. B. B. Reducing Sugar, Total Phenolic Content, and Antioxidant Potential of Nepalese Plants. *Biomed Res. Int.* **2020**, 7296859, 1-7, (2020).
 34. Eun, Y. P., Murakami, H. & Matsumura, Y. Effects of the addition of amino acids and peptides on lipid oxidation in a powdery model system. *J. Agric. Food Chem.* **53**, 8334–8341 (2005).
 35. Lelon, J., Jumba, I., Keter, J., Wekesa, C. & Oduor, F. Assessment of physical properties of gum arabic from Acacia Senegal varieties in Baringo District, Kenya. *African J. Plant Sci.* **4**, 95–98 (2010).
 36. Marklund, S. & Marklund, G. Involvement of the Superoxide Anion Radical in the Autoxidation of Pyrogallol and a Convenient Assay for Superoxide Dismutase. *Eur. J. Biochem.* **47**, 469–474 (1974).
 37. Bhushan, B. & Apte, U. Liver Regeneration after Acetaminophen Hepatotoxicity: Mechanisms and Therapeutic Opportunities. *Am. J. Pathol.* **189**, 719–729 (2019).
 38. Yeh, C.-T. & Yen, G.-C. Induction of Hepatic Antioxidant Enzymes by Phenolic Acids in Rats Is Accompanied by Increased Levels of Multidrug Resistance–Associated Protein 3 mRNA Expression. *J. Nutr.* **136**, 11–15 (2006).

39. Kouka, P. *et al.* Olive oil with high polyphenolic content induces both beneficial and harmful alterations on rat redox status depending on the tissue. *Toxicol. Reports* **7**, 421–432 (2020).
40. Kouka, P. *et al.* The Polyphenolic Composition of Extracts Derived from Different Greek Extra Virgin Olive Oils Is Correlated with Their Antioxidant Potency. *Oxid. Med. Cell. Longev.* **2019**, 1870965 (2019).
41. Merra, E., Calzaretti, G., Bobba, A., Storelli, M. M. & Casalino, E. Antioxidant role of hydroxytyrosol on oxidative stress in cadmium-intoxicated rats: different effect in spleen and testes. *Drug Chem. Toxicol.* **37**, 420–426 (2014).
42. Makri, S. *et al.* Novel feed including olive oil mill wastewater bioactive compounds enhanced the redox status of lambs. *In Vivo (Brooklyn)*. **32**, 291–302 (2018).
43. Ranneh, Y. *et al.* Crosstalk between reactive oxygen species and pro-inflammatory markers in developing various chronic diseases: a review. *Appl. Biol. Chem.* **60**, 327–338 (2017).
44. Liu, Y. *et al.* Liver NF- κ B-Inducing Kinase Promotes Liver Steatosis and Glucose Counterregulation in Male Mice With Obesity. *Endocrinology* **158**, 1207–1216 (2017).
45. Reeves, H. & Friedman, S. Activation of hepatic stellate cells - A key issue in liver fibrosis. *Front. Biosci.* **7**, d808-26 (2002).
46. Kwong, K. Y. *et al.* Differential regulation of IL-8 by IL-1 β and TNF α in hyaline membrane disease. *J. Clin. Immunol.* **18**, 71–80 (1998).
47. Bousquet, J. *et al.* Asthma: a disease remodeling the airways. *Allergy* **47**, 3–11 (1992).
48. Weber, L. W. D., Boll, M. & Stampfl, A. Hepatotoxicity and Mechanism of Action of Haloalkanes: Carbon Tetrachloride as a Toxicological Model. *Crit. Rev. Toxicol.* **33**, 105–136 (2003).
49. Kamal, E. *et al.* Gum Arabic Fibers Decreased Inflammatory Markers and Disease Severity Score among Rheumatoid Arthritis Patients, Phase II Trial. *Int. J. Rheumatol.* **2018**, 4197537 (2018).
50. Araimi, A. AL, Kharusi, A. AL, Bani Oraba, A., Kindi, I. AL & Zadjali, F. Anti-fibrotic effect of gum arabic in recovery from inflammatory bowel disease. *J Gastrointest Dig Syst* **6**, 4 (2016).
51. Dey, P. *et al.* Pharmaceutical Biology Evaluation of anti-inflammatory activity and standardisation of hydro-methanol extract of underground tuber of *Dioscorea alata* Evaluation of anti-inflammatory activity and standardisation of hydro-methanol extract of underground tub. *Pharm. Biol.* **54**, 1474–1482 (2016).
52. Elshama, S. The preventive role of Arabic gum in the treatment of Toxicity. *Open Access: Toxicology & Research*, **1**, 27–29 (2018).
53. Lykkesfeldt, J. & Svendsen, O. Oxidants and antioxidants in disease: oxidative stress in farm animals. *Vet. J.* **173**, 502–511 (2007).
54. Hemdan, D. I. & Abdulmaguid, N. Y. M. The Therapeutic Effect of Arabic Gum, Purslane and Cress Seeds on Rat Infected with Elevated Uric Acid Levels in the Blood. *Information* **21**, 1249-1260 (2018).
55. Taga, M. S., Miller, E. E. & Pratt, D. E. Chia seeds as a source of natural lipid antioxidants. *J. Am. Oil Chem. Soc.* **61**, 928–931 (1984).

56. Zhishen, J., Mengcheng, T. & Jianming, W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.* **64**, 555–559 (1999).
57. Abu-Serie, M. & Habashy, N. The ameliorating effect of the combined extract from Greek *Thymus vulgaris* and bee's honey on the hydrocortisone-induced osteoporosis in rat bone cells via modulating the bone turnover, oxidative stress, and inflammation. *RSC Adv.* **8**, 28341–28355 (2018).
58. Re, R. *et al.* Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **26**, 1231–1237 (1999).
59. Marcocci, L., Maguire, J. J., Droy-Lefaix, M. T. & Packer, L. The nitric oxide-scavenging properties of Ginkgo biloba extract EGb 761. *Biochem. Biophys. Res. Commun.* **201**, 748–755 (1994).
60. Barreira, J. C. M., Ferreira, I. C. F. R., Beatriz, M., Oliveira, P. P. & Pereira, A. Antioxidant activities of the extracts from chestnut flower, leaf, skins and fruit. *Food Chem.* **107**, 1106–1113 (2008) doi:10.1016/j.foodchem.2007.09.030.
61. Tyagi, S., Singh, A., Saxena, A. & Patel, B. In vitro Antioxidant Activity of Methanolic and Aqueous Extract of *Flacourtia indica* Merr. *J. Sci. Res.* **5**, 201–206 (2010).
62. Ikeda, H. *et al.* Sphingosine 1-phosphate regulates regeneration and fibrosis after liver injury via sphingosine 1-phosphate receptor 2. *J. Lipid Res.* (2009) doi:10.1194/jlr.M800496-JLR200.
63. Crow, J. P. Dichlorodihydrofluorescein and dihydrorhodamine 123 are sensitive indicators of peroxynitrite in vitro: implications for intracellular measurement of reactive nitrogen and oxygen species. *Nitric oxide Biol. Chem.* **1**, 145–157 (1997).
64. Ohkawa, H., Ohishi, N. & Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **95**, 351–358 (1979).
65. Kim, J. J., Shajib, M. S., Manocha, M. M. & Khan, W. I. Investigating intestinal inflammation in DSS-induced model of IBD. *J. Vis. Exp.* **60**, 1–6 (2012) doi:10.3791/3678.
66. Rotruck, J. T. *et al.* Selenium: biochemical role as a component of glutathione peroxidase. *Science* **179**, 588–590 (1973).
67. Ellman, G. L. Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* **82**, 70–77 (1959).

Figures

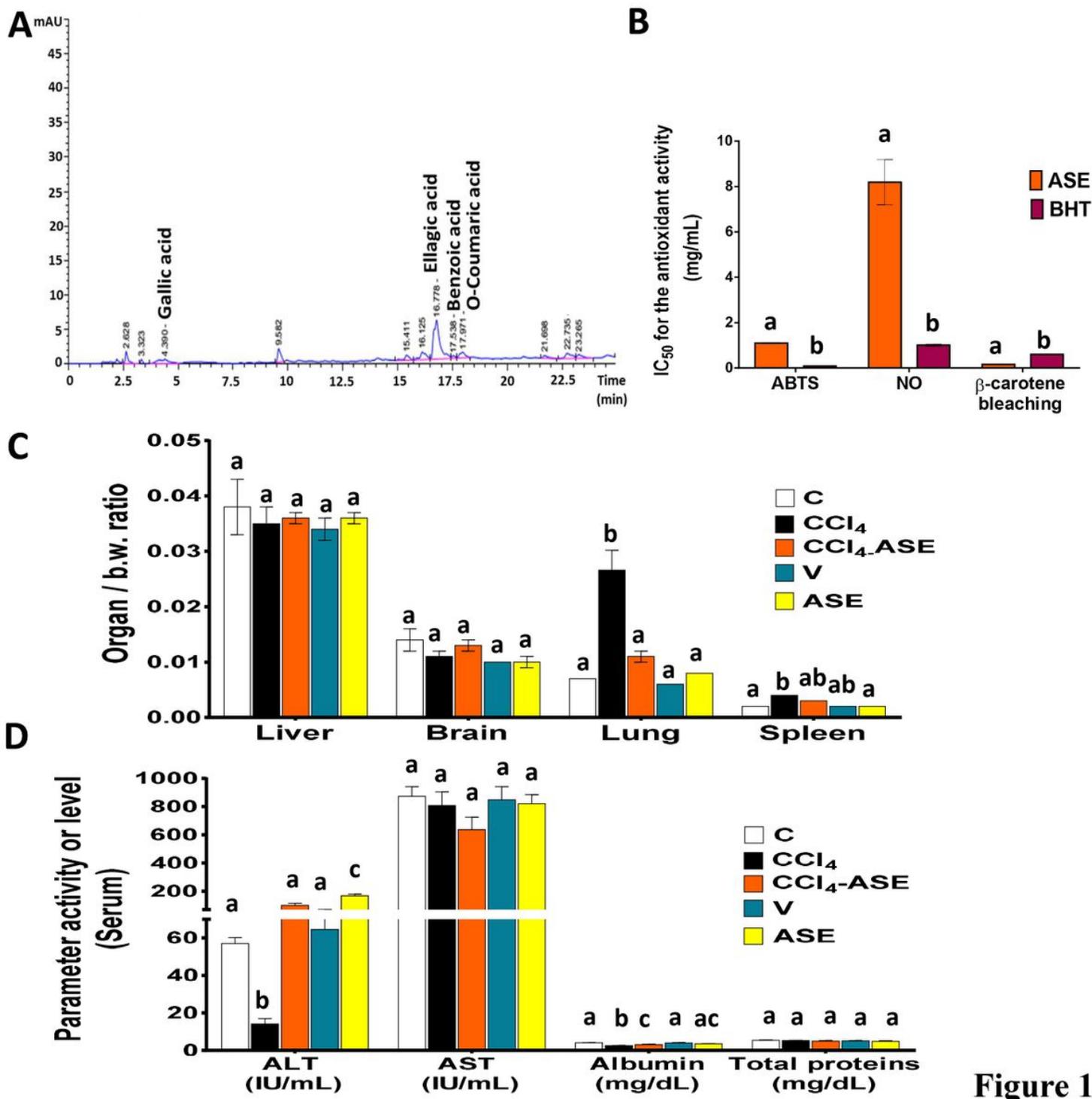


Figure 1

Figure 1

In vitro characterization of ASE, organ weights, and serum analysis of rats in all the studied groups. (A) HPLC analysis of the ASE. (B) organ weight/ body weight (b.w.) ratio. (C) liver function parameters in the serum of rats. Results are expressed as mean \pm S.E of 7 animals. C, Control untreated rats; V, olive oil (vehicle of CCl₄)-administered rats (0.5 ml/kg b.w., ip, 6 times); CCl₄, rats with systemic toxicity induced by CCl₄ injection (1 mL/kg b.w., ip, 6 times); CCl₄-ASE, rats with systemic toxicity after their oral treatment with ASE (7.5 g/kg b.w.) for 10 days. ASE, normal rats administered only ASE (7.5 g/kg b.w.) for 10 days.

Different letters refer to the significance at $p < 0.05$; CCl₄-ASE group was compared with the CCl₄ group, while V and ASE groups were individually compared with the C group. ABTS, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); ALT, alanine aminotransferase; AST, aspartate aminotransferase; BHT, butylated hydroxytoluene; IC₅₀, 50% inhibitory concentration; NO, nitric oxide radical.

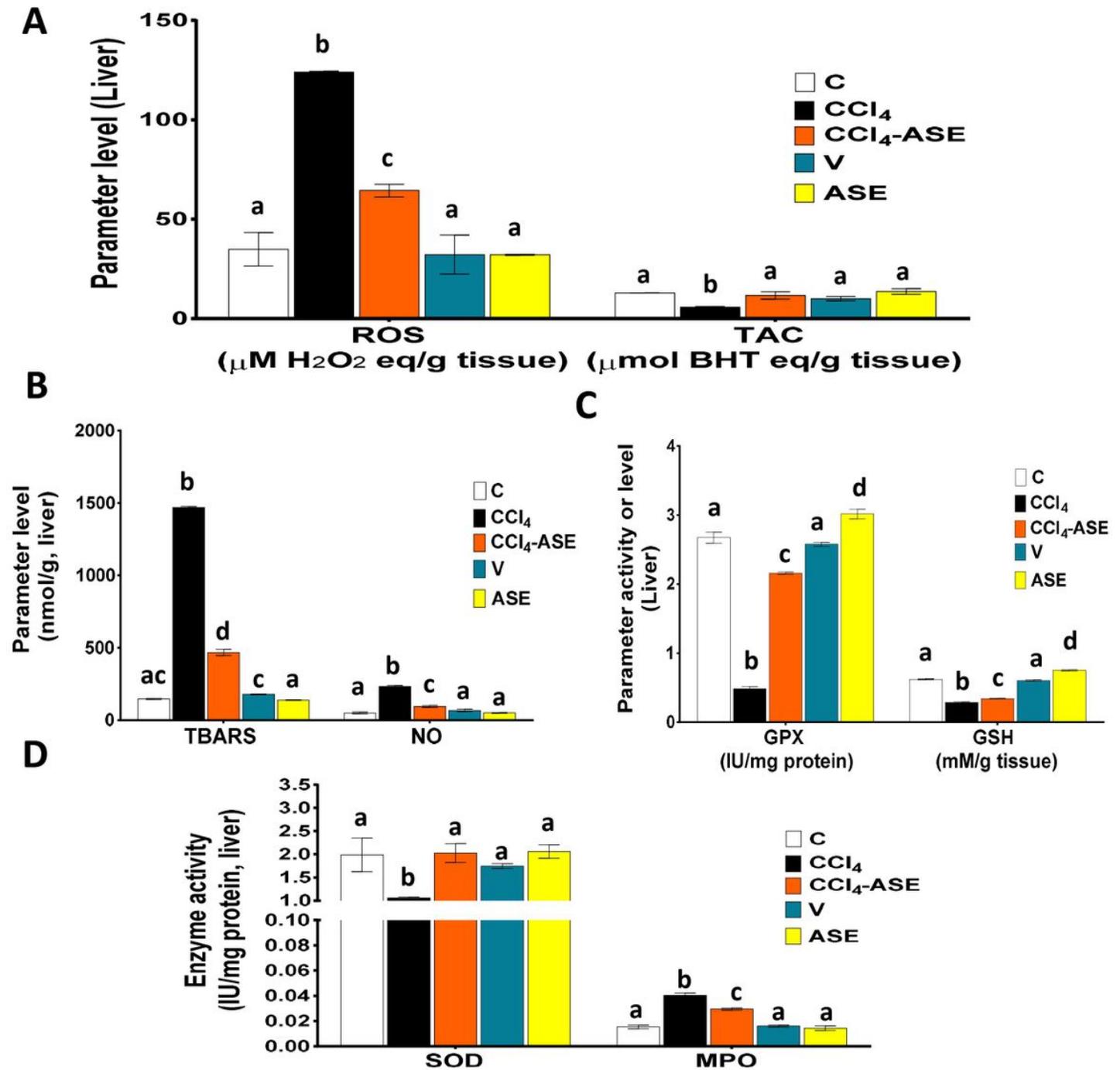


Figure 2

Figure 2

Ameliorating effect of ASE on the CCl₄-induced hepatic redox state disturbance. (A) reactive oxygen species (ROS) and total antioxidant capacity (TAC) levels. (B) thiobarbituric acid reactive substances

(TBARS) and nitric oxide (NO) levels. (C) reduced glutathione (GSH) level and glutathione peroxidase (GPX) activity. (D) superoxide dismutase (SOD) and myeloperoxidase (MPO) activities. Results are expressed as mean \pm S.E of 7 animals. BHT, butylated hydroxytoluene; C, Control untreated rats; V, olive oil (vehicle of CCl₄)-administered rats (0.5 ml/kg b.w., ip, 6 times); CCl₄, rats with systemic toxicity induced by CCl₄ injection (1 mL/kg b.w., ip, 6 times); CCl₄-ASE, rats with systemic toxicity after their oral treatment with ASE (7.5 g/kg b.w.) for 10 days. ASE, normal rats administered only ASE (7.5 g/kg b.w.) for 10 days. Different letters refer to the significance at $p < 0.05$; CCl₄-ASE group was compared with the CCl₄ group, while V and ASE groups were individually compared with the C group.

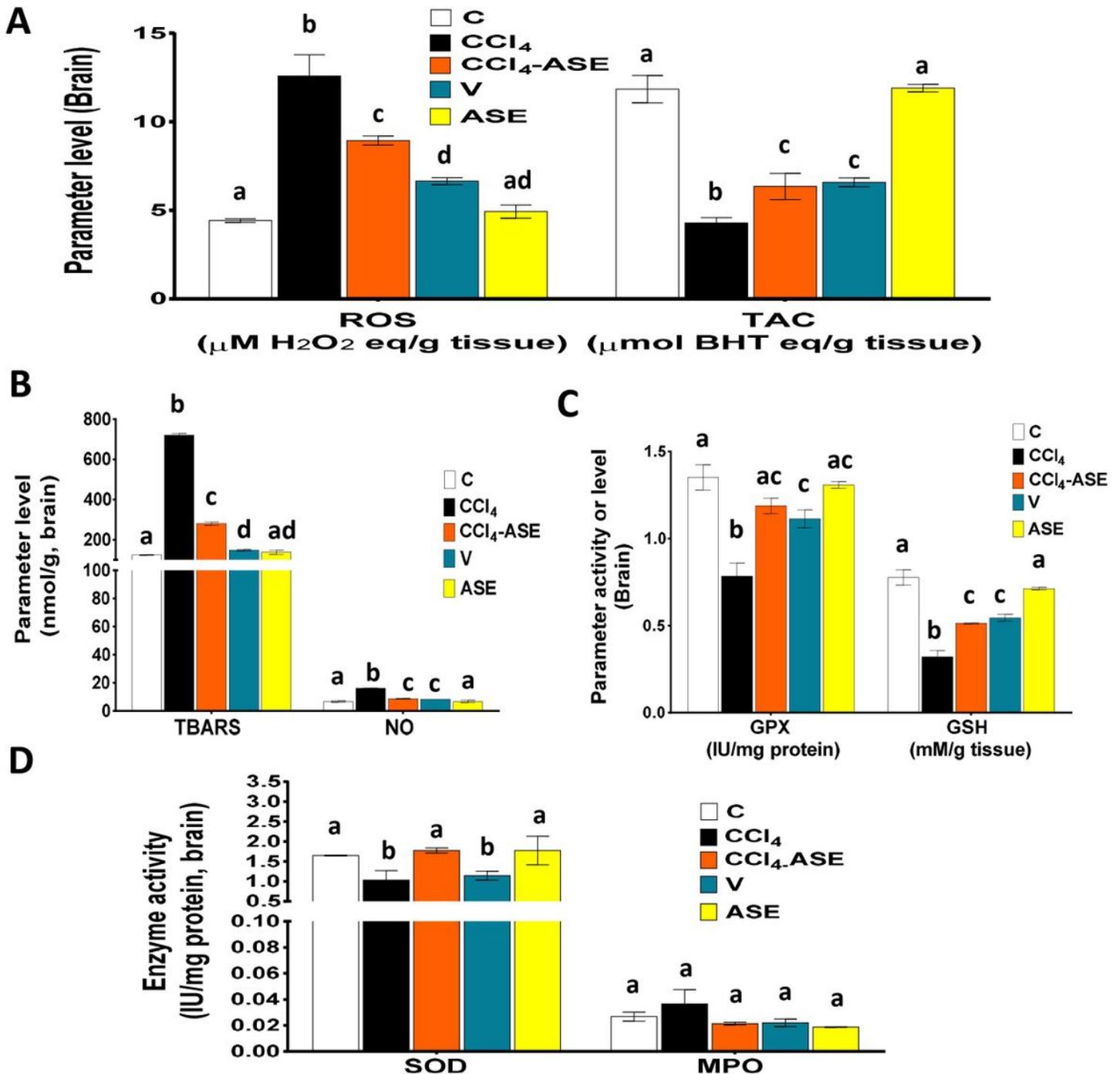


Figure 3

Figure 3

Ameliorating effect of ASE on the CCl₄-induced redox state disturbance in the brain tissue. (A) reactive oxygen species (ROS) and total antioxidant capacity (TAC) levels. (B) thiobarbituric acid reactive substances (TBARS) and nitric oxide (NO) levels. (C) reduced glutathione (GSH) level and glutathione peroxidase (GPX) activity. (D) superoxide dismutase (SOD) and myeloperoxidase (MPO) activities. Results are expressed as mean \pm S.E of 7 animals. BHT, butylated hydroxytoluene; C, Control untreated rats; V, olive oil (vehicle of CCl₄)-administered rats (0.5 ml/kg b.w., ip, 6 times); CCl₄, rats with systemic toxicity induced by CCl₄ injection (1 mL/kg b.w., ip, 6 times); CCl₄-ASE, rats with systemic toxicity after their oral treatment with ASE (7.5 g/kg b.w.) for 10 days. ASE, normal rats administered only ASE (7.5 g/kg b.w.) for 10 days. Different letters refer to the significance at $p < 0.05$, CCl₄-ASE group was compared with the CCl₄ group, while V and ASE groups were individually compared with the C group.

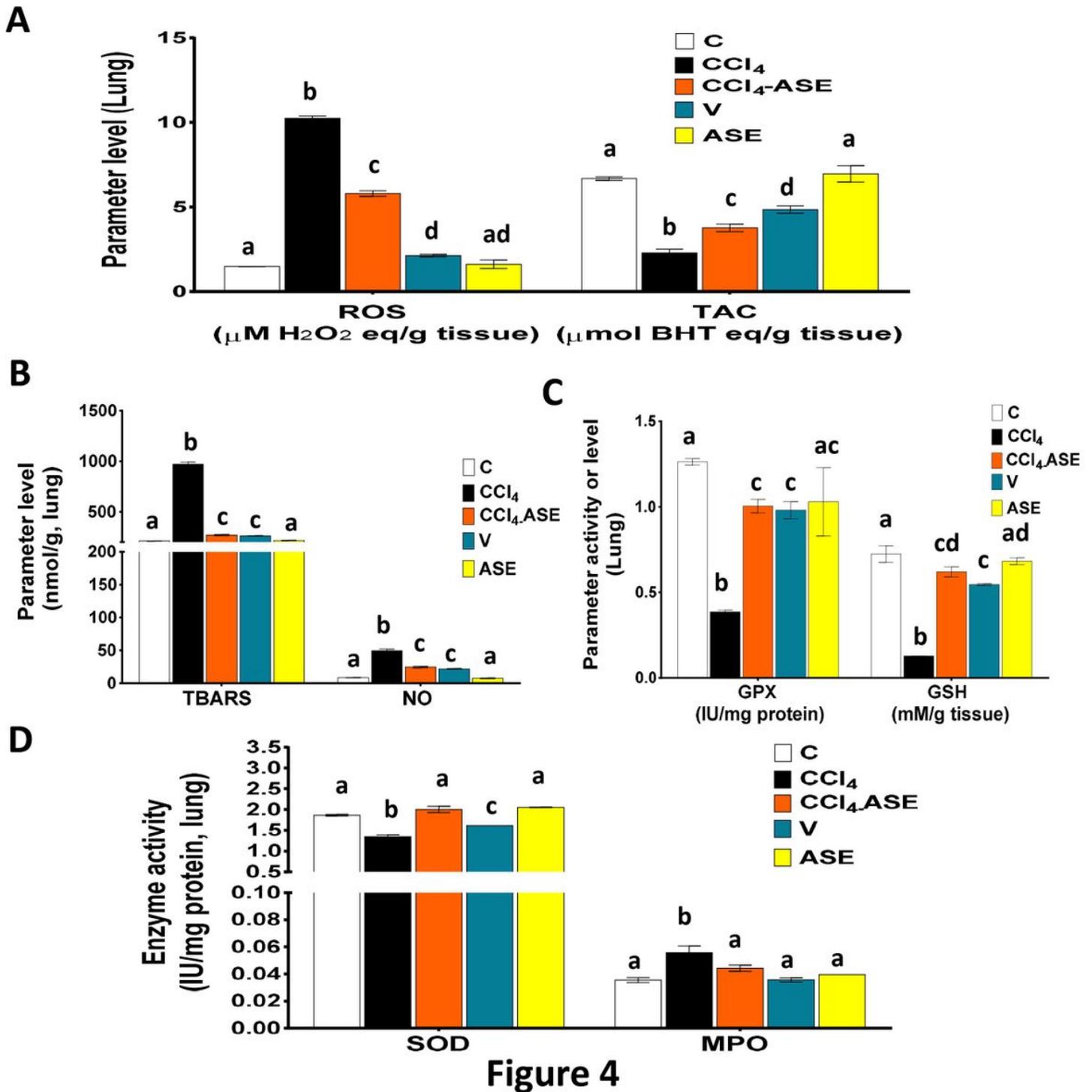


Figure 4

Figure 4

Ameliorating effect of ASE on the CCl₄-induced redox state disturbance in the lung tissue. (A) reactive oxygen species (ROS) and total antioxidant capacity (TAC) levels. (B) thiobarbituric acid reactive substances (TBARS) and nitric oxide (NO) levels. (C) reduced glutathione (GSH) level and glutathione peroxidase (GPX) activity. (D) superoxide dismutase (SOD) and myeloperoxidase (MPO) activities. Results are expressed as mean ± S.E of 7 animals. BHT, butylated hydroxytoluene; C, Control untreated rats; V, olive oil (vehicle of CCl₄)-administered rats (0.5 ml/kg b.w., ip, 6 times); CCl₄, rats with systemic

toxicity induced by CCl₄ injection (1 mL/kg b.w., ip, 6 times); CCl₄-ASE, rats with systemic toxicity after their oral treatment with ASE (7.5 g/kg b.w.) for 10 days. ASE, normal rats administered only ASE (7.5 g/kg b.w.) for 10 days. Different letters refer to the significance at *p* < 0.05, CCl₄-ASE group was compared with the CCl₄ group, while V and ASE groups were individually compared with the C group.

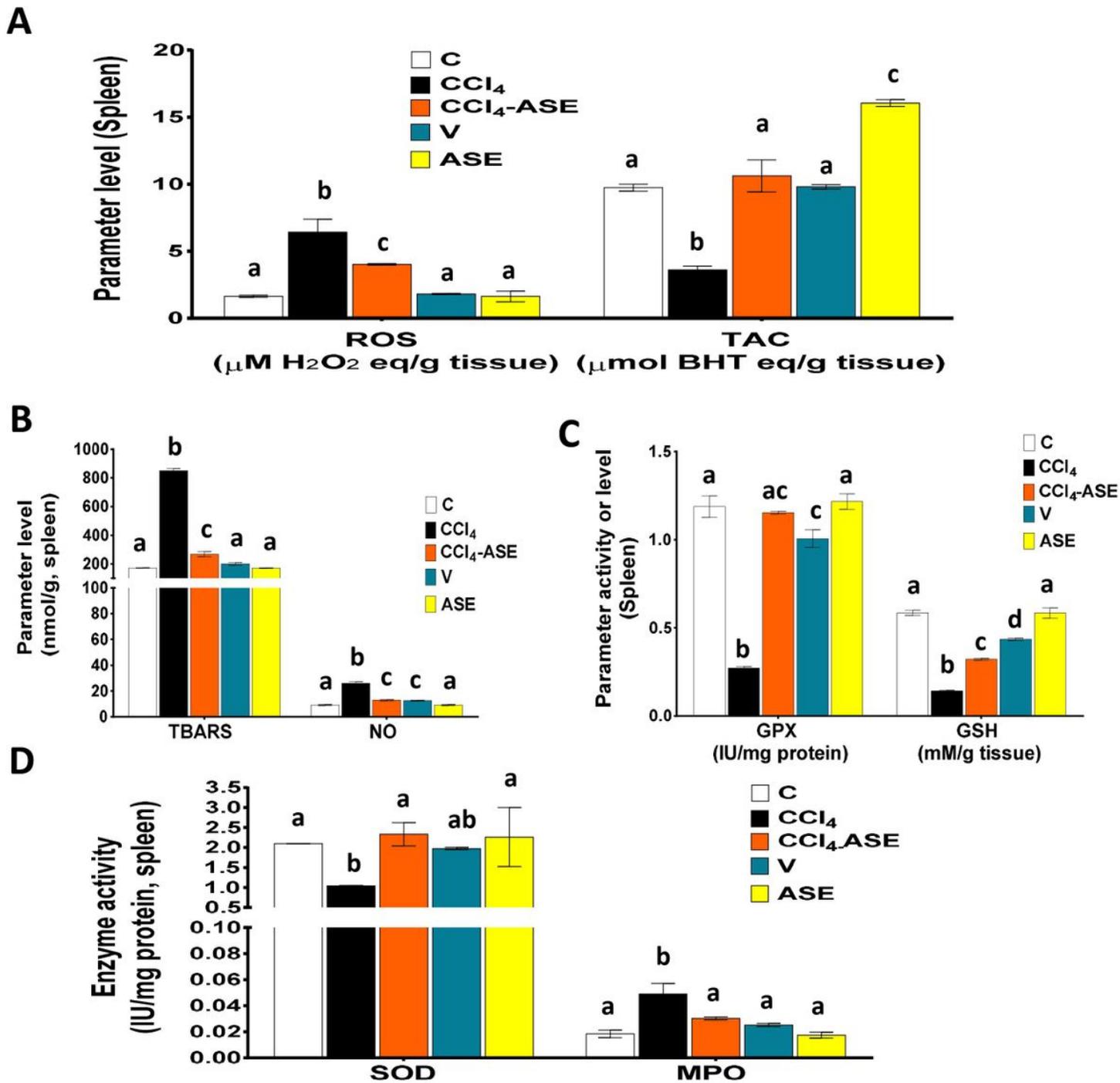


Figure 5

Figure 5

Ameliorating effect of ASE on the CCl₄-induced redox state disturbance in the spleen tissue. (A) reactive oxygen species (ROS) and total antioxidant capacity (TAC) levels. (B) thiobarbituric acid reactive

substances (TBARS) and nitric oxide (NO) levels. (C) reduced glutathione (GSH) level and glutathione peroxidase (GPX) activity. (D) superoxide dismutase (SOD) and myeloperoxidase (MPO) activities. Results are expressed as mean \pm S.E of 7 animals. BHT, butylated hydroxytoluene; C, Control untreated rats; V, olive oil (vehicle of CCl₄)-administered rats (0.5 ml/kg b.w., ip, 6 times); CCl₄, rats with systemic toxicity induced by CCl₄ injection (1 mL/kg b.w., ip, 6 times); CCl₄-ASE, rats with systemic toxicity after their oral treatment with ASE (7.5 g/kg b.w.) for 10 days. ASE, normal rats administered only ASE (7.5 g/kg b.w.) for 10 days. Different letters refer to the significance at $p < 0.05$; CCl₄-ASE group was compared with the CCl₄ group, while V and ASE groups were individually compared with the C group.

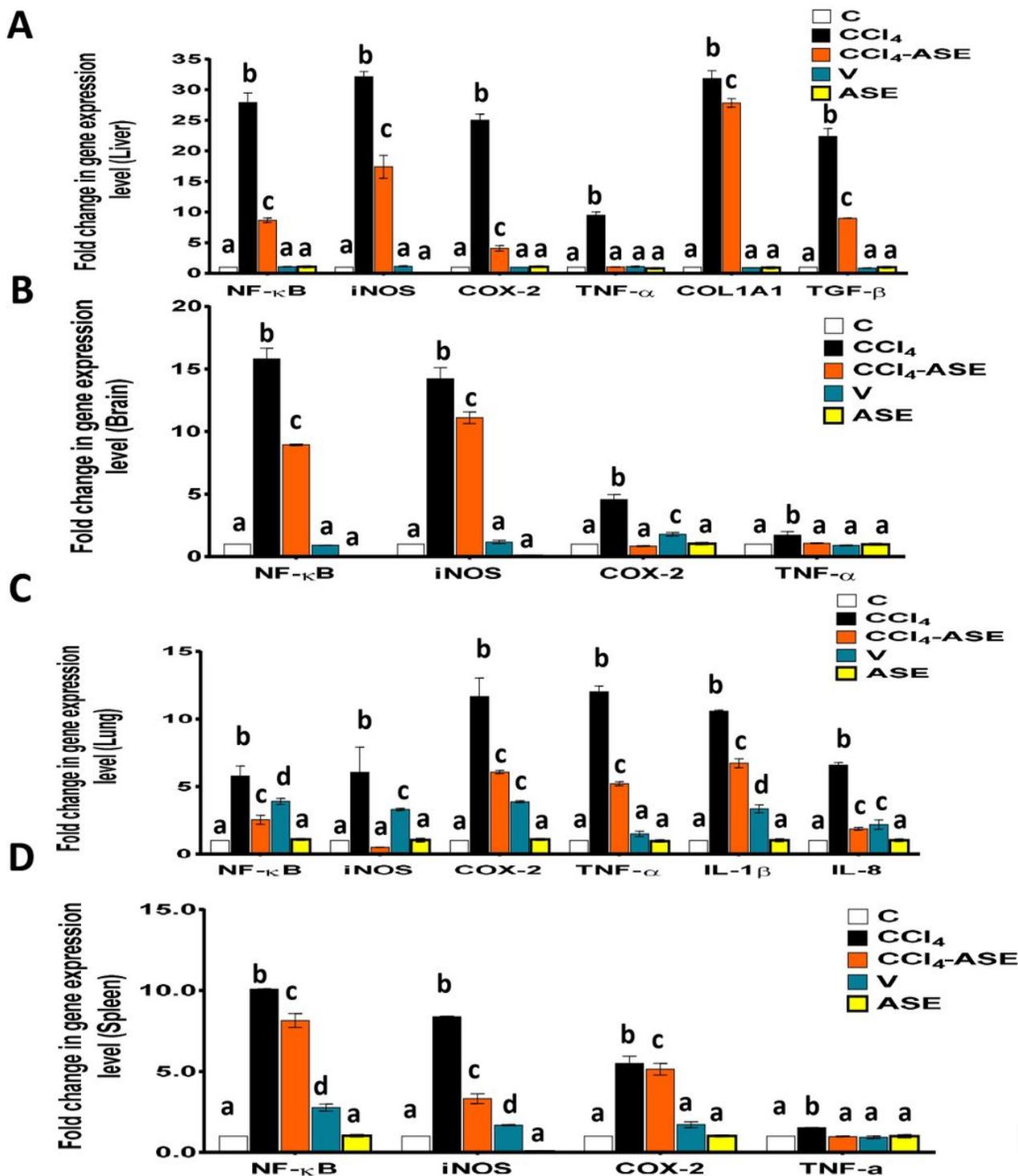


Figure 6

Figure 6

Ameliorating effect of ASE on the CCl₄-induced systemic necroinflammation. (A), (B), (C), (D) fold change in the gene expression of the pro-inflammatory mediators [nuclear factor-kappa (NF-κ)B, inducible nitric oxide synthase (iNOS), cyclooxygenase (COX)-2, and tumor necrosis factor (TNF)-α] in the liver, brain, lung, and spleen tissues, respectively. (A), (D) also show the fold change in the gene expression of the hepatic profibrotic mediators [collagen type I alpha one chain (COL1A1) and transforming growth factor (TGF)-β1] and lung-specific pro-inflammatory cytokines [interleukin (IL)-1β and IL-8], respectively. Results are expressed as mean ± S.E of 7 animals. C, Control untreated rats; V, olive oil (vehicle of CCl₄)-administered rats (0.5 ml/kg b.w., ip, 6 times); CCl₄, rats with systemic toxicity induced by CCl₄ injection (1 mL/kg b.w., ip, 6 times); CCl₄-ASE, rats with systemic toxicity after their oral treatment with ASE (7.5 g/kg b.w.) for 10 days. ASE, normal rats administered only ASE (7.5 g/kg b.w.) for 10 days. Different letters refer to the significance at $p < 0.05$; CCl₄-ASE group was compared with the CCl₄ group, while V and ASE groups were individually compared with the C group.

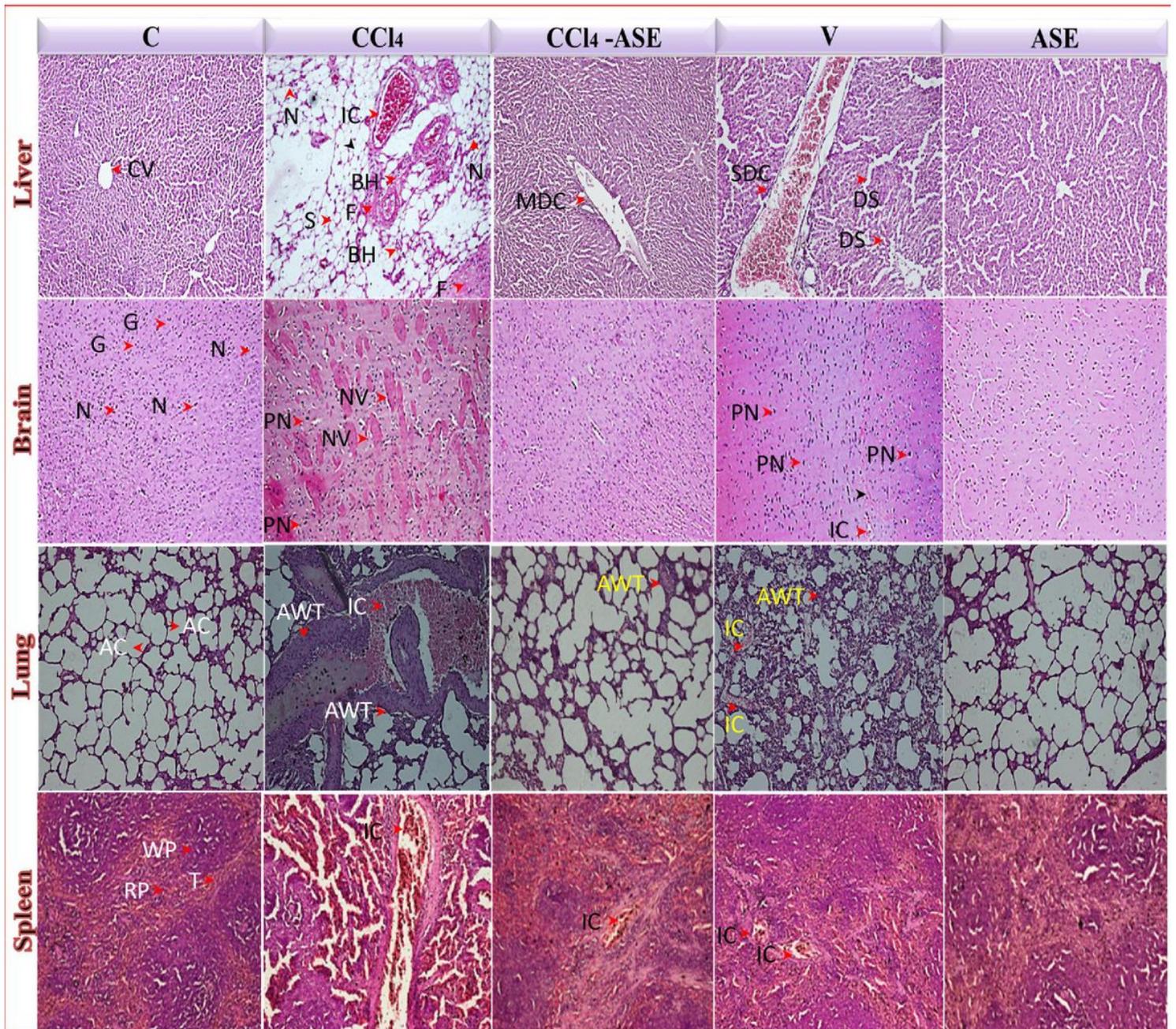


Figure 7

Figure 7

Photomicrographs (H&E stain, 200x) for liver, brain, lung, and spleen sections of the different studied groups revealed the ameliorating effects of Acacia Senegal extract (ASE) against the systemic toxicity induced by CCl₄ injection. AC: alveolar cells, AWT: alveolar wall thickness, BH: binucleated hepatocytes, CV: central vein, DS: dilation of sinusoidal cells, F: fibrous bands, G: glial cells, IC: inflammatory cells, MDC: mild dilation of central vein, N: neurons, NV: neurons with the vacuole, PN: neuron with pyknotic nuclei, RP: red pulp, SDC: severe dilation of central vein, S: steatosis, T: trabecula, WP: white pulp.

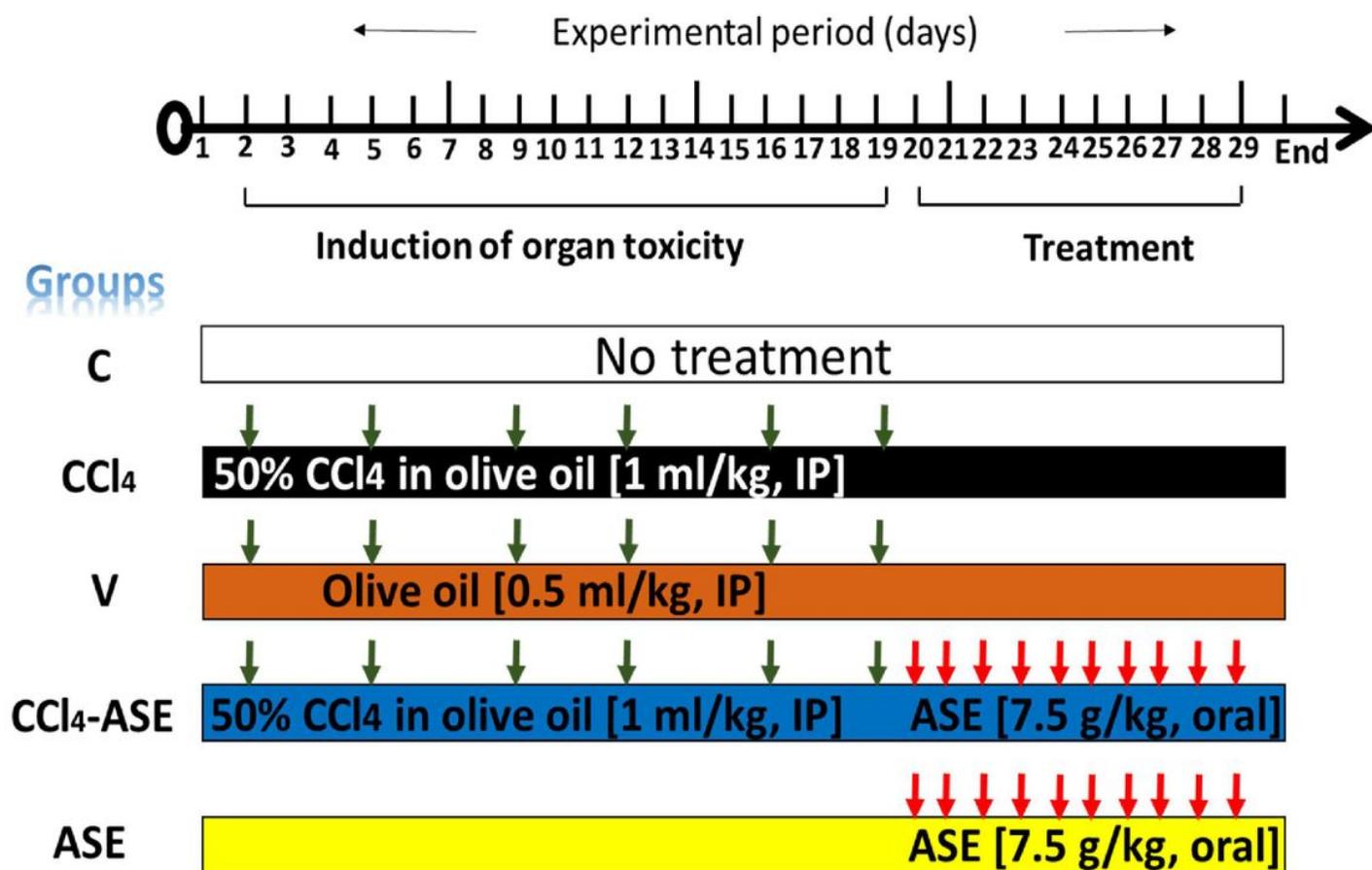


Figure 8

Figure 8

Experimental design with animal group treatments. Induction of systemic toxicity was performed by intraperitoneal (IP) injection with CCl₄ for 3 weeks (twice/week). Animals in the CCl₄-ASE group were orally gavaged with ASE for 10 days following induction of systemic toxicity. The study includes three control groups of animals, those orally administered ASE for 10 days (ASE group) and the untreated animals (C group), as well as animals in V group that were injected for 3 weeks (twice/week) with the vehicle of CCl₄ (olive oil).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryFigure1.pdf](#)
- [SupplementaryTable1.pdf](#)