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Citric acid protects against toluene-induced brain neurodegeneration and liver damage in rats

Omar M.E. Abdel-Salam[1*], Amany A. Sleem[2], Fatma A. Morsy[3]

[1]*Department of Toxicology and Narcotics, National Research Centre, Cairo, Egypt*

[2]*Department of Pharmacology, National Research Centre, Cairo, Egypt*

[3]*Department of Pathology, National Research Centre, Cairo, Egypt*

Abstract

Volatile solvent abuse is an important global health problem with serious consequences on the central nervous system. In this study, the effect of citric acid on brain oxidative stress and histopathological changes in brain and liver following exposure to toluene in rats was investigated. Rats were treated with toluene (900 mg/kg, intraperitoneally, daily) either alone or together with citric acid at 200, 400, 1000 or 2000 mg/kg for successive two days. Oxidative stress biomarkers malondialdehyde (MDA), reduced glutathione (GSH) and nitric oxide were determined. Histopathology of the brain and liver was done. Results showed that compared with the vehicle exposure to toluene caused increased brain MDA and NO along with decreased GSH content. Histopathological examination of the brain of toluene only-treated rats showed vacuolar degeneration, pyknotic and necrotic material in cerebral cortex and degeneration in the most of Purkinje cells in cerebellum. The liver exhibited distorted architecture, massive hepatocyte degeneration, and red blood cells in sinusoidal spaces. Citric acid given at 1 or 2 g/kg improved the pathological changes in cerebral cortex with most neurons being of normal shape after 2 g/kg citric acid. In cerebellum, few Purkinje cells appeared normal after citric acid administration. In the liver, citric acid at 1 or 2 g/kg almost prevented the deleterious effects induced by toluene. Collectively, these results indicate that treatment with citric acid at 1 or 2 g/kg was able to decrease neuronal damage in cerebral cortex and prevent liver damage in rats exposed to toluene.

Key words: Toluene; citric acid; neurodegeneration; liver injury; oxidative stress

1 Introduction

Volatile solvent abuse is a global health problem, especially among adolescents and young adults in both developed and developing countries [1, 2]. Toluene (methylbenzene) represents the most widely abused organic solvent because of its wide availability in gasoline, glues, adhesives, paint thinners, and household cleaners [3]. Because of its high lipid solubility, toluene is rapidly distributed to the brain [4] resulting in acute intoxication that manifests as cheerfulness, excitation followed by lethargy, motor incoordination, coma and even death due to respiratory depression [3]. Repeated and long-term exposure to toluene is associated with serious neurologic and mental disorders including leucoencephalopathy, ataxia, white matter changes, brain atrophy, and dementia [5-7]. Animal studies showed that acute exposure to toluene results in neuronal death in cerebral cortex and hippocampus and degeneration of cerebellar Purkinje cells. There was also increased immunostaining of the apoptotic marker caspase-3 in the cytoplasm of cortical and hippocampal neurons [8-10].

Oxidative stress is one of the most important mechanisms implicated in toluene-induced neuronal injury [11]. Oxidative stress is the term used to describe the state in which the balance between the formation of reactive oxygen metabolites and antioxidants in the cell is shifted towards the oxidant side. The result is oxidative modification of membrane lipids, enzymes and DNA with perturbations of cellular function and even lethal outcome. This occurs because of free radicals being generated at a high rate and/or deficient antioxidants [12]. Reactive oxygen species (ROS) are produced during metabolism; the most important site being the mitochondrial respiratory chain where leakage of electron onto molecular oxygen results in the generation of superoxide ($O_2^{\bullet-}$). The latter can be dismutated by the enzyme superoxide dismutase to form hydrogen peroxide (H_2O_2) that in turn is metabolized by catalase and glutathione reductase regenerating H_2O and O_2 or converted to the highly oxidant hydroxyl radical ($^{\bullet}OH$) via the Fenton reaction catalyzed by transition metals Cu^{+2} and Fe^{+3} [13]. Other sources of $O_2^{\bullet-}$ and H_2O_2 are flavoenzymes, e.g., xanthine oxidase activated in ischemia-reperfusion, lipoxygenase and cyclooxygenase, cytochrome P450, NADPH-dependent oxidase of phagocytes [14].

*Corresposnding author; E-mail: omasalam@hotmail.com

Mitochondrial damage by $O_2^{\bullet-}$ or environmental toxins results in increased release of reactive oxygen metabolites and further mitochondrial damage creating a vicious cycle that can lead to neuronal cell death [15, 16]. The brain tissue with its high rate of O_2 consumption and rich content of polyunsaturated fatty acids (PUFA) is considered highly vulnerable to oxidative stress [12]. An increase in the serum levels of the lipid peroxidation end product MDA has been reported in workers with toluene-containing paint thinners [17], while animal studies provided evidence for the increased generation of reactive oxygen metabolites in rat brain [18] with increased protein carbonyls [19], MDA and depletion of the antioxidant GSH [9, 20].

Citric acid (2-hydroxy-1,2,3-propanetricarboxylic acid) is an important intermediate in the tricarboxylic or Kreb's cycle in the mitochondria which is the final common pathway for the oxidation of carbohydrates, fatty acids and amino acids with provision of energy in the form of adenosine 5'-triphosphate or ATP for the metabolic requirements of the cell [21]. It is also a component of the human diet being present in large amounts in citrus fruits and fruit juices eg., lemon, orange, grape fruit, and tangerine [22]. Citric acid is used to prevent urinary calcium oxalate crystallization and stone formation [23] and citrate anticoagulation is employed during haemodialysis [24]. Citric acid was shown to attenuate the degranulation of polymorphonuclear leucocytes and the release of myeloperoxidase and the proinflammatory cytokine interleukin-1beta (IL-1β) during haemodialysis [25,26]. It was also shown to attenuate oxidative stress and protect against brain and liver damage during systemic endotoxaemia [27] and following the administration of such toxins as malathion [28], carbon tetrachloride [29, 30] or rotenone [20] in animal studies. The aim of this study is to investigate the possible protective effect of citric acid administration on the development of oxidative stress and tissue damage in the brain and liver following toluene intoxication in the rat.

2 Materials and methods

2.1 Animals

Male Sprague-Dawley rats, obtained from Animal House of the National Research Centre, Cairo, weighing between 150-170 g were group-housed under temperature- and light-controlled conditions with standard laboratory rodent chow and water provided ad libitum. Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.2 Drugs and chemicals

Toluene and citric acid (Sigma-Aldrich, St Louis, MO, USA) were used in the study. Other chemicals and reagents were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Toluene was freshly prepared in paraffin oil to obtain the required doses. Citric acid was dissolved in normal saline, freshly prepared before use and administered intraperitoneally (i.p.). The doses of toluene or citric acid used were based on other studies [31].

2.3 Study design

Rats were randomly divided into different equal groups, six rats each. Group 1 received the vehicle. Group 2 received toluene in paraffin oil (vol/vol) in a dose 900 mg/kg (2.6 ml/kg), i.p. on two successive days. Groups 3-7 received toluene (900 mg/kg, i.p) together with citric acid at doses of 200, 400, 1000 or 2000 mg/kg. Rats were then euthanized 4 h after the last treatments by decapitation under ether anesthesia for tissue collection. The brain and liver of each rat were quickly removed, washed with ice-cold phosphate buffered saline (PBS, pH 7.4), weighed, and stored at −80 °C until the biochemical analyses were carried out. The tissues were homogenized with 0.1 M phosphate buffer saline at pH 7.4 to give a final concentration of 10% w/v for the biochemical assays.

2.4 Biochemical analysis

2.4.1 Lipid peroxidation

Lipid peroxidation products in the brain homogenates was assayed by measuring the level of malondialdehyde (MDA) using the method of Ruiz-Larrea et al. [32] where the thiobarbituric acid reactive substances react with thiobarbituric acid to produce a red colored complex having a peak absorbance at 532 nm.

2.4.2 Reduced glutathione

Brain reduced glutathione (GSH) was determined in homogenates spectrophotometrically according to Ellman method [33]. The procedure is based on the reduction of Ellman´s reagent by –SH groups of GSH to form 2-nitro-s-mercaptobenzoic acid, which is intense yellow in colour and determined spectrophotometrically at 412 nm.

2.4.3 Nitric oxide

Nitric oxide measured as nitrite was determined using Griess reagent, according to the method of Moshage et al. [34] where nitrite, a stable end product of nitric oxide radical, is mostly used as indicator for the production of nitric oxide. In this assay, nitrate is converted to nitrite by nitrate reductase. Griess reagent then converts nitrite to a deep purple azo compound. The absorbance is read at 540 nm using spectrophotometer.

2.4.4 Serum liver enzymes

At the end of the experiments, blood samples were obtained from the retro-orbital venous plexus under light ether anesthesia. The activities of the liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase

(AST) in serum were measured according to Reitman–Frankel colorimetric transaminase procedure [35].

2.5 Statistics

Results are expressed as mean ± SE. Data were statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test for multiple group comparison. Statistical significance was considered at a probability value of less than 0.05. GraphPad Prism 6 for Windows (GraphPad Prism Software Inc., San Diego, CA, USA) was used.

3 Results

3.1 Biochemical results

3.1.1 Effect of citric acid on brain oxidative stress in toluene-treated rats

Rats given toluene exhibited significant increments in brain MDA and NO levels by 59.8% (28.10 ± 0.94 vs. 17.58 ± 0.76 nmol/g.tissue) and 54.5% (30.12± 1.32 vs. 19.03 ± 0.94 µmol/g.tissue) compared with the vehicle group. Meanwhile, the level of GSH was significantly decreased by 41.4% (1.88 ± 0.12 vs. 3.21 ± 0.04 µmol/g.tissue) in the brain of toluene-treated rats compared to their controls. Citric acid given at 200 or 400 mg/kg had no significant effect on brain MDA, NO or GSH in toluene-treated rats. The higher doses of 1 or 2 g/kg, however, resulted in significant decreases in MDA by 27.6% and 30.6% (20.33 ± 0.90 and 19.50 ± 0.50 vs. 28.10 ± 0.94 nmol/g.tissue) and in NO by 20.8% and 31.4% (23.85 ± 0.54 and 20.67 ± 0.89 vs. 30.12± 1.32 µmol/g.tissue). In toluene-treated rats, the administration of citric acid at 1 or 2 g/kg resulted in significant increase in GSH by 33.5% and 51.1% compared to the toluene control group (2.51 ± 0.09 and 2.84 ± 0.17 vs. 1.88 ± 0.12 µmol/g.tissue) (Fig. 1).

3.1.2 Effect of citric acid on liver oxidative stress in toluene-treated rats

In rats treated with only toluene, there was a significant increase in liver MDA and nitric oxide contents by 50.1% (40.34± 0.64 vs. 26.87± 0.94 nmol/g.tissue) and 45.3% (42.39± 0.98 vs. 29.18 ± 1.06 µmol/g.tissue). Meanwhile, GSH decreased by 34.6% (2.72 ± 0.16 vs. 4.16± 0.07µmol/g.tissue) compared with the vehicle control group. Citric acid given at 200 or 400 mg/kg had no significant effects on liver MDA, NO or GSH in toluene-treated rats. Treatment with citric acid at 1 or 2 g/kg resulted in significant decrease in live MDA by 24.7% and 28.23% (30.36± 0.65 and 28.95 ± 0.55 vs. 40.34 ± 1.56 nmol/g.tissue) and in NO by 21.3% and 29.8% (33.37± 1.0 and 29.77± 0.62 vs. 42.39 ± 0.98 µmol/g.tissue) compared to the toluene control. On the other hand, there was a significant increase in GSH content by 32.7% and 43.0% (3.61 ± 0.13 and 3.89 ± 0.06vs. 2.72 ± 0.16 µmol/g.tissue) (Fig. 2).

3.1.3 Effect of citric acid on serum enzymes

In rats treated with toluene, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activities in serum were markedly raised by 188.9% (169.6 ± 3.7 vs. 58.8 ± 2.2 U/l) and 160.5% (180.0 ± 3.4 vs. 69.1 ± 2.6 U/l) compared to their corresponding vehicle control values, respectively. The administration of citric acid at 1 or 2 g/kg resulted in significant decrease in serum ALT by 37.9% and 53% (105.5 ± 5.3 and 79.9 ± 6.0 vs. 169.6 ± 3.7 U/l) and AST by 39% and 58.7% (109.8 ± 6.0 and 74.4 ± 2.4 vs. 180.0 ± 3.4 U/l) (Fig. 3).

3.2 Brain histopathological changes

3.2.1 Cerebral cortex

The cerebral cortex of vehicle control rats showed normal architecture (Fig. 4A). Rats given toluene only exhibited area of vacuolar degeneration due to cytoplasmic destruction, many pyknotic cells, mitotic figures and necrotic material. Dilation of cerebral veins and perivascular edema, neurons with intracytoplasmic proliferation of filaments producing the neurofibrillary tangle, Lewy body, and plaque formation (Fig. 4B-D). The cerebral cortex of rats treated with toluene and citric acid at 200 mg/kg revealed no improvement of changes, vacuolation in most of sections that has been referred to as spongiform degeneration, necrotic neurons and hyalinized blood vessels were still present (Fig. 4E). Following toluene and citric acid at 400 mg/kg, the cortex still suffered from the deleterious effect of toluene in the form of degeneration of neuron, gliosis and congestion of cerebral blood vessel. The neurons showed intracytoplasmic proliferation of filaments producing the neurofibrillary tangle (Fig. 4F). Rats given toluene and citric acid at 1 g/kg showed moderate improvement in pathological changes as compared to the group treated with toluene only with absent neurofibrillary tangles, Lewy bodies, mitotic figures, or vacuolar degeneration, although pyknotic neurons, gliosis, and congested blood vessel were seen (Fig. 4G). More improvement in pathological changes was observed after treatment with citric acid at 2 g/kg where most neurons appeared normal although karyorrhexis in some neurons, gliosis, and congestion of blood vessel were observed (Fig. 4H).

3.2.2 Cerebellum

The cerebellum from the vehicle-treated rats showed normal structure (Fig. 5A). Exposure to toluene caused degeneration in the most of Purkinje cells (Fig. 5B). There was no improvement in the pathological changes after treatment with citric acid at 200 or 400 mg/kg (Figs. 5C & D). However, after the administration of 1 or 2 g/kg citric acid few Purkinje cells appeared normal (Figs. 5E & F).

3.2.3 *Liver histopathological changes*

The liver tissue sections of the vehicle control group showed a normal appearance (Fig. 6A). On the other hand, the administration of toluene induced deformity of liver architecture, massive hepatocyte degeneration, ballooning degeneration, pyknotic nuclei in some hepatocytes and red blood cells in sinusoidal spaces (Fig. 6B). Treatment with citric acid at 200 mg/kg showed no improvement in pathological changes; the liver tissue revealed ballooning degeneration, most hepatocytes appeared necrotic and red blood cells in the sinusoidal space were present (Fig. 6C). Sections from rats treated with toluene along with citric acid at 400 mg/kg revealed that most of hepatocytes appeared degenerated with cellular necrosis in most of hepatocytes in the form of vacuolated cytoplasm, hemorrhages and red blood cells in the sinusoidal space (Fig. 6D). The higher dose of citric acid (1 g/kg) resulted in improvement in pathological changes in the form of no ballooning degeneration, most of hepatocytes appeared normal although minimal vacuolation, fibrosis, congestion in portal vein and few inflammatory infiltrates were seen (Fig. 6E). Treatment with 2 g/kg citric acid decreased the deleterious effects induced by toluene; the central vein and hepatocytes appeared normal but red blood cells in the sinusoidal space were still present (Fig. 6F)

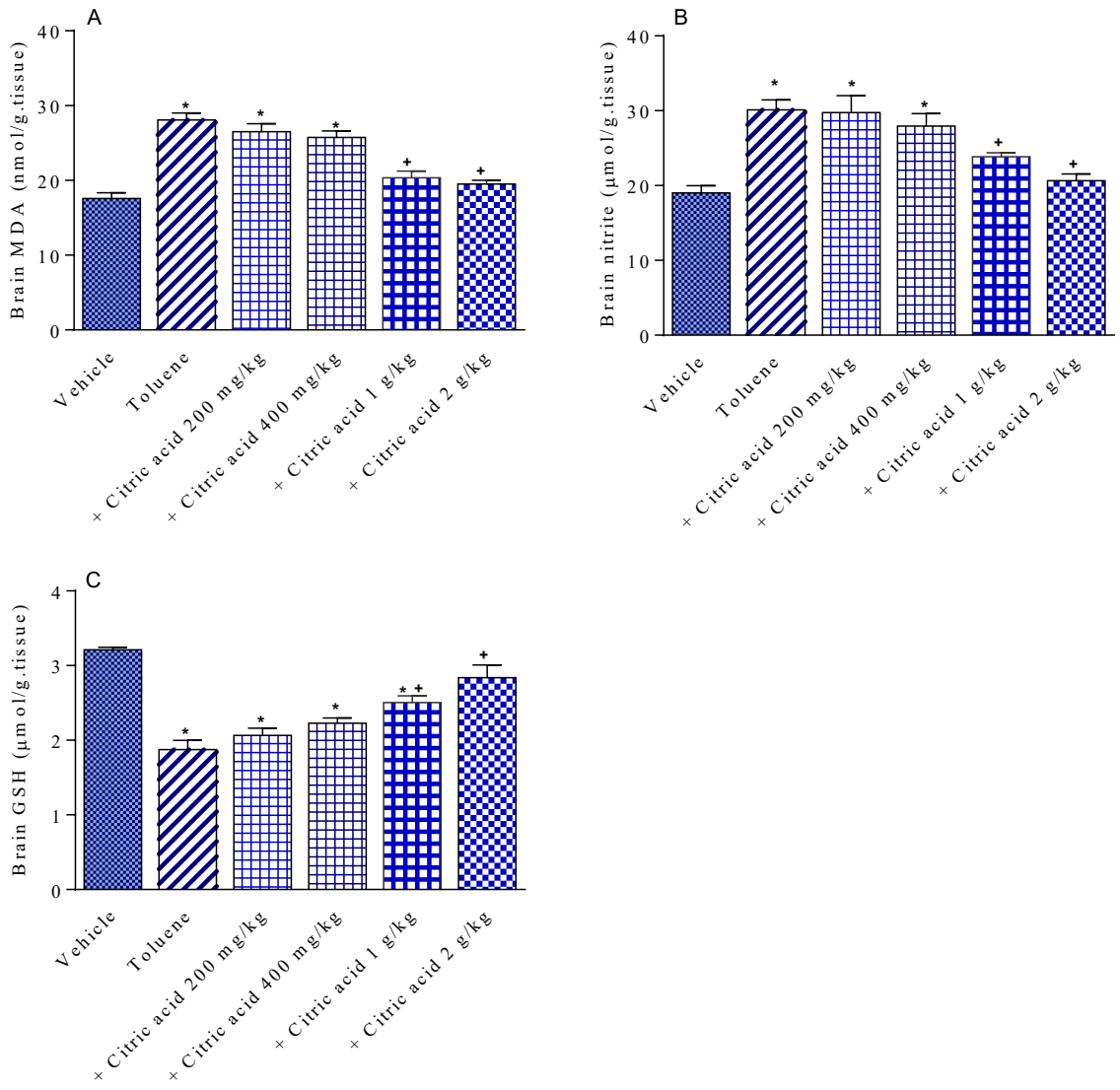

Figure 1. Effect of citric acid on brain malondialdehyde (MDA), nitrite and reduced glutathione (GSH) in toluene-treated rats. *: P<0.05 vs. vehicle. +: P<0.05 vs. toluene control.

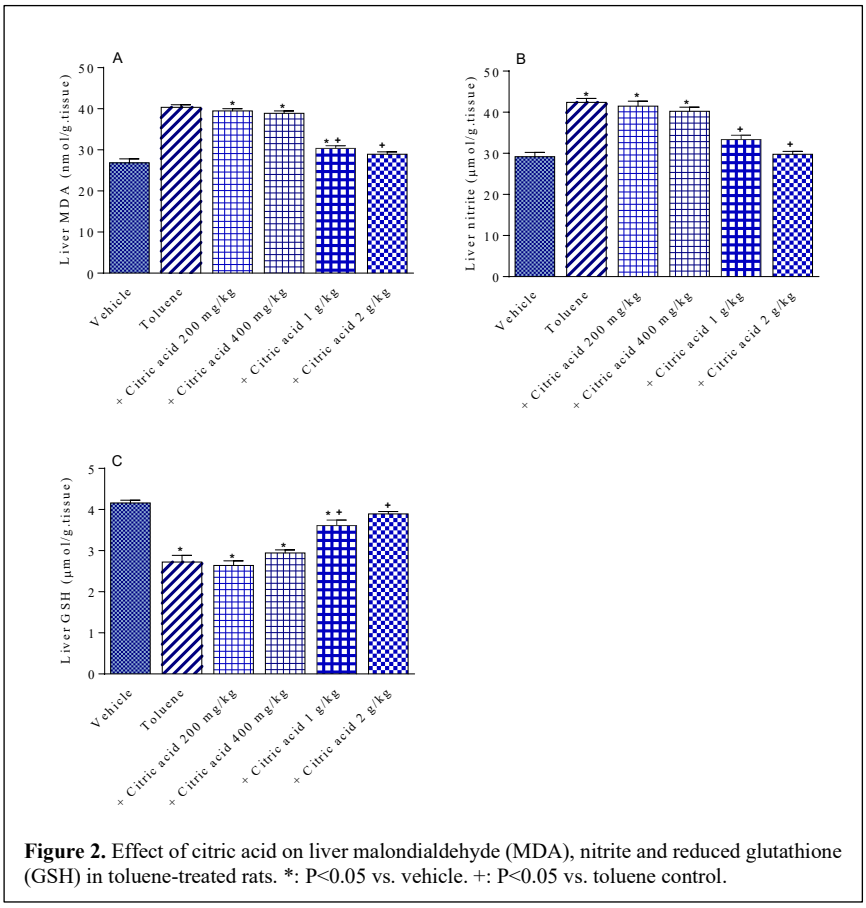

Figure 2. Effect of citric acid on liver malondialdehyde (MDA), nitrite and reduced glutathione (GSH) in toluene-treated rats. *: P<0.05 vs. vehicle. +: P<0.05 vs. toluene control.

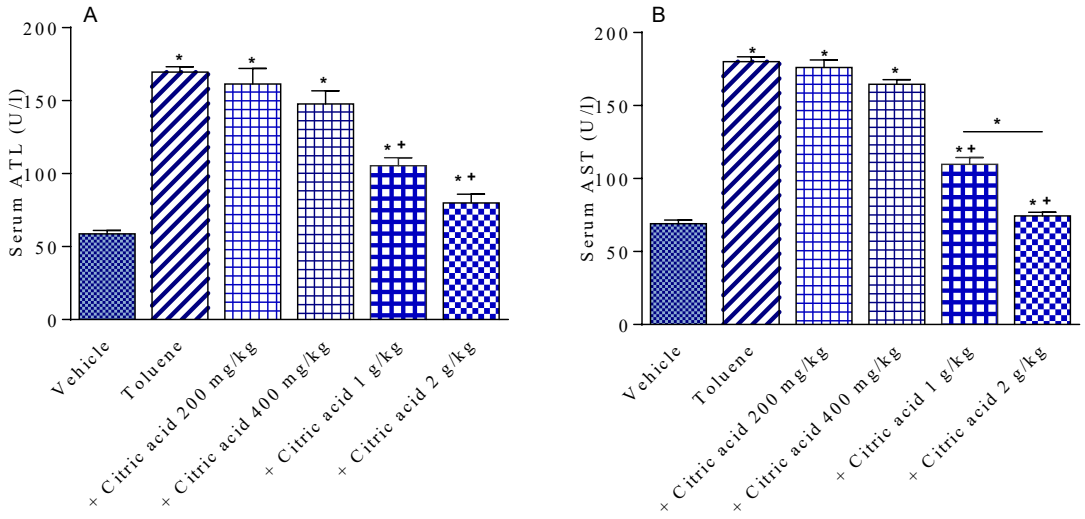

Figure 3. Effect of citric acid on serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in toluene-treated rats. *: P<0.05 vs. vehicle and between different groups as shown in the graph. +: P<0.05 vs. toluene control.

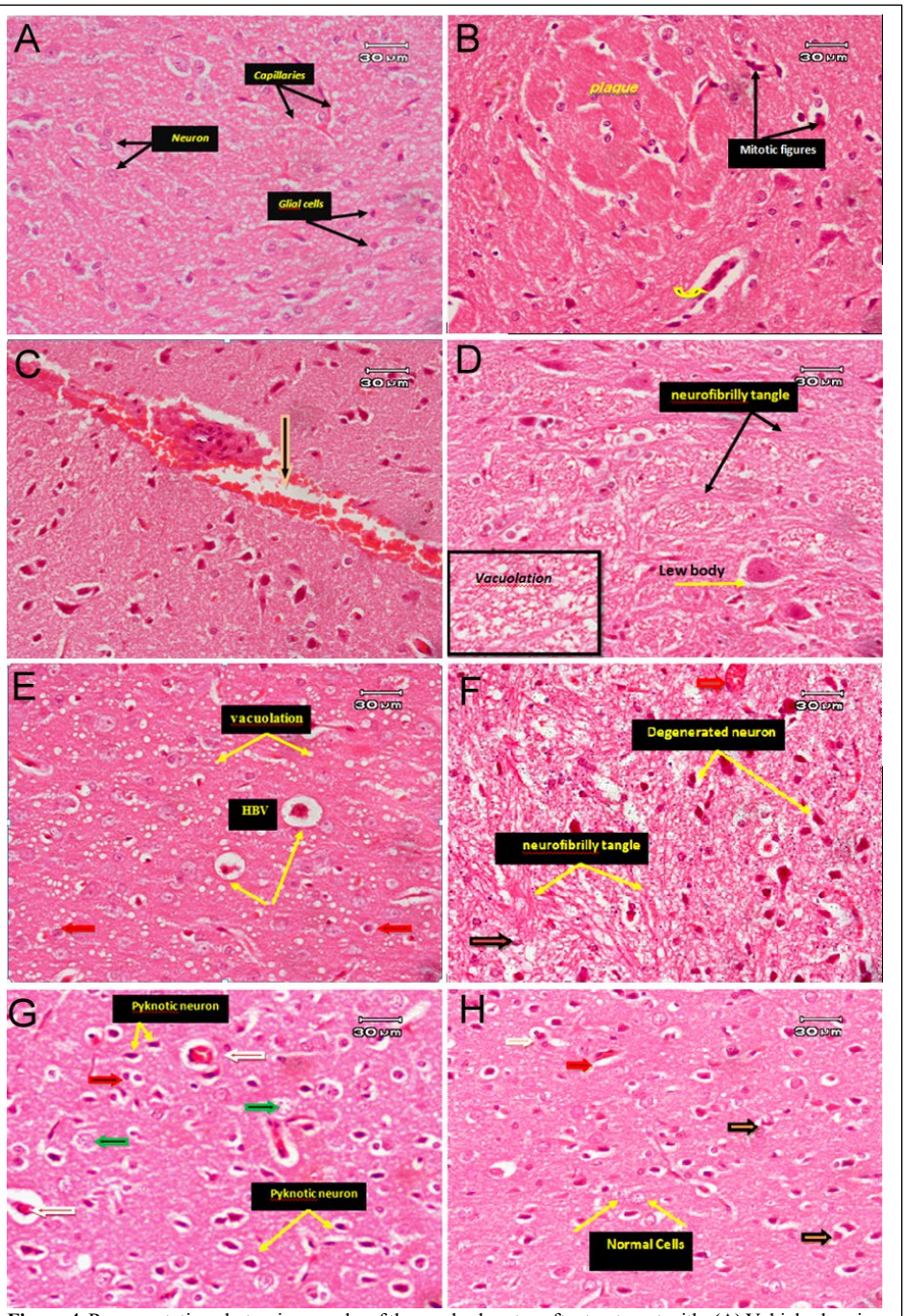

Figure 4. Representative photomicrographs of the cerebral cortex after treatment with**: (A)** Vehicle showing normal appearance of neurons, glial cells, and capillaries. **(B)** Toluene only showing mitotic figure and plaque formation and aggregate of necrotic material (yellow arrow). **(C)** Toluene only showing dilation of cerebral veins and perivascular edema (arrow). **(D)** Toluene only **(**another filed) showing deformity of normal structure, neurofibrillary tangle due to intracytoplasmic proliferation of filaments, Lewy body and vacuolation. **(E)** Toluene along with citric acid 200 mg/kg showing spongiform degeneration (vacuolation), some necrotic neurons (red arrow) and hyalinized blood vessels (yellow arrow). **(F)** Toluene along with citric acid 400 mg/kg showing degeneration of the most of neurons (yellow arrow), neurofibrillary tangle due to intracytoplasmic proliferation of filaments and gliosis (black arrow). (G) Toluene along with citric acid 1g/kg showing moderate improvement in pathological changes but some degenerated neurons (green arrow), pyknotic neurons (yellow arrow), gliosis (red arrow), and congested blood vessel (white arrow) are seen. **(H)** Toluene along with 2 g/kg citric acid showing more improvements in pathological changes, most neurons appeared normal, karyorrhexis (black arrow) in some neurons, gliosis (white arrow) and congestion of cerebral blood vessel (red arrow).

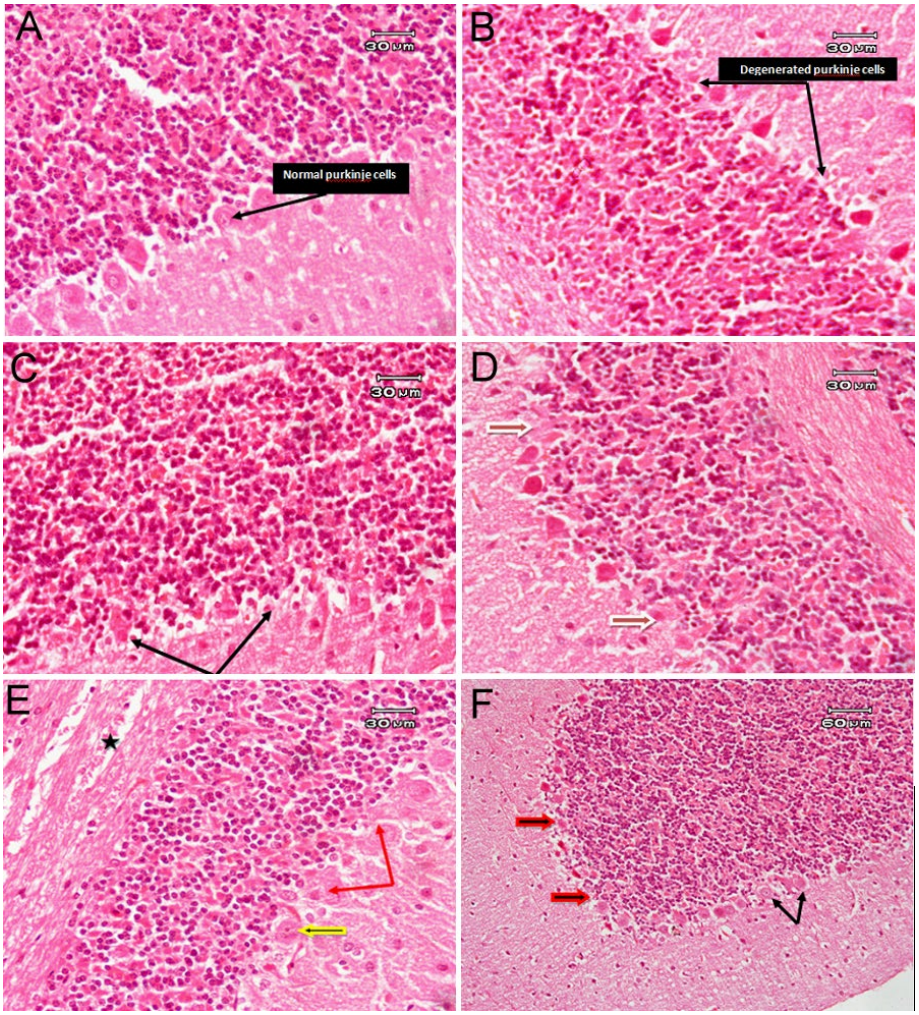

Figure 5. Representative photomicrographs of the cerebellum after treatment with**: (A)** Vehicle showing normal appearance of Purkinje cells (arrow). **(B)** Toluene only showing degeneration the most of Purkinje cells (black arrow). **(C)** Toluene and citric acid 200 mg/kg showing degeneration in most of Purkinje cell (black arrow). **(D)** Toluene and citric acid 400 mg/kg showing degeneration in most of Purkinje cell (white arrow). **(E)** Toluene and citric acid 1 g/kg showing degeneration the most of Purkinje cells (red arrow), few appeared normal (yellow arrow) and congestion of blood vessel in white matter (star). **(F)** Toluene and citric acid 2g/kg showing degeneration the most of Purkinje cell (red arrow), few appeared normal (black arrow).

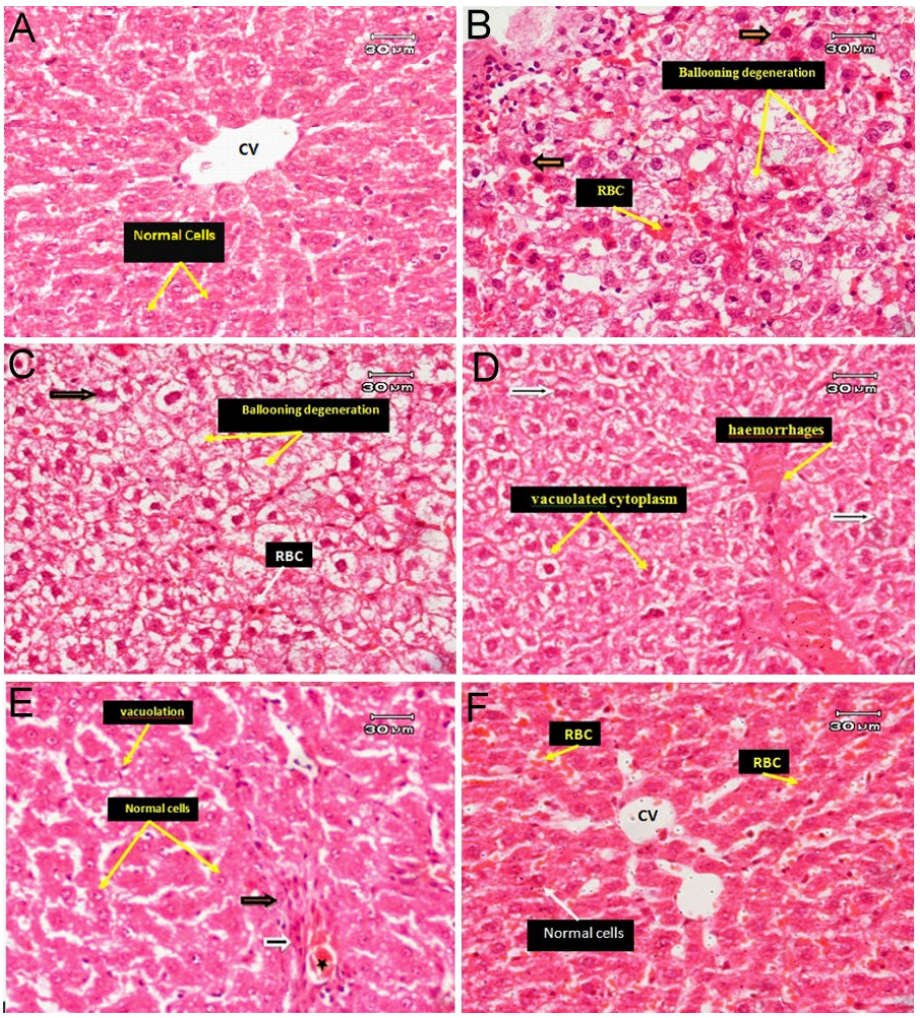

Figure 6. Representative photomicrographs of the liver tissue of rats treated with**: (A)** Vehicle showing normal histological structure of hepatic cells and central vein (CV). **(B)** Toluene only showing massive hepatocyte degeneration, pyknotic nuclei in some cells (black arrow), ballooning degeneration and red blood cells in dilated blood sinusoids. **(C)** Toluene along with citric acid 200 mg/kg showing that most cells appeared necrotic (black arrow), ballooning degeneration and red blood cells in dilated blood sinusoids. **(D)** Toluene along with citric acid 400 mg/kg showing degenerated cells (white arrow), cellular necrosis in most of hepatocytes in the form of vacuolated cytoplasm and hemorrhage in blood vessels. **(E)** Toluene along with citric acid 1 g/kg showing most cells appeared normal but minimal vacuolation, minimal fibrosis (black arrow), congestion in portal vein (star) and few inflammatory infiltrate (white arrow) are still present. **(F)** Toluene along with citric acid 2 g/kg showing more improvement in pathological changes in the form of normal central vein (CV) and normal hepatocytes, but red blood cells in sinusoidal space are still present.

4 Discussion

The findings in the present study provides the first evidence that the administration of citric acid was able to ameliorate the toluene induced cerebral- and hepato-toxicity. In this study, acute exposure to toluene was associated with significant increase in brain lipid peroxidation assessed by measuring MDA, indicative of increased generation of ROS and consequent attack on membrane PUFA [36]. There was also significant decrease in reduced glutathione (GSH), an important antioxidant and free radical scavenger molecule. By shuttling between the reduced (GSH) and the oxidized (GSSG) forms, the tripeptide glutathione (γ-L-glutamyl-L-cysteinylglycine) acts to maintain the redox balance in the cell [37]. The observed decrease in the level of GSH, therefore, is indicative of increased consumption of glutathione by the excessive generation of ROS in the brain of toluene-intoxicated rats. The observed decrease in GSH could also be caused by toluene-inducing mitochondrial damage and consequent decrease in energy supply, resulting in decreased synthesis of glutathione or failure of GSH regeneration. Our results are thus in agreement with other studies indicating increased lipid peroxidation, and decreased cellular antioxidants following exposure to toluene in rats [8, 20, 27, 38, 39]. Increased levels of protein carbonyls indicative of protein oxidation in the brain have also been reported in rats exposed to toluene [19]. The notion that oxidative stress plays a significant role in toluene

neurotoxicity derives further support from studies reporting decreased oxidative stress and brain neurodegeneration in toluene intoxicated rats after treatment with the antioxidants omega-3, vitamin C, and vitamin E [8, 38].

Similar to its effects on the brain, toluene induced significant increase in lipid peroxidation besides depletion of GSH in the liver tissue which is in agreement with our previous observations [10, 20] and which highlights increased oxidative stress as an important mechanism in the toluene-induced tissue injury. Other studies reported increased 8-hydroxy-2′-deoxyguanosine in the liver and kidney indicative of oxidative DNA damage in rats exposed to toluene [40]. In this study, treatment with citric acid at 1 or 2 g/kg was associated with a significant decrease in lipid peroxidation in brain and liver tissues, which is possibly due to an antioxidant effect of citric acid. There was also markedly increased GSH content in the brain and liver, indicate of lowered levels of ROS and/or increased regeneration of GSH by improved energy supply to the cell. Similar antioxidant effects of citric acid were observed in rotenone intoxicated rats where its administration decreased MDA, and increased total antioxidant capacity in brain and liver tissue [27].

We also observed significant increase in NO in brain and liver of rats following toluene injection, which is in agreement with previously reported studies [9, 10, 20, 27]. Other researchers have reported increased 3-nitrotyrosine, a marker of protein nitration in brain of toluene-treated rats [41]. The increase in NO could therefore be involved at least in part in the neurotoxic effects of toluene. The excessive generation in NO during inflammatory and toxic states can result in neuronal death. This is attributable to the generation of the highly reactive peroxynitrite ($ONOO^-$) from the reaction of NO and $O_2^{\bullet-}$ causing oxidation, nitration of protein tyrosine residues, and nitrosylation of thiols in proteins or GSH [42,43]. In this study, the toluene-induced increase in brain and liver NO levels were ameliorated by administering citric acid. In a previous study, the increase in inducible nitric oxide synthase (iNOS) immunostaining in the livers of mice during lipopolysaccharide-induced endotoxaemia was decreased by treatment with 1 or 2 g/kg of citric acid [27]. This suggests that a decrease in iNOS-derived NO could be involved at least in part in the protective effect of citric acid.

Our results showed that the cerebral cortex of toluene-intoxicated rats suffered vacuolar degeneration and necrosis. Neurons with neurofibrillary tangle, Lewy body, and plaque formation were observed. Other studies reported the presence of dead and apoptotic neurons, perineuronal vacuolation, glial cells and cerebral oedema in brain from rats treated with the solvent [8, 9, 20, 38, 44]. There was also degeneration of cerebellar Purkinje cells which is consistent with our previously published studies [10, 39] and in line with the reported cerebellar symptoms in those who were exposed to the solvent [45]. We also demonstrated that toluene caused distorted liver architecture, massive hepatocyte degeneration, ballooning degeneration, and red blood cells in sinusoidal spaces. An earlier study showed vacuolar degeneration, massive inflammatory infiltrate and hemorrhage in rats given the solvent at 900 mg/kg for two successive days. In the present study, the administration of citric acid at 1 or 2 g/kg was found to improve the toluene-induced pathological changes in cerebral cortex and liver in a dose-dependent manner. In a previous study, liver necrosis, inflammatory cell infiltration and DNA fragmentation during systemic endotoxaemia in mice was almost prevented by treatment with 1 or 2 g/kg of citric acid [27]. In the Parkinson's disease model caused by rotenone injection in rats, citric acid at 200 or 400 mg/kg, however, prevented the histopathological changes in cortex, striatum, hippocampus and liver [20].

5 Conclusion

In summary, the findings in the present study provided the first evidence that treatment with citric acid prevents the toluene induced cerebral- and hepato-toxicity. The protective effect of citric acid was demonstrated at both the biochemical and histological levels. The mechanism (s) by which citric acid prevents the toluene-induced tissue damage could involve a decrease in oxidative and nitrosative stress. By stimulation of Kreb's cycle and provision of reducing equivalents for the energy demands of the cell, citric acid is also able to counteract the bioenergetic deficit induced by toluene.

Conflict of interest

The authors declare no conflicts of interest

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References

1. Filley CM. Toluene abuse and white matter a model of toxic leukoencephalopathy. Psychiatr Clin N Am. 2013; 36: 293–302. http://dx.doi.org/10.1016/j.psc.2013.02.008
2. Elkoussi A, Bakheet S. Volatile substance misuse among street children in Upper Egypt. Subst Use Misuse. 2011; 46 (Suppl 1): 35-39.
3. Lubman DI, Yucel M, Lawrence AJ. Inhalant abuse among adolescents: neurobiological considerations. Br J Pharmacol 2008; 154: 316–326.
4. Gerasimov MR. Brain uptake and biodistribution of [11C] toluene in nonhuman primates and mice. Methods Enzymol 2004; 385: 334–349.
5. Rosenberg NL, Grigsby J, Dreisbach J, Busenbark D, Grigsby P. Neuropsychologic impairment and MRI abnormalities associated with chronic solvent abuse. J Toxicol Clin Toxicol 2002; 40(1):21–34.
6. Fornazzari L, Pollanen MS, Myers V, Wolf A. Solvent abuse-related toluene leukoencephalopathy. J Clin Forensic Med 2003; 10(2), 93–95. doi: 10.1016/S1353-1131(03)00035-X
7. Filley CM, Halliday W, Kleinschmidt-DeMasters BK. The effects of toluene on the central nervous system. J Neuropathol Exp Neurol. 2004; 63(1):1–12.
8. Atef MM, Galal AF, Abdel-Salam OME, Shafee N, Tadros MG, Khalifa AE. Neurobehavioral and neurochemical changes

in toluene-treated rats and the effect of antioxidants. World J Pharmaceutical Res. 2015; 4(12):309–356.
9. Abdel-Salam OME, Youness ER, Morsy FA, Yassen NN, Mohammed NA, Sleem AA. Methylene blue protects against toluene-induced brain damage: involvement of nitric oxide, NF-κB, and caspase-3. Reactive Oxygen Species. 2016; 2(5): 371–87. doi: 10.20455/ros.2016.855.
10. Abdel-Salam OME, Sleem AA, Youness ER, Morsy FA. Exacerbation of toluene's neuro- and hepato-toxicity by amiodarone or chlorpropamide: Involvement of oxidative stress. Reactive Oxygen Species 2019; 8(24):358–371.
11. Burmistrov SO, Arutyunyan AV, Stepanov MG, Oparina TI, Prokopenko VM. Effect of chronic inhalation of toluene and dioxane on activity of free radical processes in rat ovaries and brain. Bull Exp Biol Med 2001; 132(3): 832-836.
12. Halliwell B. Reactive oxygen species and the central nervous system. J Neurochem 1992; 59(5):1609–23. doi: 10.1111/j.1471-4159.1992.tb10990.x.
13. Aruoma OI. Free radicals, oxidative stress, and antioxidants in human health and disease. JAOCS 1998; 75: 199–212.
14. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature 2000; 408: 239-249.
15. Jacobson J, Duchen MR. Mitochondrial oxidative stress and cell death in astrocytes – requirement for stored Ca2+ and sustained opening of the permeability transition pore. J Cell Sci 2002 ;115(Pt 6):1175-88.
16. Zorov D, Juhaszova M, Sollott SJ. Mitochondrial ROS-induced ROS release: An update and review. Biochimica et Biophysica Acta 2006; 1757: 509–517.
17. Halifeoglu I, Canatan H, Ustundag B, Ilhan N, Inanc F. Effect of thinner inhalation on lipid peroxidation and some antioxidant enzymes of people working with paint thinner. Cell Biochem Funct 2000; 18(4): 263–267.
18. Mattia CJ, Adams JD Jr, Bondy SC. Free radical induction in the brain and liver by products of toluene catabolism. Biochem Pharmacol 1993;46(1):103–110. doi: 10.1016/0006-2952(93)90353-x.
19. Kodavanti PR, Royland JE, Moore-Smith DA, Besas J, Richards JE, Beasley TE, et al. Acute and subchronic toxicity of inhaled toluene in male Long-Evans rats: oxidative stress markers in brain. Neurotoxicology 2015; 51:10–9. doi: 10.1016/j.neuro.2015.09.001.
20. Abdel-Salam OME, Morsy SMY, Youness ER, Yassen NN, Shaffie N, Sleem AA. Citric acid protects dopaminergic cells against rotenone-induced neurodegeneration. Reactive Oxygen Species 2020; 9(27):118–135.
21. Fromm HJ, Hargrove MS. The tricarboxylic acid cycle. In: Essentials of biochemistry. Berlin Heidelberg: Springer-Verlag; 2012, p. 205-221.
22. Penniston KL, Nakada SY, Holmes RP, Assimos DG. Quantitative assessment of citric acid in lemon juice, lime juice, and commercially-available fruit juice products. J Endourol 2008; 22(3): 567-570.
23. Robinson MR, Leitao VA, Haleblian GE, Scales CD, Jr., Chandrashekar A, Pierre SA, et al. Impact of long-term potassium citrate therapy on urinary profiles and recurrent stone formation. J Urol 2009; 181(3):1145–50. doi: 10.1016/j.juro.2008.11.014.
24. Stucker F, Ponte B, Tataw J, Martin PY, Wozniak H, Pugin J, Saudan P. Efficacy and safety of citrate-based anticoagulation compared to heparin in patients with acute kidney injury requiring continuous renal replacement therapy: a randomized controlled trial. Critical Care 2015; 19(1): 91.
25. Gabutti L, Ferrari N, Mombelli G, Keller F, Marone C. The favorable effect of regional citrate anticoagulation on interleukin-1beta release is dissociated from both coagulation and complement activation. J Nephrol 2004; 17(6):819–25.
26. Gritters M, Grooteman MP, Schoorl M, Schoorl M, Bartels PC, Scheffer PG, et al. Citrate anticoagulation abolishes degranulation of polymorphonuclear cells and platelets and reduces oxidative stress during haemodialysis. Nephrol Dial Transpl 2006; 21(1):153-159.
27. Abdel-Salam OM, Youness ER, Mohammed NA, Morsy SM, Omara EA, Sleem AA. Citric acid effects on brain and liver oxidative stress in lipopolysaccharide-treated mice. J Med Food 2014; 17(5):588–98. doi: 10.1089/jmf.2013.0065.
28. Abdel-Salam OM, Youness ER, Mohammed NA, Yassen NN, Khadrawy YA, El-Toukhy SE, et al. Novel neuroprotective and hepatoprotective effects of citric acid in acute malathion intoxication. Asian Pac J Trop Med 2016; 9(12):1181–94. doi: 10.1016/j.apjtm.2016.11.005.
29. Abdel-Salam OME, Sleem AA, Shaffie NM. Hepatoprotective effects of citric acid and aspartame on carbon tetrachloride-induced hepatic damage in rats. EXCLI J 2008; 8:41–9. doi: 10.17877/DE290R-15693
30. Abdel Salam OME, Sleem AA, Shaffie NM. Protection against carbon tetrachloride-induced liver damage by citric acid. Cell Biol: Res Ther 2015, 4:1. doi: 10.4172/2324-9293.1000116
31. Abdel-Salam OME, Shaffie NM, Omara EA, Yassen NN. Citric Acid an Antioxidant in Liver. In: Vinood Patel, editors: The Liver: Oxidative Stress and Dietary Antioxidants, Chennai: Academic Press; 2018, p. 183-198.
32. Ruiz-Larrea MB, Leal AM, Liza M, Lacort M, de Groot H. Antioxidant effects of estradiol and 2-hydroxyestradiol on iron-induced lipid peroxidation of rat liver microsomes. Steroids 1994; 59(6):383–8. doi: 10.1016/0039-128x(94)90006-x.
33. Ellman GL: Tissue sulfhydryl groups. Arch Biochem 1959;82: 70–77.
34. Moshage H, Kok B, Huizenga JR. Nitrite and nitrate determination in plasma: a critical evaluation. Clin Chem 1995;41:892–896.
35. Crowley LV. The Reitman-Frankel colorimetric transaminase procedure in suspected myocardial infarction. Clin Chem 1967; 13: 482-487.
36. Gutteridge JM. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin Chem 1995; 41(12 Pt 2):1819–28.
37. Wu G, Fang YZ, Yang S, Lupton JR, Turner ND. Glutathione metabolism and its implications for health. J Nutr 2004; 134: 489–492.
38. Meydan S, Altas M, Nacar A, Ozturk OH, Tas U, Zararsiz I, Sarsilmaz M. The protective effects of omega-3 fatty acid against toluene-induced neurotoxicity in prefrontal cortex of rats. Hum Exp Toxicol 2012; 31(11):1179–85. doi: 10.1177/0960327112457187.
39. Abdel-Salam OME, Sleem AA, Khadrawy YA, Morsy FA. Prevention of toluene-induced brain neurodegeneration by atropine and neostigmine. J Basic Pharmacol Toxicol 2020;4(1):1-9.
40. Tokunaga I, Gotohda T, Ishigami A, Kitamura O, Kubo S. Toluene inhalation induced 8-hydroxy-2'-deoxyguanosine formation as the peroxidative degeneration in rat organs. Leg Med (Tokyo) 2003; 5(1):34–41.
41. Riegel AC, Ali SF,Torinese S, French ED. Repeated exposure to the abused inhalant toluene alters levels of neurotransmitters and generates peroxynitrite in nigrostriatal and mesolimbic nuclei in rat. Ann NY Acad Sci 2004; 1025: 543–551. doi: 10.1196/annals.1316.079
42. Wink DA, Feelisch M, Vodovotz Y, Fukuto J, Grisham MB. The chemical biology of nitric oxide. In: Gilbert, Colton, editors. Reactive oxygen species in biological systems. New York: Kluwer Academic/Plenum Publishers; 1999, p. 245-291.
43. Brown GC. Nitric oxide and neuronal death. Nitric Oxide 2010; 23(3):153–65. doi: 10.1016/j.niox.2010.06.001.
44. Tumer AR, Atilla P, Onbasilar I, Biberoglu G, Muftuoglu S. Protective role of vitamin E on the harmful effects of toluene on brain tissue. Neurosciences 2007; 12 (3): 191-197.

45. Isa MFM, Zain NRM, Gaillard F, Chee FKY. Toluene dependency, psychosis, and cerebellar syndrome. J Neuropsychiatry Clin Neurosci 2013; 25:2, E42-43.