DRUG DISCOVERY

The protective effects of methylene blue on thioacetamide-induced acute liver and brain injury

Omar ME Abdel-Salam[1*], **Eman R Youness**[2], **Fatma A Morsy**[3], **Amany Ameen Sleem**[4]

To Cite:
Abdel-Salam OME, Youness ER, Morsy FA, Sleem AA. The protective effects of methylene blue on thioacetamide-induced acute liver and brain injury. *Drug Discovery* 2023; 17: e32dd1951
doi: https://doi.org/10.54905/disssi.v17i40.e32dd1951

Author Affiliation:
[1]Department of Toxicology and Narcotics, National Research Centre, Cairo, Egypt
[2]Department of Medical Biochemistry, National Research Centre, Cairo, Egypt
[3]Department of Pathology, National Research Centre, Cairo, Egypt
[4]Department of Pharmacology, National Research Centre, Cairo, Egypt

[*]**Corresponding author**
Department of Toxicology and Narcotics, National Research Centre, Cairo,
Egypt
Email: omasalam@hotmail.com

Peer-Review History
Received: 05 July 2023
Reviewed & Revised: 08/July/2023 to 11/September/2023
Accepted: 14 September 2023
Published: 18 September 2023

Peer-Review Model
External peer-review was done through double-blind method.

Drug Discovery
pISSN 2278–540X; eISSN 2278–5396

DISCOVERY SCIENTIFIC SOCIETY

ABSTRACT

This study aimed to investigate the potential protective effects of methylene blue (MethyB) on thioacetamide (TAA)-induced acute liver and brain injury in rats. TAA (300 mg/kg) was administrated intraperitoneally (i.p.) with or without MethyB at 10, 20, or 40 mg/kg for two successive days, and rats were euthanized 24 hours after the last treatments. Markers of oxidative stress, including malondialdehyde (MDA), reduced glutathione (GSH), and nitric oxide, were determined in liver and brain tissues. Histological examination of liver tissue and different brain regions such as the cerebral cortex, hippocampus, and cerebellum were carried out. Results indicated that compared with the respective saline controls, MDA and nitric oxide levels were significantly raised in the TAA control group, but GSH levels were significantly decreased in the liver and brain tissues. Administration of MethyB to TAA-treated rats resulted in a significant and dose-related decrease in lipid peroxidation (MDA) and nitric oxide. At the same time, the levels of GSH increased in the liver and brain compared with the TAA control group. TAA evoked marked liver tissue damage with micro and macrovesicular steatosis, swelling and apoptosis of hepatocytes, and inflammatory cell aggregates. In addition, TAA caused neuronal cell loss, necrosis, apoptosis of neurons, vacuolar degeneration in cortex and focal gliosis. The hippocampus suffered structural deformity, and shrinkage of large pyramidal cells, with darkened nuclei. In the cerebellum, widely displaced or emptiness of most Purkinje cells, and vacuolation of white matter were observed. MethyB prevented the pathological changes caused by TAA in the brain and liver in a dose-dependent manner, with the highest dose of 40 mg/kg providing a remarkable protective effect. The study indicated that MethyB prevented the harmful effects of acutely administered TAA in the liver and brain of rats. These effects of MethyB involved lowered levels of oxidative stress.

Keywords: Thioacetamide; methylene blue; liver damage; hepatic encephalopathy; oxidative stress

1. INTRODUCTION

The liver is the largest organ in the body, which plays an essential role in carbohydrate, protein, and fat metabolism. It is also the central site for detoxifying many drugs, toxins and xenobiotics. This function makes the liver susceptible to injury by the active metabolites, free radicals and reactive oxygen metabolites resulting from the biotransformation and metabolism of these drugs, and xenobiotics, which can damage liver cells or exacerbate an existing liver pathological condition (Chiang, 2014). When the production of these reactive species exceeds the capacity of the cell's antioxidants, a state of oxidative stress is said to be present in which there is oxidative modification of many critical cellular biomolecules causing oxidation of membrane phospholipids (lipid peroxidation), damage to the mitochondria or cellular proteins, DNA base mutation, and depletion of cellular antioxidants, thereby resulting in serious cellular derangement and possibly lethal outcome (Halliwell, 1996; Halliwell and Gutteridge, 1997). Oxidative stress plays a significant role in the onset and progression of a variety of liver disorders since the use of antioxidants has proved to be effective in their treatment (Mari et al., 2010).

Thioacetamide (TAA) is an organosulfur compound, previously used as a fungicide, and which is known for its ability to cause serious toxic liver injury. In rats, single high doses of TAA cause acute liver failure with centrilobular necrosis and encephalopathy, while its chronic administration at low doses can induce liver fibrosis (Túnez et al., 2005; Uskukovic-Markovic et al., 2007). These effects of TAA make the toxin a widely used experimental tool for studying the pathogenetic mechanisms of acute liver failure and encephalopathy, and for screening potential therapeutic interventions. The toxic action of TAA on liver cells results from its oxidative bioactivation by hepatic cytochrome P450 enzymes or FAD-containing monooxygenases into its metabolites TAA-S-oxide and TAA-S, S-dioxide. These chemically reactive metabolites of TAA cause oxidative stress and glutathione depletion and modify critical cellular components by covalently binding to proteins and lipids, resulting in lipid peroxidation, centrilobular necrosis, and inflammation (Wallace et al., 2015).

The phenothiazine dye, methylene blue (MethyB) is utilized in the treatment of several medical disorders, including methemoglobinemia, cyanide poisoning Clifton and Leikin, (2003), malaria Meissner et al., (2006), and encephalopathy occurring in patients undergoing chemotherapy with ifosfamid (Muñoz, 2006). It is also an effective rescue treatment in vasoplegic shock following cardiac surgery (McCartney et al., 2018). Recent research in MethyB focuses on its potential to treat central nervous system disorders, e.g., depression and Alzheimer's disease (Naylor et al., 1986; Medina et al., 2011; Alda et al., 2017). MethyB was reported to protect brain neurons in experimentally-induced Huntington's disease Sontag et al., (2012), and traumatic brain injury (Talley-Watts et al., 2014).

It protected the brain neurons and liver cells against toxic chemical damage by the pesticide rotenone, the organophosphorus compound malathion Abdel-Salam et al., (2016a), and the organic solvent toluene Abdel-Salam et al., (2016b) in rats, by the virtue of an antioxidant and anti-inflammatory actions. In this study, we examined the impact of MethyB on the development of oxidative stress, brain neuronal injury, and liver cell damage in the rat model of acute liver toxicity induced by TAA. In addition, we carried out a detailed histological investigation of the effect of TAA on different brain regions besides its impact on the liver tissue.

2. MATERIALS AND METHODS

Animals

Male Sprague-Dawley strain rats (170-180 g: National Research Centre, Cairo) were used in the experiments. Rats were housed under a standard 12-h light/dark cycle and had free food and tap water access. The animal experiments followed the recommendations of the Institute Ethics Committee and the Guide for Care and Use of Laboratory Animals of the US National Institutes of Health (Publication No. 85-23, revised 1996).

Drugs and chemicals

Thioacetamide and methylene blue were purchased from Sigma-Aldrich (St Louis, MO, USA). Other chemicals and reagents were from Sigma Chemical Co. (St Louis, MO, USA).

Experimental groups

Rats were randomly allocated into four equal groups (6 rats each).

Group 1: Received i.p. saline (served as negative control).

Group 2: Treated with i.p. thioacetamide at 300 mg/kg for two successive days (served as positive control)

Group 3: Treated with i.p. thioacetamide at 300 mg/kg and MethyB at 10 mg/kg for two successive days.

Group 4: Treated with i.p. thioacetamide at 300 mg/kg and MethyB at 20 mg/kg for two successive days.
Group 5: Treated with i.p. thioacetamide at 300 mg/kg and MethyB at 40 mg/kg for two successive days.

Rats had free access to food and tap water during the study. 24h after the last treatments, blood samples were obtained from the retro-orbital venous plexus, under light ether anesthesia. The rats were then killed by cervical decapitation while under ether anesthesia. The brain and liver of each rat then were quickly removed, washed with ice-cold phosphate-buffered saline (PBS, pH 7.4), weighed, and stored at −80°C for the biochemical analyses. The tissues were homogenized in 0.1 M phosphate-buffered saline at pH 7.4 to give a final concentration of 10 % w/v for the biochemical assays.

Biochemical analyses

Determination of lipid peroxidation

Malondialdehyde, an end product of lipid peroxidation was determined by measuring thiobarbituric reactive substances (TBAS) using the method by Ruiz-Larrea et al., (1994) in which TBAS react with thiobarbituric acid forming TBA-MDA adduct, and the absorbance is read at 532 nm with the use of a spectrophotometer.

Determination of nitric oxide

Griess reagent was used for the determination of total nitrate and nitrite concentration, indicative of nitric oxide production in the tissue. In this assay, nitrate is converted to nitrite by nitrate reductase. The Griess reagent then reacts with nitrite forming a deep purple azo compound. The absorbance is read at 540 nm with a spectrophotometer (Moshage et al., 1995).

Determination of reduced glutathione

Reduced glutathione (GSH) was determined using Ellman´s reagent (DTNB (5, 5'-dithiobis (2-nitrobenzoic acid)), which is reduced by the free sulfhydryl group on the GSH molecule, forming 5-thio-2-nitrobenzoic acid. The latter has a stable yellow color and is determined by reading absorbance at 412 nm (Ellman, 1959).

Serum liver enzyme assay

The activities of the liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum were measured according to Reitman–Frankel colorimetric transaminase procedure (Crowley, 1967).

Histological studies

Liver and brain samples were dehydrated in ascending grades of alcohol, cleared in xylene, and embedded in paraffin. Sections were cut at five μm with a microtome, mounted on glass slides, and stained with hematoxylin and eosin (Hx & E) (Drury and Wallington, 1980). For histological assessment of damage to the liver and brain, sections were investigated under the light microscope: Olympus Cx 41 with DP12 Olympous digital camera (Olympus Optical Co. Ltd, Tokyo, Japan).

Statistical analysis

Data are expressed as mean ± SEM. Statistical analysis of the data was performed using one-way ANOVA and Tukey's multiple comparisons test. GraphPad Prism 6 for Windows (GraphPad Prism Software Inc., San Diego, CA, USA) was used. Statistical significance was considered at a probability value of less than 0.05.

3. RESULTS

Effect of MethyB on TAA-induced liver oxidative stress

Rats treated with TAA, exhibited a significant increase in liver MDA levels by 108.7% compared to the saline control group (67.0 ± 3.0 *vs.* 32.1 ± 1.9 nmol/g.tissue). The level of nitric oxide increased significantly by 64.9% from 37.0 ± 1.6 μmol/g.tissue in the saline group to 61.0 ± 4.0 μmol/g.tissue in the TAA group. In addition, liver GSH content dropped by 54.6% in TAA-treated rats compared to their saline controls (2.9 ± 0.15 *vs.* 6.4 ± 0.3 μmol/g.tissue).

In TAA-treated rats, co-administration of MethyB at 20 or 40 mg/kg resulted in significant decrease in liver MDA by 30.0% and 46.3% (47.0 ± 1.8 and 36.0 ± 1.5 *vs.* control value of 67.0 ± 3.0 nmol/g.tissue). There was also a significant decrease in nitric oxide levels by 29.5% and 34.9% by 20 or 40 mg/kg MethyB compared to the TAA control (43.0 ± 2.8 and 39.7 ± 2.7 *vs.* control value of 61.0 ± 4.0 μmol/g.tissue). In addition, MethyB at the above doses caused a significant increase in liver GSH by 58.6% and 79.3% from TAA control value of 2.9 ± 0.15 to 4.6 ± 0.2 and 5.2 ± 0.3 μmol/g.tissue, respectively (Figure 1).

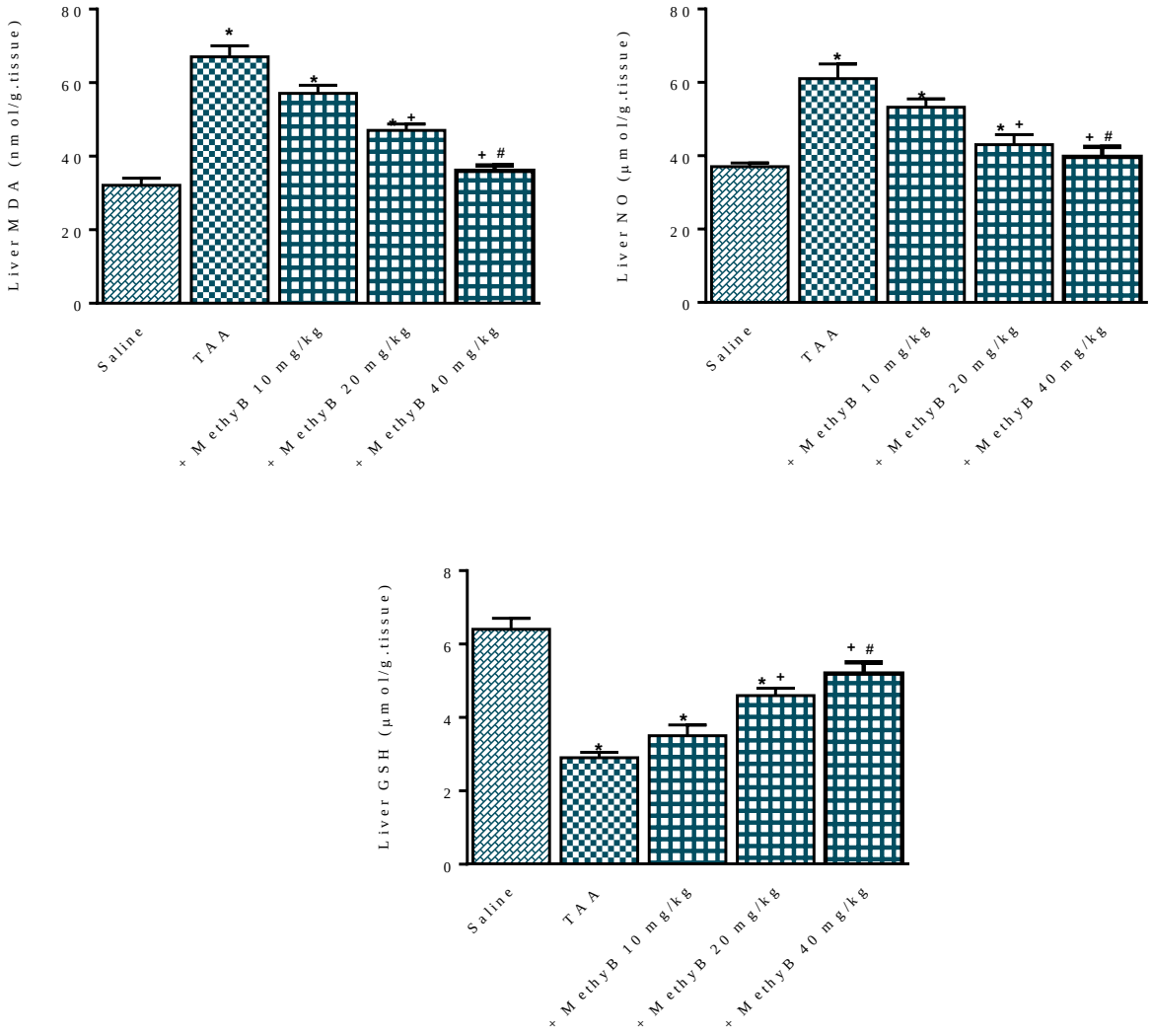

Figure 1 Effect of MethyB on malondialdehyde (MDA), nitric oxide (NO), and reduced glutathione (GSH) in the liver tissue of thioacetamide (TAA)-treated rats. *: P<0.05 *vs.* saline control. +: P<0.05 *vs.* TAA only group. #: P<0.05 *vs.* TAA + MethyB 10 mg/kg.

Effect of MethyB on serum liver enzymes

In the TAA control group, the activities of ALT and AST in serum were significantly increased by 130% (121.0 ± 8.3 *vs.* 52.6 ± 2.1 U/l), and 115.3% (176.3 ± 10.0 *vs.* 81.9 ± 3.1 U/l), respectively, compared to the respective saline control values. The treatment with MethyB at 20 or 40 mg/kg caused a significant decrease in serum ALT and AST. Serum ALT decreased by 33.1% and 44.5% from 121.0 ± 8.3 U/l in TAA control group to 81.0 ± 5.8 U/l and 67.1 ± 3.0 U/l in the TAA/MethyB treated acid groups. Meanwhile, serum AST decreased by 34.4% and 45.3% from control value of 176.3 ± 10.0 U/l to 115.7 ± 8.3 U/l and 96.5 ± 4.1 U/l, respectively (Figure 2).

Effect of MethyB on TAA-induced brain oxidative stress

Administration of TAA significantly increased brain MDA levels by 130.8% (45.0 ± 2.7 *vs.* 19.5 ± 1.2 nmol/g.tissue). It also significantly increased nitric oxide level by 75.6% (38.1 ± 2.0 *vs.* 21.7 ± 1.0 µmol/g, $p < 0.05$). In addition, a significant decrease in the level of GSH by 42.9% was observed (2.0 ± 0.03 *vs.* 3.5 ± 0.06 µmol/g.tissue).

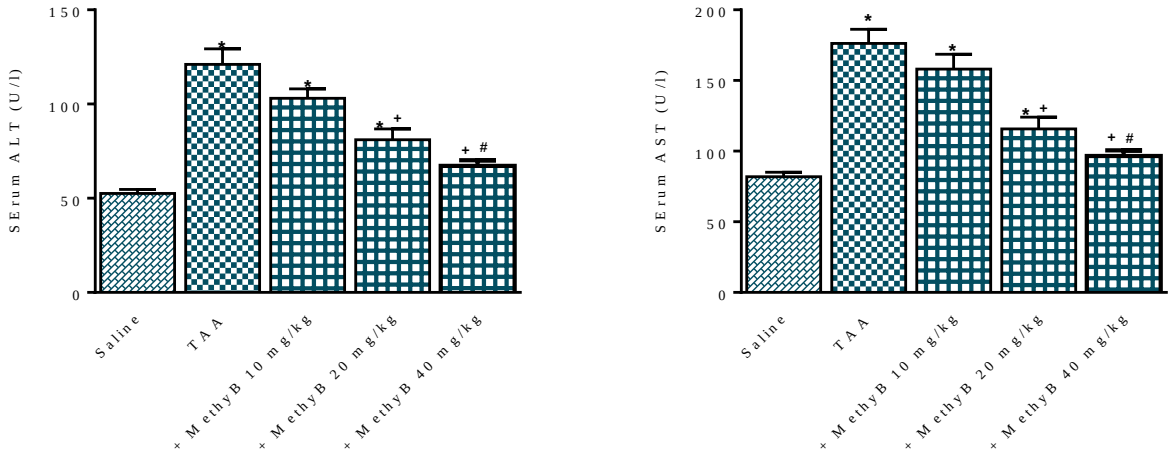

Figure 2 Effect of MethyB on serum liver enzymes in thioacetamide (TAA)-treated rats. *: P<0.05 *vs.* saline control. +: P<0.05 *vs.* TAA only group. #: P<0.05 *vs.* TAA + MethyB 10 mg/kg.

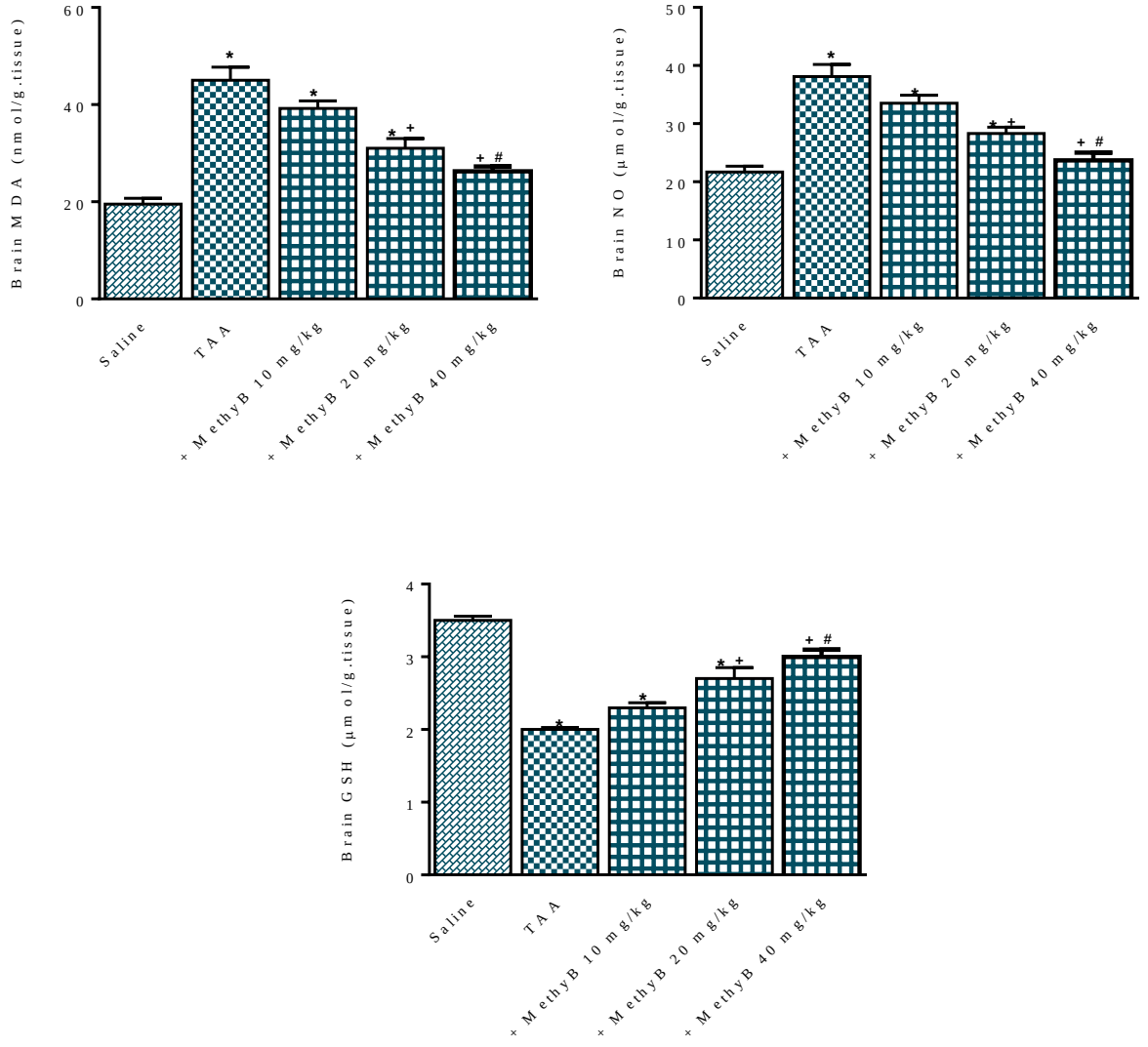

Figure 3 Effects of MethyB on malondialdehyde (MDA), nitric oxide (NO), and reduced glutathione (GSH) in brain tissue of thioacetamide (TAA)-treated rats. *: P<0.05 *vs.* saline control. +: P<0.05 *vs.* TAA only group. #: P<0.05 *vs.* TAA + MethyB 10 mg/kg.

In TAA/MethyB-treated groups, the level of MDA significantly decreased by 31.0% and 41.6% after treatment with 20 and 40 mg/kg MethyB, respectively (from 45.0 ± 2.7 to 31.0 ± 2.0 and 26.3 ± 1.0 nmol/g. tissue). Meanwhile, nitric oxide levels were significantly decreased by 26.0% and 37.8% by the above doses of MethyB (from 38.1 ± 2.0 in the TAA control group to 28.2 ± 1.1 and 23.7 ± 1.3 μmol/g. tissue in the TAA/MethyB-treated groups). Moreover, MethyB given at doses of 20 or 40 mg/kg to TAA-treated rats resulted in a significant increase in GSH levels by 35% and 50%, respectively, compared to the TAA control group (from 2.0 ± 0.03 to 2.7 ± 0.15 and 3.0 ± 0.1 μmol/ g. tissue) (Figure 3).

Histological changes in the liver

The liver tissue of the saline control rats revealed the typical architecture (Figure 4A, 4B). The liver tissue of rats treated with TAA only showed micro and macrovesicular steatosis, congestion of the portal vein, inflammatory cells aggregates, fibrosis, swelling of some hepatocytes, apoptosis of others, congestion of sinusoidal spaces and interstitial edema (Figure 4C, 4D). Liver sections in rats treated with TAA and 10 mg/kg MethyB showed few improvements in the pathological changes in the form of some normal hepatocytes.

However, microvacuolar degeneration, dilated and congested portal vein, inflammatory cells, hyperplasia of bile ducts, and fibrosis were still present (Figure 4E). Sections from rats treated with TAA and MethyB at 20 mg/kg showed moderate degree of improvement in the form of most of hepatocytes appeared normal, and few cells with vacuolated cytoplasm. The portal vein appeared dilated, and congested with thickening of the portal vein vascular wall, fibrosis, and hyperplasia of the bile duct (Figure 4F). The liver of rats treated with TAA and MethyB at 40 mg/kg revealed good preserved hepatic architecture, and most hepatocytes appeared normal. The hepatocytes were arranged in cords, but few vacuolations in the cytoplasm of some cells, and few dilatations in the sinusoidal space, little inflammatory infiltrates and Kupffer cells were seen (Figure 4G).

Histological changes in the brain

Cerebral cortex

The cerebral cortex of the saline controls showed a normal appearance (Figure 5A). In contrast, the histological findings in rats after administration of only TAA showed neuronal cell loss due to apoptosis, micro and macrovacuolar degeneration in the frontal cortex (spongiosis), pyknosis, necrosis of neurons and focal gliosis. Neurons showed intracytoplasmic proliferation of filaments, producing the neurofibrillary tangle. Diffuse cerebral edema and degeneration of pyramidal cells were observed (Figure 5B, 5C, 5D). Sections of cerebral cortex from rats treated with TAA and MethyB at 10 mg/kg showed that the brain tissue still suffered from pathological changes in the form of massive neuronal cell necrosis with intensely eosinophilic shrunken neuronal cells. Macro and microvacuolation characterized by multiple small rounded vacuoles within the neuropil of the cortex were seen. There were also dilated congested cerebral blood vessels and focal gliosis. Signs of neurodegeneration in the form of karyolysis and karyorrhexis were still present (Figure 5E, 5F).

As shown in Figure 6 (A & B) administration of TAA and MethyB at 20 mg/kg resulted in some improvement in the histological changes in the form of some neurons that appeared normal, no neurofibrillary tangle, no cerebral edema, and no apoptotic neurons. However, microvacuolation characterized by multiple small rounded vacuoles within the cortex, pyknosis, karyorrhexis of neurons, focal gliosis, and dilated congested cerebral blood vessels were still present. Brain sections in rats administrated TAA and MethyB at 40 mg/kg showed improvement of the harmful changes induced by TAA. The cortical tissues showed normal neuronal cells. However, some neurons appeared vacuolated, and a few pyknosis, necrosis, and congestion of cerebral blood vessels were seen. The pyramidal cells were irregular in shape and eosinophilic (Figure 6C, 6D).

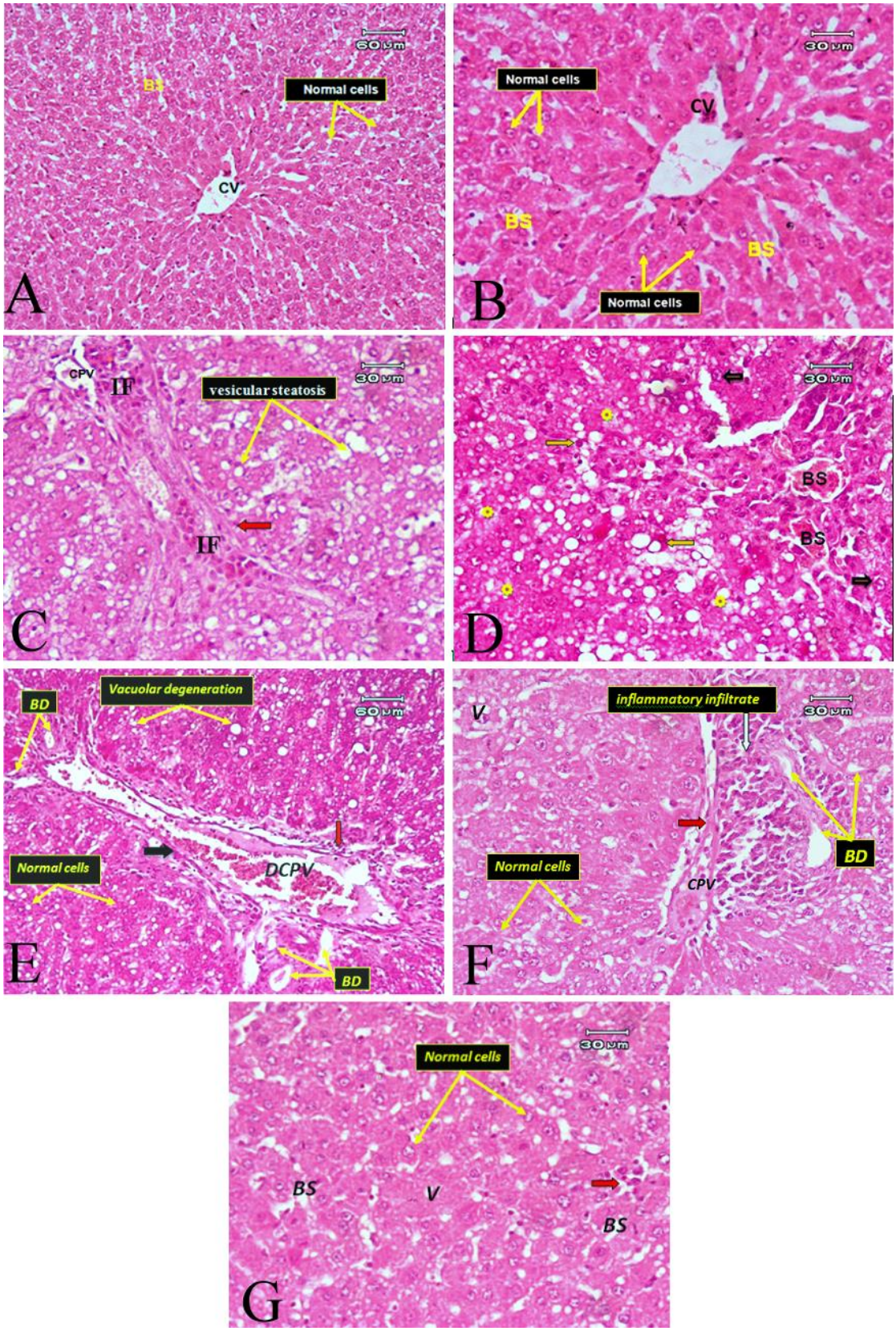

Figure 4 Representative photomicrographs of Hx & E-stained liver sections after treatment with (A) Saline showing the general appearance of the hepatic lobules (Normal cells). Notice the central vein (CV) and normal blood sinusoids (BS); (B) Saline (high power); (C) TAA only showing micro and macrovesicular steatosis (yellow arrow), congestion of portal vein (CPV), fibrosis (red arrow) and inflammatory infiltrate (IF); (D) TAA only (another field) showing dilated, congested in blood sinusoids (BS), interstitial edema (star), swelling in some hepatocytes (black arrow) and apoptosis (orange arrow); (E) TAA and 10 mg/kg MethyB showing microvacuolar degeneration (yellow arrow), dilated and congested portal vein (DCPV), hyperplasia of the bile duct, fibrosis (black

arrow), inflammatory cells (red arrow) and some normal hepatocytes; (F) with TAA and 20 mg/kg MethyB showing moderate improvement. Most hepatocytes appeared normal, although few vacuolations in the cytoplasm of some hepatocytes (V), congested portal vein (CPV), with thickening vein vascular wall, fibrosis (red arrow), hyperplasia of the bile duct (BD), pre portal inflammatory infiltrate were seen; (G) TAA and 40 mg/kg MethyB showing more or less preserved hepatic architecture in the form of most hepatocytes appeared normal, although few vacuolations in the cytoplasm of some hepatocytes (V), few dilatations in the sinusoidal space (BS), and few inflammatory infiltrates (red arrow) were still present.

Hippocampus

Sections from the saline controls revealed the typical normal architecture of the hippocampal tissue (Figure 7A). In contrast, the hippocampus of rats treated with TAA only showed marked pathological changes in all regions in the form of disorganization of the histological features, shrinkage in the size of large pyramidal cells, with darkened nuclei. The granular cell layer also showed marked vacuolations. The molecular layer exhibited vacuolated neurons (Figure 7B). Concerning rats administrated TAA along with MethyB at 10 mg/kg, examination of the hippocampus showed some improvement. Some neurons appeared normal, but most of the neurons were shrunken, with hyperchromatic nuclei and vacuolated cytoplasm. Signs of degeneration of neurons in the form of karyorrhexis were observed. In contrast, other neurons appeared pyknotic (Figure 7C). On the other hand, the hippocampus of rats given TAA and 20 mg/kg MethyB showed moderate tissue changes as mild vacuolar degeneration, shrunken, and pyknotic neurons. Some normal pyramidal neurons, with vesicular nuclei were observed (Figure 7D). The hippocampus of rats treated with TAA and 40 mg/kg MethyB showed a reasonable degree of improvement. Most pyramidal neurons appeared normal. However, some pyramidal cells appeared degenerated and vacuolated (Figure 7E).

Cerebellum

The cerebellum of saline control rats showed the typical architecture of Purkinje cells (Figure 8A, 8B). Treatment of rats with TAA only induced widely displaced or emptiness of most of Purkinje cells, area of degeneration in the granular layer cells, congestion of blood vessels, vacuolation of white matter, distribution of fibers in the molecular layer, and hemorrhage in meninges above the surface (Figure 8C). Sections from rats treated with TAA and 10 mg/kg MethyB showed that the Purkinje layer exhibited loss of some cells or degeneration, vacuolation in empty spaces and white matter (Figure 8D). In rats treated with TAA and 20 mg/kg MethyB, there was some improvement in the pathological changes. Few Purkinje cells appeared normal, but others were degenerated. Areas devoid of cells, and vacuolation in white matter were seen (Figure 8E). Sections of the cerebellum from rats treated with TAA and MethyB at 40 mg/kg revealed moderate improvement in the histological changes. Some Purkinje cells appeared normal. Others appeared degenerated, with empty spaces in some cells between the granular layers, few nerve fibers in the molecular layer (Figure 8F).

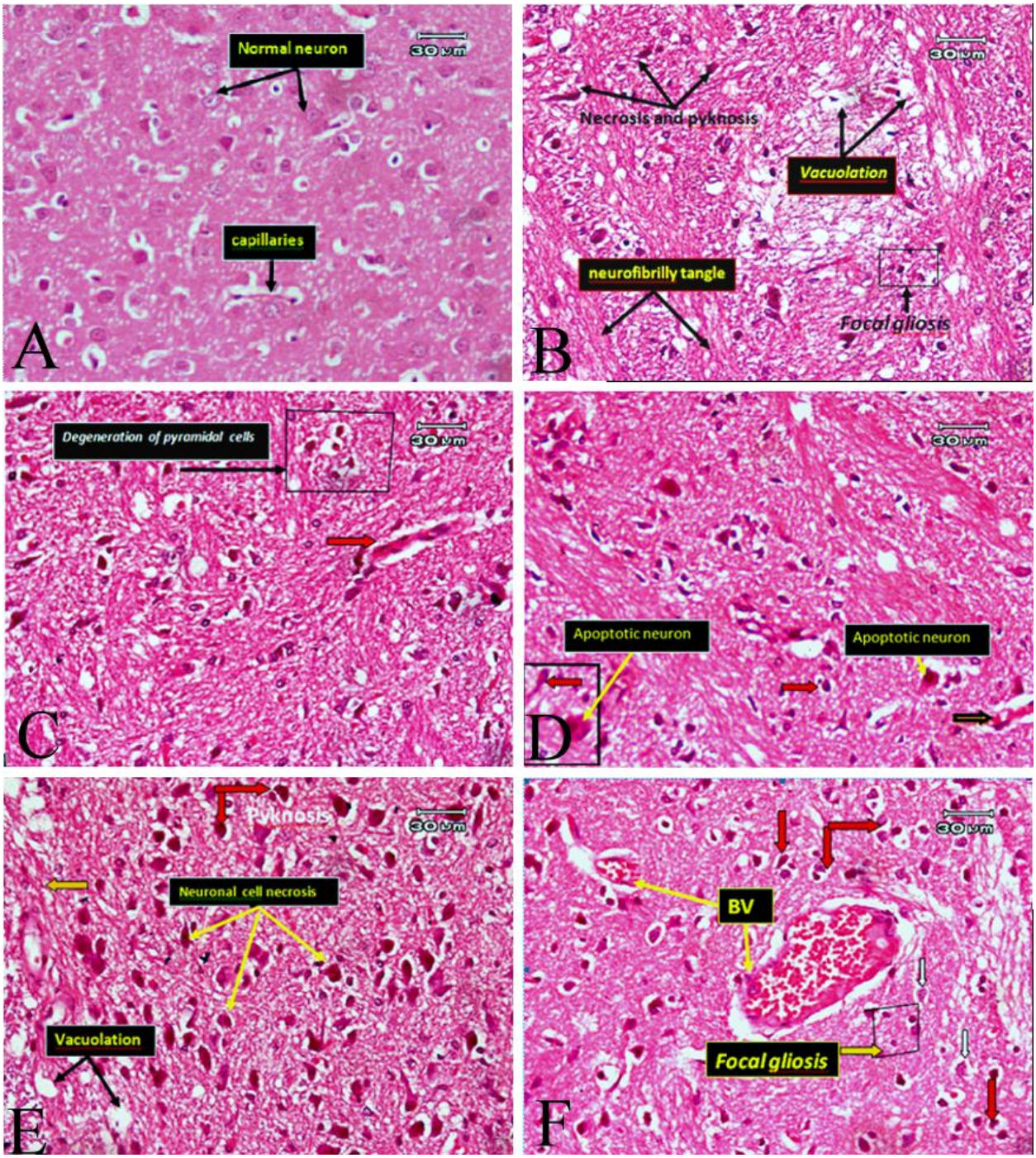

Figure 5 Representative photomicrographs of Hx & E stained cerebral cortex sections after treatment with (A) Saline showing typical appearance of neurons, capillaries in cerebral cortex; (B) TAA only showed deformity of normal structure, pyknosis, necrosis of neurons, micro and macrovacuolar degeneration (spongiosis), neurofibrillary tangle, and focal gliosis; (C) TAA only (another field) showing degeneration of pyramidal cells, and congested of cerebral blood vessels (red arrow); (D) TAA only (another field) showing apoptotic neurons (yellow arrow), some mitotic neurons (red arrow), and cell loss; (E) TAA and MethyB at 10 mg/kg showing neuronal cell necrosis, with intensely eosinophilic shrunken neuronal cells, vacuolation (black arrow), and neurofibrillary tangle (orange arrow); (F) TAA and MethyB at 10 mg/kg (another field) showing signs of degeneration in the form of karyolysis (white arrow), and karyorrhexis (red arrow), focal gliosis (orange arrow), dilated and congested cerebral blood vessels (BV).

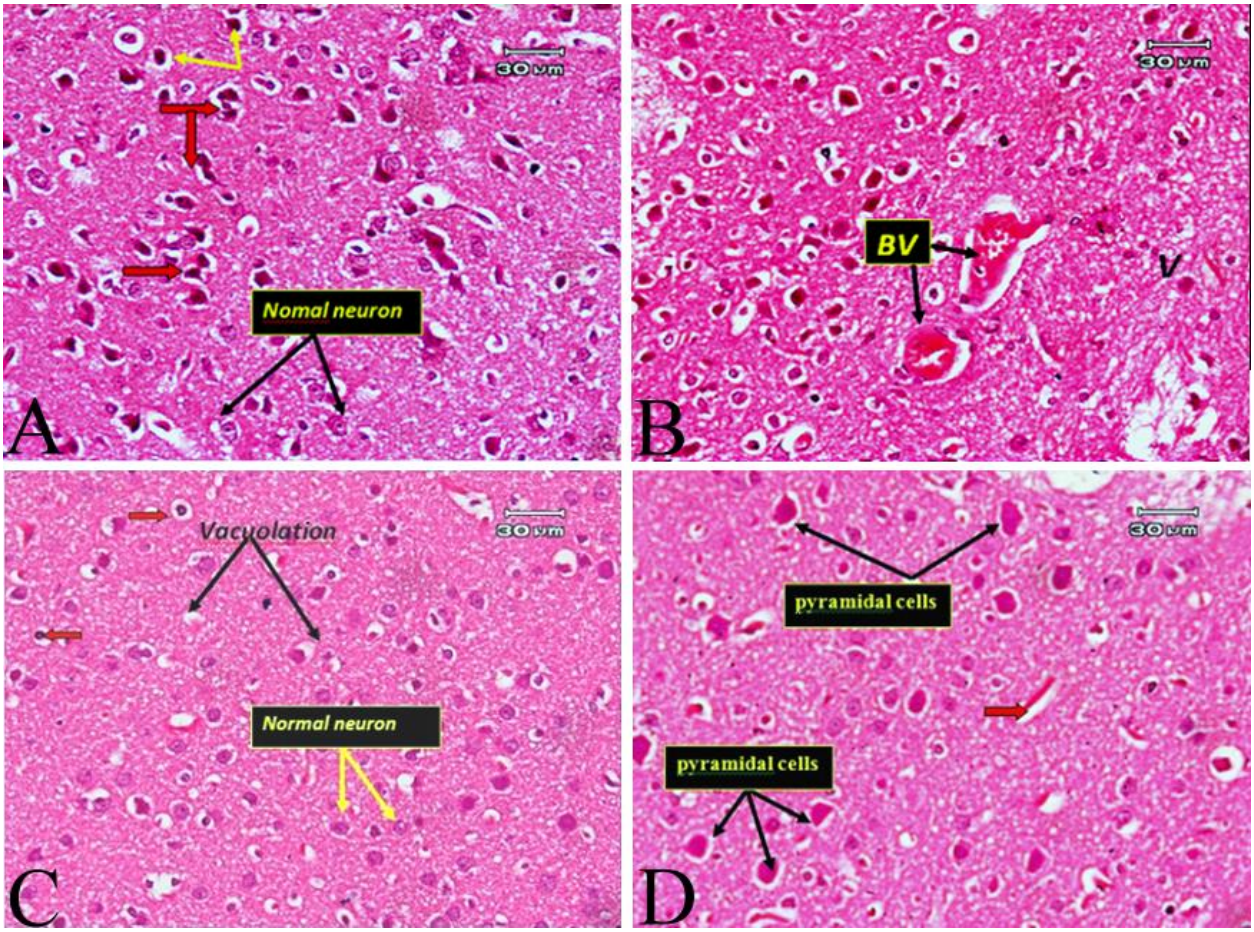

Figure 6 Cerebral cortex. (A) TAA and MethyB at 20 mg/kg showed improvement in the form of some normal neurons (black arrow). There were still signs of degeneration of neurons in the form of pyknosis (yellow arrow), and karyorrhexis (red arrow); (B) TAA and MethyB at 20 mg/kg (another field) showing dilated and congested cerebral blood vessel (BV), small rounded vacuoles in the cortex (V); (C) TAA and MethyB at 40 mg/kg showing more improvement in the pathological changes. Most neurons appeared normal (yellow arrow), some vacuolated (black arrow), and pyknosis of some neurons; (D) TAA and MethyB at 40 mg/kg (another field) showing the pyramidal cells irregular in shape, eosinophilic and congested cerebral blood vessel.

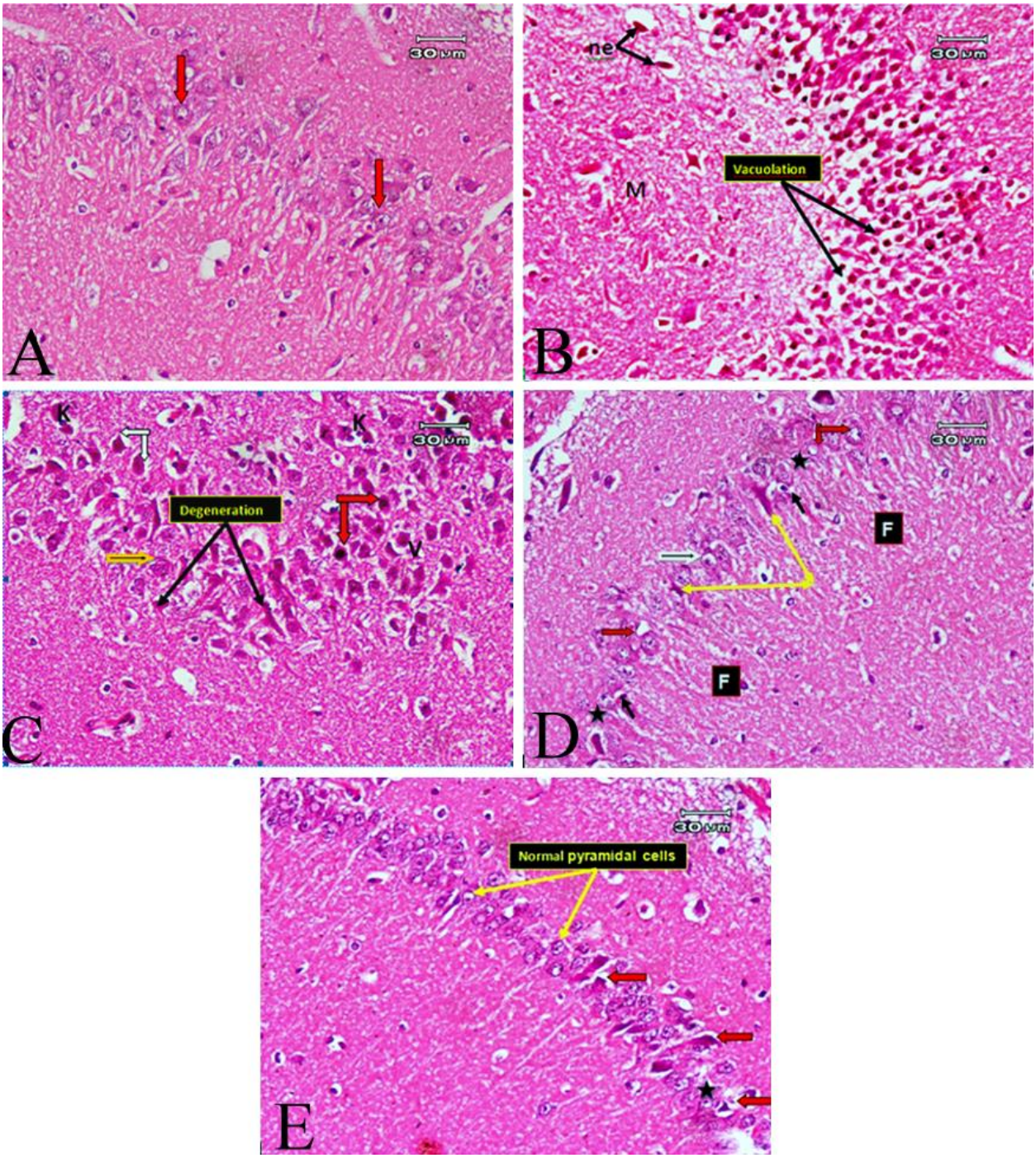

Figure 7 Representative photomicrographs of Hx & E-stained sections of the hippocampus after treatment with (A) Saline showing the typical appearance of pyramidal cells (red arrow). The cells have vesicular nuclei, with prominent nucleoli; (B) TAA showing deformity of the histological features, shrinkage in the size of large pyramidal cells with darkened nuclei. The granular cell layer also showed marked vacuolations. The molecular layer (M) exhibited vacuolated neurons (ne), and excess glial cells; (C) TAA and 10 mg/kg MethyB showing some improvement. Few neurons appeared normal, but most of pyramidal cells were shrunken with hyperchromatic nuclei (white arrow), and vacuolation of the cytoplasm (V), signs of degeneration of the neurons in the form of necrosis, pyknosis (red arrow), karyorrhexis (K); (D) TAA and 20 mg/kg MethyB showing some improvement in the form of some normal pyramidal cells with vesicular nuclei (white arrow), few pyramidal cells with pyknotic nuclei and vacuolated cytoplasm (black arrows). Some neurons were degenerated, vacuolated (red arrow), others were shrunken (yellow arrow). Few areas were devoid of pyramidal neurons (star); (E) TAA and MethyB at 40 mg/kg showing more improvement in the histological changes. Most pyramidal cells appeared normal. Some pyramidal cells, however, appeared degenerated, and vacuolated, and an area devoid of pyramidal neurons was seen (star).

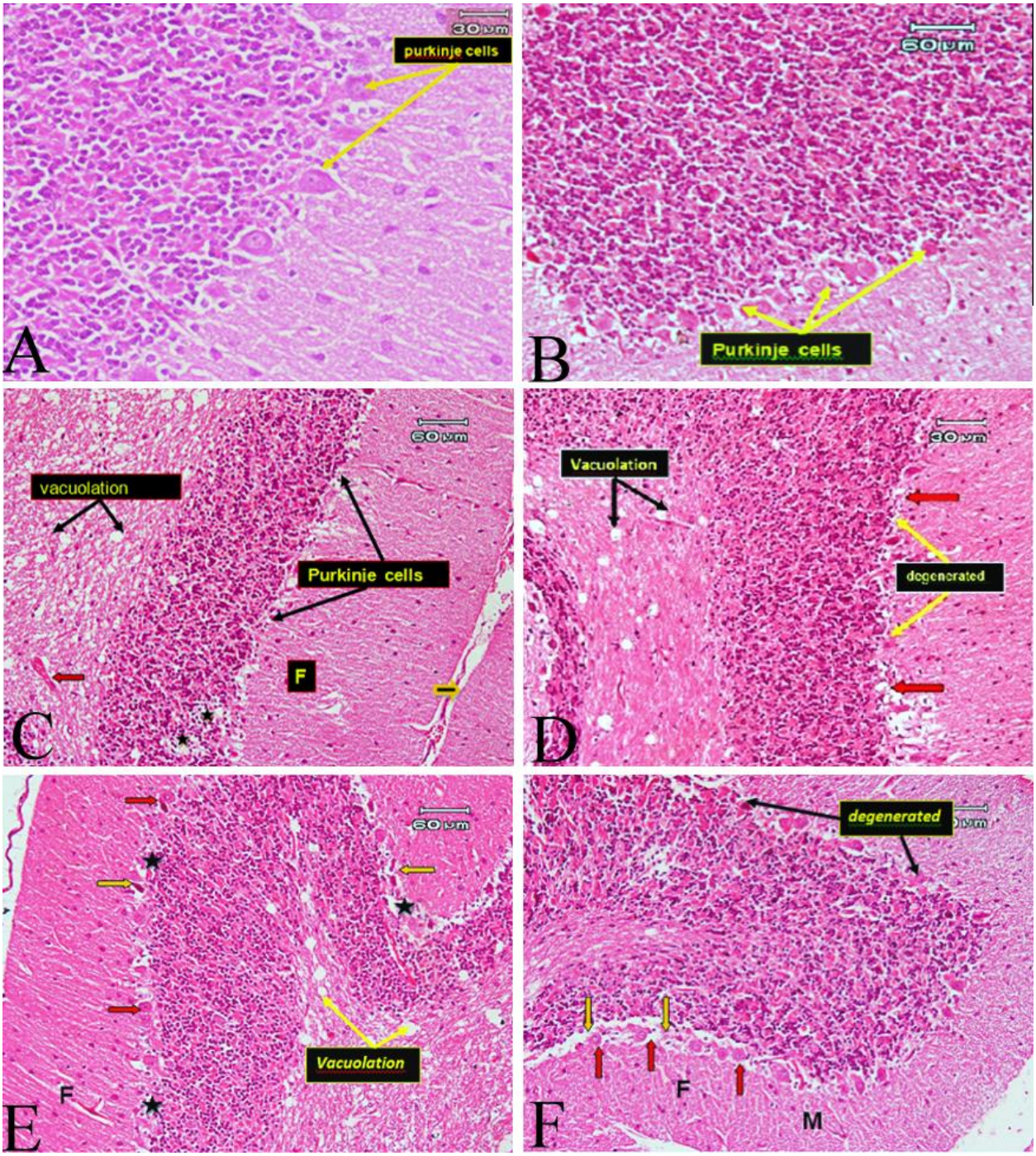

Figure 8 Representative photomicrographs of Hx & E stained sections of the cerebellum after treatment with: (A & B) Saline showing normal Purkinje cells; (C) TAA only showing widely displaced or emptiness of all Purkinje cells (black arrow), vacuolation (black arrow) and congestion of blood vessels in white matter (red arrow), hemorrhage in meninges above the surface (orange arrow), and few fibers in molecular layer (F), degenerated area of granular layer (star); (D) TAA and 10 mg/kg MethyB showing degeneration of Purkinje cells (yellow arrow). Purkinje cells were widely displaced and vacuolated (red arrow). Vacuolation in white matter (black arrow); (E) TAA and 20 mg/kg MethyB showing few Purkinje cells that appeared normal (red arrow), others degenerated (orange arrow), some areas devoid of cells (star), and vacuolation in white matter (yellow arrow). The molecular layer is comprised of many vacuolated nerve fibers (F); (F) TAA and 40 mg/kg MethyB showing moderate improvement. Some Purkinje cells appeared normal (red arrow). Degenerated neurons were still present, with empty spaces in some cells between the granular layers (orange arrow). The molecular layer (M) is formed of a few nerve fibers (F).

4. DISCUSSION

Oxidative stress is involved in the development of cell death and tissue damage in several liver disorders and hepatic encephalopathy (Vascotto and Tiribelli, 2015). The liver is involved in the biotransformation and metabolism of drugs, environmental toxins, and xenobiotics, which results in the generation of reactive metabolites and free radical species (Chiang, 2014). In the liver, reactive oxygen and nitrogen species are produced by cytochrome P450 reductase, lipooxygenase, and by nitric oxide synthase, and xanthine oxidase from the mitochondria, Kupffer cells, and infiltrating neutrophils (Vascotto and Tiribelli, 2015). The toxic action of TAA on liver cells is mediated by its reactive metabolites TAA-S-oxide and TAA-S, S-dioxide, which binds covalently to membrane lipids and proteins, causing lipid peroxidation, and liver necrosis (Wallace et al., 2015). TAA, therefore, represents an established model of toxin-induced acute liver failure and hepatic encephalopathy (Lee et al., 2011).

In this study, the administration of TAA at a high dose for two successive days caused significant oxidative stress in both the liver and brain tissue, as indicated by markedly increased levels of MDA. The latter is an end-product of lipid peroxidation, and increased levels, therefore, indicates an increased production of oxidant free radicals and the consequent oxidation of the cell membrane lipids. In addition, TAA caused a marked increase in tissue levels of nitric oxide. In the cell, low levels of nitric oxide produced by the endothelial and neuronal nitric oxide synthases (NOS), are involved in cell signaling and maintaining vascular tone. In contrast, during toxic and inflammatory conditions, a high concentration of nitric oxide produced by the inducible NOS (iNOS) in phagocytic and other inflammatory cells can be damaging to the cell, owing to the formation of the potent oxidant peroxynitrite radical and other reactive nitrogen species (Brown, 2010).

Our results also showed that TAA administration was associated with a significant and marked depletion of the liver and brain contents of the antioxidant and free radical scavenger reduced glutathione. This finding indicates the increased release of free radicals, and consequently the consumption, or possibly the reduced synthesis of the antioxidant. In addition, a marked increase in the plasma levels of the hepatocellular enzymes ALT and AST in TAA-treated rats was observed. These enzymes are released into the circulation following hepatocellular injury, and their levels correlate with the extent of damage to the liver tissue (Giannini et al., 2005). The above observations are supported by the findings from other studies that showed increased serum transaminases, liver oxidative stress, and necroinflammatory response in the liver after TAA administration. There was also upregulation of iNOS and tumor necrosis factor-α (TNF-α) in liver and increased levels of TNF-α, interleukin-1β and interleukin-6 in serum, indicative of an inflammatory response (Wang et al., 2004; Luo et al., 2015).

Our histological study indicated the harmful effects of TAA on the integrity of the liver and brain tissues. The liver exhibited distorted architecture, micro and macrovesicular steatosis, swelling, and apoptosis of hepatocytes, fibrosis, and inflammatory infiltrates, thereby, supporting our previous observations using single i.p. dose of 300 mg/kg (Abdel-Salam et al., 2013). Other studies reported necrosis and inflammatory cell infiltration following two i.p. injections of TAA at 350 mg/kg De-David et al., (2011) or after a single intravenous dose of TAA at 280 mg/ kg in the rat (Chen et al., 2008). In a study in which TAA was administered in a low dose of 50 mg/kg twice weekly, for two weeks, the presence of degenerated neurons with dark small nuclei in the brain was observed. The present study attempted a detailed investigation of TAA effects on different brain regions. We have shown that TAA caused marked neuronal cell loss in the cerebral cortex due to apoptosis, necrosis and vacuolar degeneration, degeneration of pyramidal cells and focal gliosis. The hippocampus exhibited structural deformity, and shrinkage of large pyramidal cells, while the cerebellum showed widely displaced or emptiness of most Purkinje cells.

In the present study, the potential protective effects of MethyB were examined in the TAA model of acute brain and liver injury. The study provided evidence that MethyB exerted dose-dependent amelioration of oxidative stress in both the brain and liver of TAA-treated rats, resulting in significant decreases in lipid peroxidation and nitric oxide levels, and preventing the depletion in reduced glutathione. This indicates the reduced release of free radicals, and consequently, the sparing and possibly replenishing of the brain and liver contents of reduced glutathione. In addition, the increase in plasma levels of the hepatocellular enzymes ALT and AST in TAA-treated rats was reduced dose-dependently by MethyB. The decline in activities of serum transaminases by MethyB, therefore, reflects reduced extent of liver tissue damage by the dye. These protective actions of MethyB were confirmed by the histological study, where the pathological changes evoked by TAA in the liver and brain were ameliorated by MethyB. The effects are dose-dependent, with the highest dose of 40 mg/kg, resulting in a remarkable protection against the TAA-induced damage.

The mechanism by which MethyB exerts its neuro- and hepatic-protective effects is not entirely clear. MethyB is a redox-cycling dye, where its blue oxidized form is reduced by NADPH or thioredoxin to the uncharged and colorless leucoMethyB, which is then re-oxidized by O2 or by iron-containing compounds e.g., methemoglobin (Miclescu and Wiklund, 2010). MethyB is an antioxidant, and was reported to prevent the formation of oxygen-free radicals by the mitochondria (Atamna et al., 2008). The antioxidant effect

of MethyB was shown in the present study, and also in several previous studies Abdel-Salam et al., (2016a), Abdel-Salam et al., (2016b), and is likely to have accounted, at least in part, for its protective effects. Another essential property of MethyB is its ability to inhibit nitric oxide synthases, and guanylate cyclase enzyme (Mayer et al., 1993a; Mayer et al., 1993b).

This action of MethyB is suggested to underlie its beneficial effects in vasoplegic shock occurring after cardiac surgery, in which an excessive release of nitric oxide is primarily thought to account for the reduced systemic vascular resistance and hypotension (Manghelli et al., 2015). The reduction in nitric oxide generation, therefore, may have a role at least partly in the neuro– and hepatic protective effects of MethyB observed in this study as well as in other models of toxin-induced tissue damage (Abdel-Salam et al., 2016a; Abdel-Salam et al., 2016b). In this context, MethyB decreased brain and serum nitric oxide levels and exerted neuroprotective Wiklund et al., (2007), Miclescu et al., (2010), Abdel-Salam et al., (2016b) and hepatoprotective effects (Abdel-Salam et al., 2020a). MethyB was also shown to attenuate the increase in the transcription factor nuclear factor kappa B Abdel-Salam et al., (2020b), and decrease TNF-α and caspase-3 activity (Abdel-Salam et al., 2016b).

5. CONCLUSIONS

The present study shows that MethyB afforded protection against the damaging effects of TAA on the brain neuronal and liver cells. These effects of MethyB involved a decrease in tissue oxidative stress levels.

Authors' Contributions

OMEAS: Study conception and design and data analysis; AAS: Experimental studies and data analysis; ERY: Biochemical analysis; FAM: Histological studies and their interpretation; OMEAS, AAS, FAM, ERY: Manuscript preparation, revision and final approval of the version to be published

Acknowledgment

This work was not supported by research grants.

Informed consent

Not applicable.

Ethical approval

The animal experiments followed the recommendations of the Institute Ethics Committee and the Guide for Care and Use of Laboratory Animals of the US National Institutes of Health (Publication No. 85-23, revised 1996).

Conflicts of interests

The authors declare that there are no conflicts of interest.

Funding

The study has not received any external funding.

Data and materials availability

All data associated with this study are present in the paper.

REFERENCES AND NOTES

1. Abdel-Salam OME, Mohammed NA, Sleem AA, Farrag AR. The effect of antidepressant drugs on thioacetamide-induced oxidative stress. Eur Rev Med Pharmacol Sci 2013; 17(6):735-44.
2. Abdel-Salam OME, Sleem AA, Sayed MA, Youness ER, Shaffie N. Methylene blue protects against pentylenetetrazole-induced seizures, oxidative stress and neuronal injury. Comp Clin Pathol 2020a; 29:341–354.
3. Abdel-Salam OME, Youness ER, Esmail RSE, Mohammed NA, Khadrawy YA, Sleem AA. Methylene blue as a novel neuroprotectant in acute malathion intoxication. React Oxygen Species 2016a; 1(2):165–177.
4. Abdel-Salam OME, Youness ER, Morsy FA, Sleem AA. Protection by methylene blue against haloperidol-induced neuronal and liver damage. J Basic Pharmacol Toxicol 2020b; 4 (1):17-27.

5. 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. React Oxygen Species 2016b; 2(5):371–387.
6. Alda M, McKinnon M, Blagdon R, Garnham J, MacLellan S, O'Donovan C, Hajek T, Nair C, Dursun S, MacQueen G. Methylene blue treatment for residual symptoms of bipolar disorder: randomised crossover study. Br J Psychiatry 2017; 210(1):54-60. doi: 10.1192/bjp.bp.115.173930
7. Atamna H, Nguyen A, Schultz C, Boyle K, Newberry J, Kato H, Ames BN. Methylene blue delays cellular senescence and enhances key mitochondrial biochemical pathways. FASEB J 2008; 22(3):703–712. doi: 10.1096/fj.07-9610com
8. Brown GC. Nitric oxide and neuronal death. Nitric Oxide 2010; 23(3): 153-65. doi: 10.1016/j.niox.2010.06.001
9. Chen TM, Subeq YM, Lee RP, Chiou TW, Hsu BG. Single dose intravenous thioacetamide administration as a model of acute liver damage in rats. Int J Exp Pathol 2008; 89(4):223-31. doi: 10.1111/j.1365-2613.2008.00576.x
10. Chiang J. Liver Physiology: Metabolism and Detoxification. In: Linda M. McManus, Richard N. Mitchell, editors. Pathobiology of Human Disease. San Diego: Elsevier, 2014; 1770-1782.
11. Clifton J, Leikin JB. Methylene blue. Am J Ther 2003; 10(4):289–91. doi: 10.1097/00045391-200307000-00009
12. Crowley LV. The Reitman-Frankel colorimetric transaminase procedure in suspected myocardial infarction. Clin Chem 1967; 13(6):482–7.
13. De-David C, Rodrigues G, Bona S, Meurer L, González-Gallego J, Tuñón MJ, Marroni NP. Role of quercetin in preventing thioacetamide-induced liver injury in rats. Toxicol Pathol 2011; 39(6):949-57. doi: 10.1177/0192623311418680
14. Drury RA, Walligton EA. Carleton's Histological Technique, 5th edition. Oxford University Press, New York, 1980; 195.
15. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959; 82(1):70–7. doi: 10.1016/0003-9861(59)90090-6
16. Giannini ED, Testa R, Savarino V. Liver enzyme alteration: A guide for clinicians. CMAJ 2005; 172(3):367-379. doi: 10.1503/cmaj.1040752
17. Halliwell B, Gutteridge JM. Lipid peroxidation in brain homogenates: the role of iron and hydroxyl radicals. J Neurochem 1997; 69(3):1330-1. doi: 10.1046/j.1471-4159.1997.69031330.x
18. Halliwell B. Free radicals, protein and DNA: oxidative damage versus redox regulation. Biochem Soc Trans 1996; 24(4):1023-7. doi: 10.1042/bst0241023
19. Lee SW, Kim SH, Min SO, Kim KS. Ideal experimental rat models for liver diseases. Korean J Hepatobiliary Pancreat Surg 2011; 15(2):67-77. doi: 10.14701/kjhbps.2011.15.2.67
20. Luo M, Dong L, Li J, Wang Y, Shang B. Protective effects of pentoxifylline on acute liver injury induced by thioacetamide in rats. Int J Clin Exp Pathol 2015; 8(8):8990-8996.
21. Manghelli J, Brown L, Tadros HB, Munfakh NA. A Reminder of methylene blue's effectiveness in treating vasoplegic syndrome after on-pump cardiac surgery. Tex Heart Inst J 2015; 42(5):491-4. doi: 10.14503/THIJ-14-4470
22. Marí M, Colell A, Morales A, von-Montfort C, Garcia-Ruiz C, Fernández-Checa JC. Redox control of liver function in health and disease. Antioxid Redox Signal 2010; 12(11):1295-331. doi: 10.1089/ars.2009.2634
23. Mayer B, Brunner F, Schmidt K. Inhibition of nitric oxide synthesis by methylene blue. Biochem Pharmacol 1993a; 45(2):367-74. doi: 10.1016/0006-2952(93)90072-5
24. Mayer B, Brunner F, Schmidt K. Novel actions of methylene blue. Eur Heart J 1993b; 14(Suppl I): 22-26.
25. McCartney SL, Duce L, Ghadimi K. Intraoperative vasoplegia: methylene blue to the rescue! Curr Opin Anaesthesiol 2018; 31(1):43-49. doi: 10.1097/ACO.0000000000000548
26. Medina DX, Caccamo A, Oddo S. Methylene blue reduces abeta levels and rescues early cognitive deficit by increasing proteasome activity. Brain Pathol 2011; 21(2):140–9. doi: 10.1111/j.1750-3639.2010.00430.x
27. Meissner PE, Mandi G, Coulibaly B, Witte S, Tapsoba T, Mansmann U, Rengelshausen J, Schiek W, Jahn A, Walter-Sack I, Mikus G, Burhenne J, Riedel KD, Schirmer RH, Kouyaté B, Müller O. Methylene blue for malaria in Africa: results from a dose-finding study in combination with chloroquine. Malar J 2006; 5:84. doi: 10.1186/1475-2875-5-84
28. Miclescu A, Sharma HS, Martijn C, Wiklund L. Methylene blue protects the cortical blood-brain barrier against ischemia/reperfusion-induced disruptions. Crit Care Med 2010; 38(11):2199–2206. doi: 10.1097/CCM.0b013e3181f26b0c
29. Miclescu A, Wiklund L. Methylene blue, an old drug with new indications? Rom J Anaesth Int Care 2010; 17:35-41.
30. Moshage H, Kok B, Huizenga JR, Jansen PL. Nitrite and nitrate determinations in plasma: a critical evaluation. Clin Chem 1995; 41(6 Pt 1):892–6.
31. Muñoz AS. Treatment and prophylaxis of ifosfamide-induced encephalopathy with intravenous methylene blue. Oncología 2006; 29(3):140–1.
32. Naylor GJ, Martin B, Hopwood SE, Watson Y. A two-year double blind crossover trial of the prophylactic effect of methylene blue in manic-depressive psychosis. Biol Psychiatry 1986; 21(10):915–920. doi: 10.1016/0006-3223(86)90265-9
33. 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

34. Sontag EM, Lotz GP, Agrawal N, Tran A, Aron R, Yang G, Necula M, Lau A, Finkbeiner S, Glabe C, Marsh JL, Muchowski PJ, Thompson LM. Methylene blue modulates huntingtin aggregation intermediates and is protective in Huntington's disease models. J Neurosci 2012; 32(32):11109–11119. doi: 10.1523/JNEUROSCI.0895-12.2012
35. Talley-Watts L, Long JA, Chemello J, Van-Koughnet S, Fernandez A, Huang S, Shen Q, Duong TQ. Methylene blue is neuroprotective against mild traumatic brain injury. J Neurotrauma 2014; 31(11):1063–1071. doi: 10.1089/neu.2013.3193
36. Túnez I, Muñoz MC, Villavicencio MA, Medina FJ, de-Prado EP, Espejo I. Hepato- and neurotoxicity induced by thioacetamide: protective effects of melatonin and dimethylsulfoxide. Pharmacol Res 2005; 52:223–238.
37. Uskukovic-Markovic S, Milenkovic M, Topic A, Kotur-Stevuljevic J, Stefanovic A, Antic-Stankovic J. Protective effects of tungstophosphoric acid and sodium tungstate on chemically induced liver necrosis in Wistar rats. J Pharm Pharm Sci 2007; 10(3):340–349.
38. Vascotto C, Tiribelli C. Oxidative stress, antioxidant defenses, and the liver. In: Albano E, Parola M. Studies on Hepatic Disorders, Oxidative Stress in Applied Basic Research and Clinical Practice, Springer International Publishing, Switzerland, 2015; 41-64.
39. Wallace MC, Hamesch K, Lunova M, Kim Y, Weiskirchen R, Strnad P, Friedman SL. Standard operating procedures in experimental liver research: thioacetamide model in mice and rats. Lab Anim 2015; 49(1 Suppl):21-9. doi: 10.1177/0023677215573040
40. Wang CH, Chen YJ, Lee TH, Chen YS, Hung KS, Lu CN, Liu JK. Protective effect of MDL28170 against thioacetamide-induced acute liver failure in mice. J Biomed Sci 2004; 11(5):571-8. doi: 10.1007/BF02256121
41. Wiklund L, Basu S, Miclescu A, Wiklund P, Ronquist G, Sharma HS. Neuro- and cardioprotective effects of blockade of nitric oxide action by administration of methylene blue. Ann N Y Acad Sci 2007; 1122:231–244. doi: 10.1196/annals.1403.016