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RESEARCH ARTICLE

Exacerbation of Toluene's Neuro- and Hepato-Toxicity by Amiodarone or Chlorpropamide: Involvement of Oxidative Stress

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ABSTRACT | Volatile solvent abuse is an important health problem and results in serious injury to the central nervous system. Neuroprotective effects were reported for the sulfonylurea group of drugs and for the anti-arrhythmic agent amiodarone, and these drugs have a K^+ channel-blocking effect. The K^+ channels are of interest for their ability to modulate brain damage. The aim of this study was to investigate the effect of amiodarone and chlorpropamide on the development of oxidative stress and brain and liver damage induced by toluene injection in rats. Toluene (900 mg/kg) was intraperitoneally (ip) administered alone or in combination with amiodarone or chlorpropamide (18 or 36 mg/kg, orally) once a day for 2 consecutive days. The brain and liver content of malondialdehyde (MDA), reduced glutathione (GSH), and nitric oxide (NO), and the activity of paraoxonase-1 (PON-1) were determined. In addition, butyrylcholinesterase (BChE) and the concentration of the anti-apoptotic protein (Bcl-2) were determined in brain homogenates. Histopathological examination of brain and liver sections was also performed. Results showed that compared to controls, toluene resulted in increased oxidative stress in the brain and liver tissues. MDA and NO concentrations were markedly raised along with decreased levels of GSH and PON-1 activity. Toluene also inhibited BChE activity and decreased Bcl-2 level in the brain tissue. The biochemical changes induced by toluene were aggravated by the administration of either amiodarone or chlorpropamide. Rats treated with toluene exhibited dead neurons, perineuronal vacuolations, infiltrative cells, glial cells, and degeneration of some Purkinje cells. In the liver, massive hepatic inflammatory infiltrate, hemorrhage, vacuolar degeneration, apoptosis, and degeneration of hepatocytes were observed. The co-administration of either amiodarone or chlorpropamide caused dose-dependent exacerbation of the toluene-induced pathological changes. These findings suggest the involvement of oxidative stress in the neuro- and hepato-toxicity by toluene and imply a greater toxicity in toluene abusers treated with either amiodarone or chlorpropamide.

KEYWORDS | Amiodarone; Chlorpropamide; Glutathione; Hepatotoxicity; Neurotoxicity; Nitric oxide; Oxidative stress; Solvent abuse; Toluene

ABBREVIATIONS | BChE, butyrylcholinesterase; GSH, reduced glutathione; MDA, malondialdehyde; NO, nitric oxide; PON-1, paraoxonase-1; ROS, reactive oxygen species

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CONTENTS

1. INTRODUCTION

Solvent abuse is an important health problem worldwide, especially among the youth [1, 2]. One such substance is toluene or methylbenzene, a widely used industrial solvent in the formulation of paints, glues, adhesives, paint strippers, leather finishing, gasoline manufacturing, and many others [3]. Toluene is one of the most commonly abused inhalants [4]. It is rapidly absorbed and being highly lipid soluble, readily enters the central nervous system, where acute administration results in cheerfulness followed by relaxation and lethargy, ataxia, confusion, loss of consciousness, coma, and even death [5, 6]. The solvent has been shown to exert neurotoxic actions. Studies in humans revealed that chronic abuse of toluene results in diffuse cerebral, cerebellar, and brainstem atrophy [7]. Leukoencephalopathy with dementia and prominent prefrontal dysfunction has been described after chronic use of the solvent [8]. White matter structures and periventricular/subcortical regions are mainly affected with impairments in cognitive and memory processing [9]. White matter abnormalities have also been described in rats after chronic exposure to toluene [10]. The mechanism by which this organic solvent induces its neurotoxicity involves excitotoxicity, oxidative stress, and neuroinflammation [3, 11]. When inhaled, toluene resulted in damage of the spinal cord neurons, accompanied by a decrease in the neurotrophic factors such as brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor [12]. Oxidative stress ensues when reactive oxygen species (ROS) are produced at a rate exceeding the capacity of the natural antioxidant defenses in the cell. In this respect, the brain appears to be particularly susceptible to free radical attack, because of the high metabolic rate and its rich content of polyunsaturated

fatty acids [13]. Studies indicated that toluene (0.5–1.5 g/kg, ip) increased the generation of ROS in crude mitochondrial fractions from rat cerebellum, striatum, and hippocampus [14]. Protein carbonyls increased in frontal cortex and cerebellum of rats following weeks of toluene inhalation [15]. Oxidative DNA damage was also detected in the liver and kidney of rats after toluene inhalation for 7 days [16].

There has been much interest in the role of K^+ channels in the modulation of brain damage [17]. Neuroprotective effects have been reported for the sulfonylurea tolbutamide, glyburide, and glipizide against neuronal cell death induced by kainic acid in the hippocampus [18]. These agents operate via a similar mechanism that is inhibition of sulfonylurea receptor 1 (Sur1) of the K^+_{ATP} channel [17]. Several studies also pointed to a beneficial and a neuroprotective effect for glibenclamide in pathological states [19]. Moreover, glibenclamide exerted a beneficial effect in patients with traumatic brain injury where it decreased the contusion expansion rate [20]. Amiodarone is one of the class III anti-arrhythmic drugs and these agents block K^+ channels as their main anti-arrhythmic effect [21]. Amiodarone (50 mg/kg) was shown to decrease infarct size and improve functional outcome in mice subjected to middle cerebral artery occlusion [22]. There is also evidence that the drug might be of value in the treatment of Alzheimer's disease for it inhibited beta-secretase cleavage in vitro [23].

Therefore, the aim of this study was to investigate whether chlorpropamide or amiodarone, could protect against the toluene-induced neurotoxicity by virtue of their K^+ channels blocking properties. The study was extended to evaluate the effect of both drugs on the development of hepatic injury in toluene-treated rats.

2. MATERIALS AND METHODS

2.1. Animals

Male Sprague-Dawley rats, obtained from the Animal House of the National Research Centre, (Cairo, Egypt), weighing between 130 and 140 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 U.S. National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.2. Drugs and Chemicals

Toluene (Sigma-Aldrich, St Louis, MO, USA), amiodarone (Sanofi Pharma, Egypt), and chlorpropamide (Kahira Pharm & Chem Ind, Cairo, Egypt) were used. Other chemicals and reagents were obtained from Sigma-Aldrich. Toluene was freshly prepared in paraffin oil to obtain the required doses. Amiodarone or chlorpropamide was dissolved in normal saline, freshly prepared before use and administered intraperitoneally (ip). The doses of amiodarone and chlorpropamide used were based on other studies [22, 24].

2.3. Study Design

Rats were randomly divided into six equal groups: six rats each. Group 1 received the vehicle (0.2 ml saline, ip) daily. Group 2 received toluene in paraffin oil (vol/vol) in a dose 900 mg/kg (2.6 ml/kg, ip) on two successive days. Groups 3–6 received toluene (900 mg/kg, ip) in combination with either amiodarone or chlorpropamide (18 or 36 mg/kg, by oral gavage). Toluene and test drugs were administered once a day for two successive days. Rats were then euthanized under ether anesthesia 4 h after the second day of treatments 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 in 0.1 M phosphate-buffered 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 in the brain homogenates was assayed by measuring the level of malondialdehyde (MDA) using the method of Ruiz-Larrea et al. [25] where the thiobarbituric acid-reactive substances react with thiobarbituric acid to produce a red colored

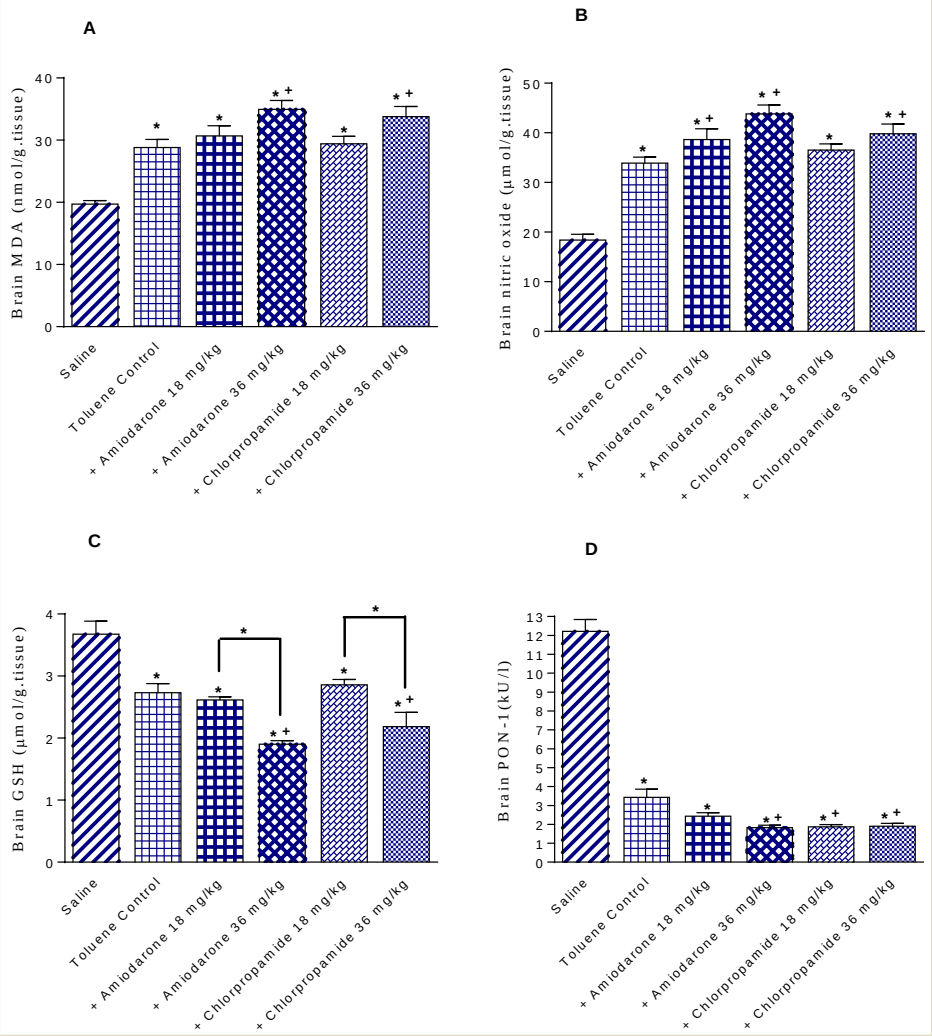

FIGURE 1. The effect of amiodarone or chlorpropamide on brain MDA (A), NO (B), GSH (C), and PON-1 (D) in rats treated with toluene. *, $p < 0.05$ vs. saline and between different groups as indicated in the figure. +, $p < 0.05$ vs. toluene control.

complex, which is determined spectrophotometrically at 532 nm.

2.4.2. Reduced Glutathione (GSH)

Brain GSH content was determined in homogenates spectrophotometrically according to Ellman et al. [26]. The procedure is based on the reduction of the Ellman's reagent by sulfhydryl groups of GSH to form 2-nitro-5-mercaptobenzoic acid, which is determined spectrophotometrically at 412 nm.

2.4.3. Nitric Oxide (NO)

The level of NO, measured as nitrite, was determined using the Griess reagent according to the procedure described by Moshage et al. [27]. Nitrite, a stable end-product of nitric oxide, is used as an indicator of the production of NO. In this assay, nitrate is converted to nitrite by nitrate reductase. The Griess reagent then react with nitrite forming a deep purple azo compound. The absorbance is read at 540 nm using a spectrophotometer.

2.4.4. Paraoxonase-1 (PON-1) Activity

Arylesterase activity of PON-1 was measured spectrophotometrically in supernatants using phenylacetate as a substrate and the formation of phenol was measured by monitoring the increase in absorbance at 270 nm and 25°C with a spectrophotometer. One unit of arylesterase activity is defined as 1 µmol of phenol produced per minute. Enzyme activity is calculated based on the extinction coefficient of phenol (1,310 M^{-1} cm^{-1} at 270 nm, pH 8.0, and 25°C) and expressed as kilo international units/liter (kU/L) [28].

2.4.5. Cholinesterase Activity

Butyrylcholinesterase (BChE) activity was measured spectrophotometrically in supernatants using a commercially available kit (Biodiagnostics, Egypt). BChE catalyzes the hydrolysis of butyrylthiocholine as a substrate into butyrate and thiocholine. The latter reacts with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) to produce a yellow chromophore which then could be quantified using a spectrophotometer [29].

2.4.6. Quantification of Bcl-2

The level of Bcl-2 in the brain tissue was determined using a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) kit (Glory Science, Del Rio, TX, USA) according to the manufacturer's instructions.

2.5. Histopathology

Brain tissues were fixed in 10% buffered formalin, dehydrated in graded ethanol, and embedded in paraffin using standard procedures. Sections of 5 µm thickness were stained with hematoxylin and eosin (H&E) for histopathological examination using a light microscope.

2.6. Statistical Analysis

Data are expressed as mean ± SE. Data were analyzed by one-way analysis of variance, followed by Duncan's multiple range test for post-hoc comparison of group means. Effects with a probability of $p < 0.05$ were considered statistically significant.

3. RESULTS

3.1. Biochemical Results

3.1.1. Lipid Peroxidation

Compared with the saline control group, toluene caused a significant increase in MDA level in brain and liver tissues by 46.2% (28.8 ± 1.3 vs. 19.7 ± 0.6 nmol/g tissue) and 40.6% (36.1 ± 1.2 vs. 26.7 ± 2.4 nmol/g tissue), respectively (**Figures 1A** and **2A**). In toluene-treated rats, there was a significant increase in brain MDA by 21.1% and 17.4% after treatment with the higher dose of either amiodarone and chlorpropamide, respectively, compared with the toluene only control group (34.9 ± 1.4 and 33.8 ± 1.6 vs. 28.8 ± 1.3 nmol/g tissue) (**Figure 1A**). There were also significant increments in liver MDA by 26.0% and 28.5% after 18 and 36 mg/kg amiodarone, respectively (45.5 ± 2.0 and 46.4 ± 1.2 vs. 36.1 ± 1.2 nmol/g tissue), and by 20.0% after 36 mg/kg chlorpropamide (43.4 ± 2.4 vs. 36.1 ± 1.2 nmol/g tissue) (**Figure 2A**).

3.1.2. NO

In toluene only-treated rats, NO significantly increased in the brain and liver tissues by 84.2% and 40.7%, respectively, compared with the corresponding saline control values (33.9 ± 1.2 vs 18.4 ± 1.1, and 33.5 ± 0.8 vs. 23.8 ± 1.3 µmol/g tissue) (**Figures 1B** and **2B**). In rats given toluene, NO in the brain was increased by 14.2 % and 29.2 % by administering 18 and 36 mg/kg amiodarone, respectively (38.7 ± 2.1 and 43.8 ± 1.8 vs. 33.9 ± 1.2 µmol/g tissue), and by 17.4% after 36 mg/kg chlorpropamide (39.8 ± 2.0 vs. 33.9 ± 1.2 µmol/g tissue) (**Figure 1B**). Liver NO content was also increased by 39.7% after 36 mg/kg amiodarone (46.8 ± 1.4 vs. 33.5 ± 0.8 µmol/g tissue) and by 18.0% and 41.8% after 18 and 36 mg/kg chlorpropamide, respectively (39.5 ± 2.1 and 47.5 ± 3.3 vs. 33.5 ± 0.8 µmol/g tissue) (**Figure 2B**).

3.1.3. GSH

Toluene administration caused significant decreases in brain and liver GSH by 25.6% and 27.8%, respectively, compared to their corresponding saline control values (2.73 ± 0.14 vs. 3.67 ± 0.21 and 3.16 ± 0.11 vs. 4.38 ± 0.09 µmol/g tissue) (**Figures 1C** and

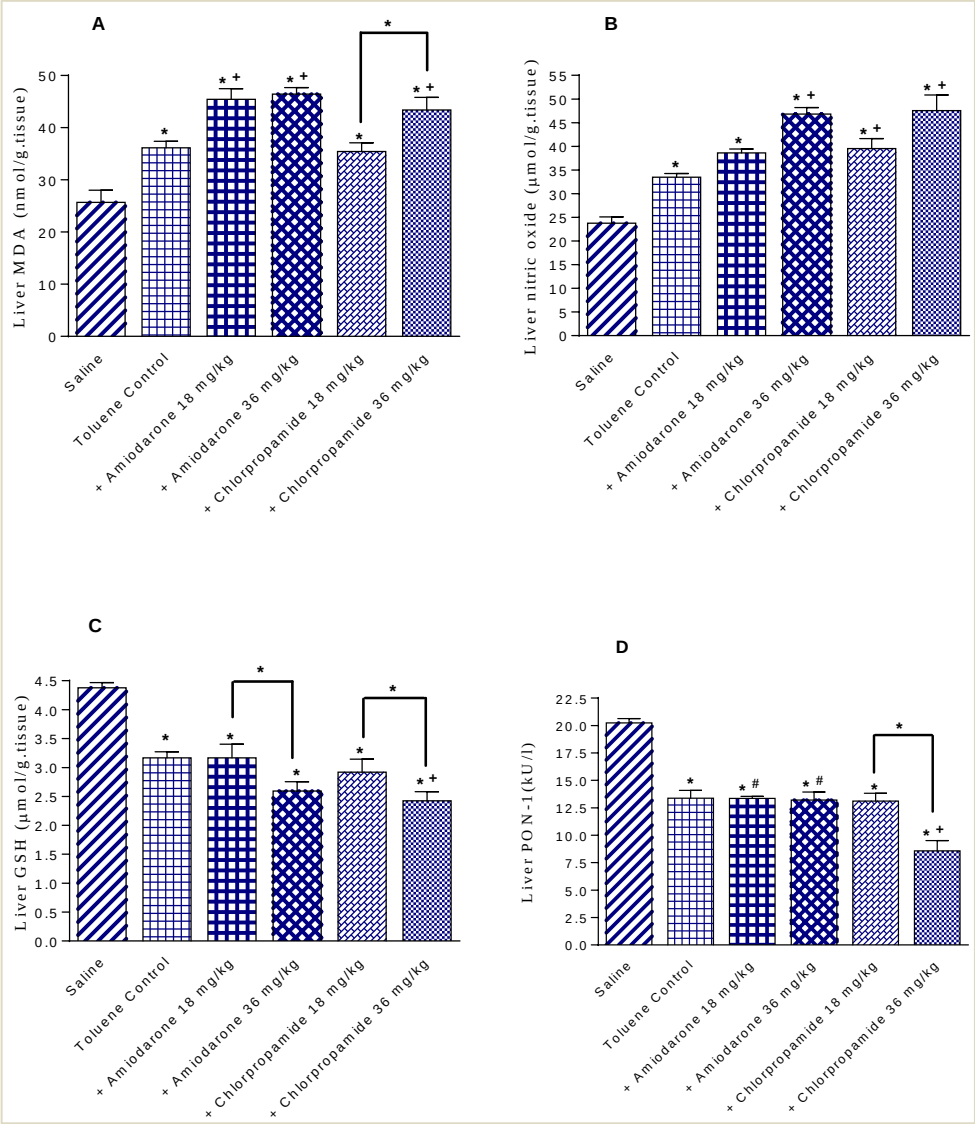

FIGURE 2. The effect of amiodarone or chlorpropamide on liver MDA (A), NO (B), GSH (C), and PON-1 (D) in rats treated with toluene. *, $p < 0.05$ vs. saline and between different groups as indicated in the figure. +, $p < 0.05$ vs. toluene control. #, $p < 0.05$ vs. toluene + chlorpropamide 36 mg/kg group.

2C). In toluene-treated rats, brain GSH showed further decreases by 30.4% and 20.1% after the higher dose of amiodarone and chlorpropamide, respectively (1.9 ± 0.6 and 2.18 ± 0.23 vs. 2.73 ± 0.14 µmol/g tissue) (**Figure 1C**). Liver GSH showed a significant decrease by 23.1% after the higher dose of chlorpropamide (2.43 ± 0.15 vs. 3.16 ± 0.11 µmol/g tissue) (**Figure 2C**).

3.1.4. PON-1

Toluene caused significant inhibition of PON-1 activity in brain and liver tissue by 72.1% and 33.7%, respectively, compared to their corresponding saline control values (3.4 ± 0.4 vs. 12.2 ± 0.6 and 13.4 ± 0.7 vs. 20.2 ± 0.4 kU/L) (**Figures 1D** and **2D**). In toluene-treated rats, PON-1 activity showed a further

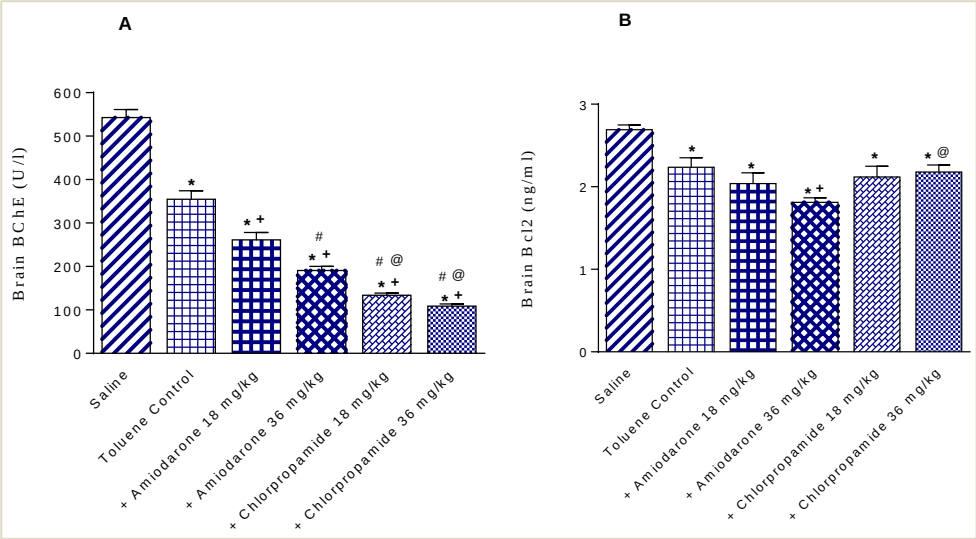

FIGURE 3. The effect of amiodarone or chlorpropamide on brain BChE (A) and Bcl-2 (B) in rats treated with toluene. *, $p < 0.05$ vs. saline. +, $p < 0.05$ vs. toluene. #, $p < 0.05$ vs. toluene + amiodarone 18 mg/kg group. @, $p < 0.05$ vs. toluene + amiodarone 36 mg/kg group.

decrease by 46.2% after the higher dose of amiodarone (1.83 ± 0.16 vs. 3.4 ± 0.41 kU/L), and by 45.0% and 44.1% after 18 and 36 mg/kg chlorpropamide, respectively (1.87 ± 0.12 and 1.9 ± 0.15 vs. 3.4 ± 0.41 kU/L) (**Figure 1D**). In the liver, PON-1 activity decreased by 35.8% after administering 36 mg/kg chlorpropamide as compared with the toluene only group (8.6 ± 0.9 vs. 13.4 ± 0.7 kU/L) (**Figure 2D**).

3.1.5. BChE

Brain BChE activity was significantly inhibited after toluene exposure by 34.6% (354.7 ± 19.3 vs. saline control value of 542.8 ± 8.4 U/L). In toluene-treated animals, BChE activity showed further decreases by 26.4% and 46.2% amiodarone at 18 and 36 mg/kg, respectively (261.2 ± 16.9 and 190.8 ± 9.3 vs. 354.7 ± 19.3 U/L), and by 47.4% and 69.3% after 18 and 36 mg/kg chlorpropamide, respectively (134.0 ± 4.9 and 108.9 ± 5.0 vs. 354.7 ± 19.3 U/L) (**Figure 3A**).

3.1.6. Bcl-2

The administration of toluene resulted in a significant decrease in brain Bcl-2 concentration by 16.7% (2.24 ± 0.11 vs. 2.69 ± 0.06 ng/ml). In toluene-treated rats, a further 19.2% decrease in brain Bcl-2 was observed after administering 36 mg/kg amiodarone (1.81 ± 0.06 vs. 2.24 ± 0.11 ng/ml) (**Figure 3B**).

3.2. Histopathological Results of the Brain

3.2.1. Cerebral Cortex

H&E-stained sections of the cerebral cortex from the vehicle-treated group showed a normal appearance, with neurons having either single or double nuclei with prominent nucleoli surrounded with basophilic cytoplasm (**Figure 4A**). Sections from rats treated with toluene only revealed many apoptotic neurons and some dead neurons. Many infiltrative cells, glial cells, and perineuronal vacuolations were seen. Thrombotic vessel shows a vessel with membrane bound vacuoles (**Figure 4B** and **4C**). Rats treated with toluene and amiodarone at 18 mg/kg showed highly necrotic, degenerated, and eosinophilic neurons (**Figure 4D**). Rats receiving the higher dose of amiodarone revealed vacuolations referred to as spongiform degeneration, neuronal loss with gliosis, eosinophilic neuronal degeneration, and pyknotic nuclei (**Figure 4E** and **4F**). Rats given toluene and chlorpropamide at 18 mg/kg exhibited neuronal de-

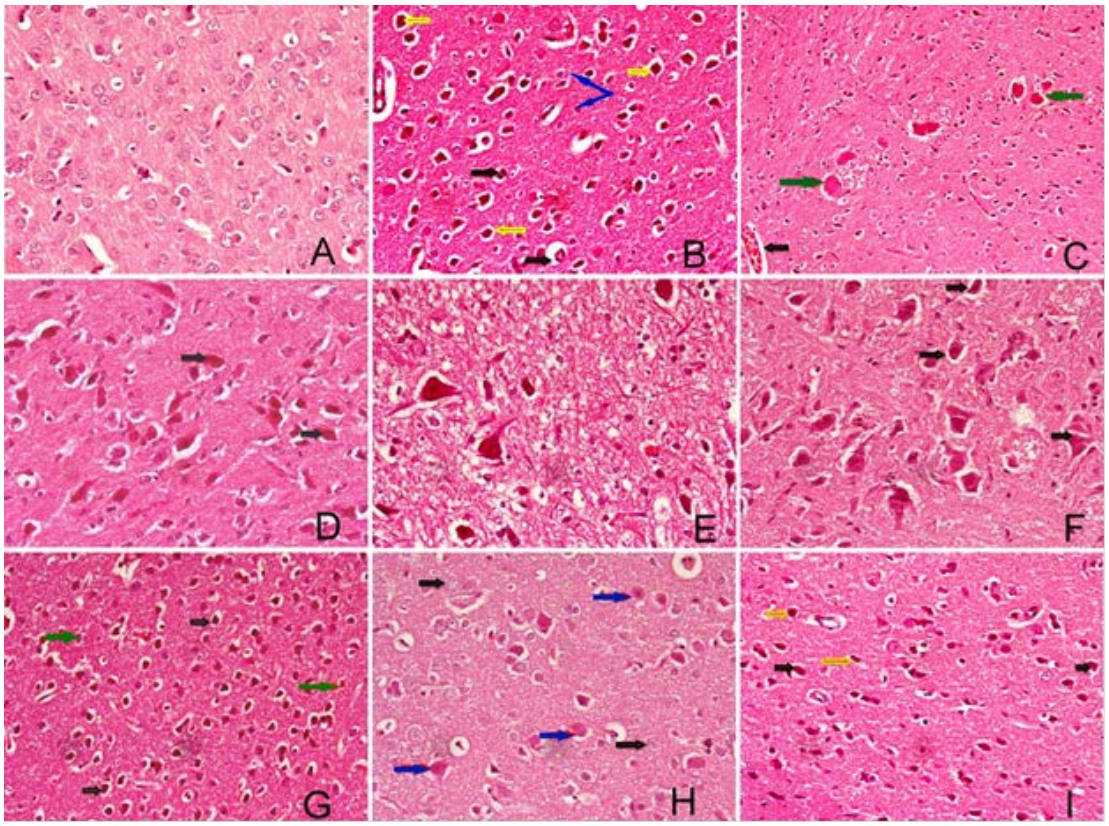

FIGURE 4. Microphotographs of representative sections of the cerebral cortex of rats in various treatment groups. (A) Saline control showing normal histological structure. (B) Toluene only showing neurodegenerative changes with pyknotic (yellow arrow) and apoptotic nuclei (black arrow) surrounded by perineuronal vacuolations with gliosis (blue arrow). (C) Toluene only showing some dead neurons (green arrow). Thrombotic vessels show a vessel with membrane bound vacuoles (black arrow). (D) Toluene + amiodarone 18 mg/kg showing highly necrotic, degenerated and eosinophilic neurons. (E) Toluene + amiodarone 36 mg/kg showing vacuolation that has been referred to as spongiform degeneration and neuronal loss with gliosis. (F) Toluene + amiodarone 36 mg/kg showing eosinophilic neuronal degeneration, and some appeared with pyknotic nuclei (arrow). (G) Toluene + chlorpropamide 18 mg/kg showing degenerated neurons (green arrow) but most of neuronal cells appear pyknotic (black arrow). (H) Toluene + chlorpropamide 36 mg/kg showing neuronal necrosis (black arrow). Affected neurons become shrunken and eosinophilic (blue arrow). (I) Toluene + chlorpropamide 36 mg/kg showing pyknotic darkly stained neurons (orange arrow), and some apoptotic neurons (black arrow). H&E staining with magnification for A, B, D, E, F, and H: of 400×; for C, G, and I: of 200×.

generation, but most of neuronal cells appeared pyknotic (**Figure 4G**). The higher dose of chlorpropamide induced neuronal necrosis. Some neurons appeared pyknotic and others apoptotic. Affected neurons became shrunken and eosinophilic with mild edema (**Figure 4H** and **4I**).

3.2.2. Cerebellum

Sections of the cerebellar cortex from the vehicle-treated group showed a normal appearance (**Figure 5A**). In rats treated with only toluene, degeneration of some Purkinje cells in cerebellum was evident

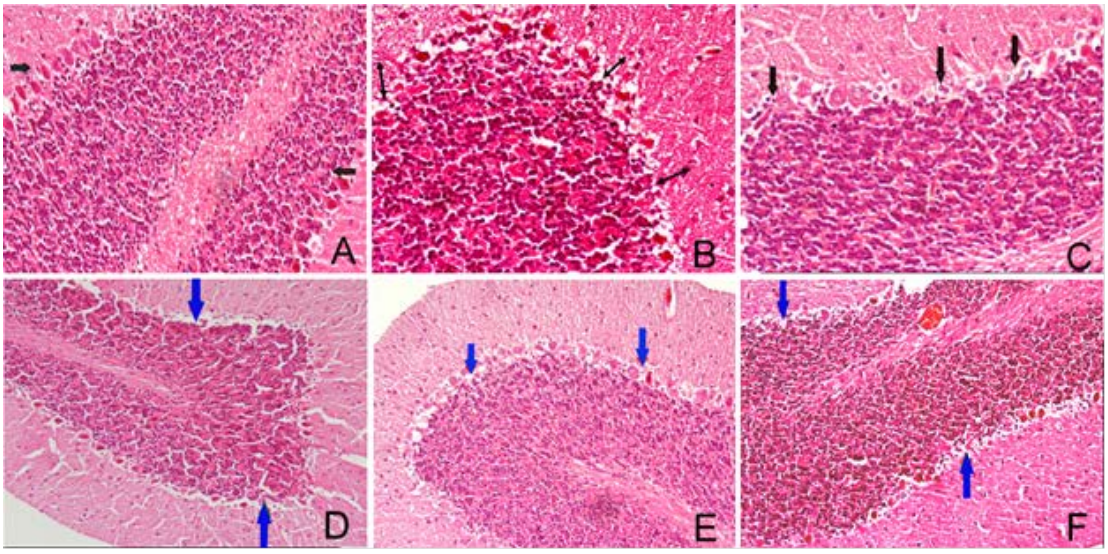

FIGURE 5. Microphotographs of representative sections of the cerebellum of rats in various treatment groups. (A) Saline control showing normal appearance of Purkinje cells (arrow). (B) Toluene only showing marked degeneration of Purkinje cells (black arrow). (C) Toluene + amiodarone 18 mg/kg showing degeneration of Purkinje cells. (D) Toluene + amiodarone 36 mg/kg showing degeneration of Purkinje cells. (E) Toluene + chlorpropamide 18 mg/kg showing degenerated Purkinje cells. (F) Toluene + chlorpropamide 36 mg/kg showing most Purkinje cells appeared degenerated (blue arrows). H&E staining with magnification of 400×.

(**Figure 5B**). In toluene and amiodarone 18 mg/kg-treated rats, some Purkinje cells in cerebellum appeared degenerated (**Figure 5C**). With the higher dose, more degenerated Purkinje cells in cerebellum were seen (**Figure 5D**). Rats given toluene and chlorpropamide at 18 mg/kg showed degenerated Purkinje cells (**Figure 5E**). In rats given toluene and chlorpropamide at 36 mg/kg, most of Purkinje cells in cerebellum appeared degenerated (**Figure 5F**).

3.3. Histopathological Results of the Liver

The liver of control rats revealed the normal characteristic architecture (**Figure 6A**). Sections from rats treated with toluene only revealed massive inflammatory infiltrate and hemorrhage, vacuolar degeneration, apoptosis in some cells, and sings of degeneration in the form of karyolysis and karyorrhexis (**Figure 6B** and **6C**). Rats treated with toluene and amiodarone at 18 mg/kg showed dilated and congested portal vein, dilated bile ducts, hemorrhage in blood sinusoids, and fibrosis. Most of hepatocytes showed vacuolated cytoplasm with pyknotic, karyorrhectic, and karyolytic nuclei (**Figure 6D**). Rats given toluene and amiodarone at 36 mg/kg showed swollen hepatocytes with accumulation of macrovesicular and microvesicular fat droplets within the cytoplasm, karyorrhexis, karyolysis, and coagulative necrosis. There were also dilated and congested blood sinusoids (**Figure 6E** and **6F**). Rats subjected to toluene and chlorpropamide at 18 mg/kg showed dilated and congested central blood vessels and thickening of wall. Aggregates of inflammatory cell infiltrate, vacuolar degeneration, and fibrosis were seen (**Figure 6G** and **6H**). The liver from rats treated with toluene and chlorpropamide at 36 mg/kg showed massive cytoplasmic vacuoles, hemorrhage in sinusoidal space, and hydropic degeneration. Some hepatocytes appeared with hyperchromatic nuclei and others with apoptotic nuclei (**Figure 6I**).

4. Discussion

Toluene abuse is a recognized health problem with serious neurological and neuropsychiatric conse-

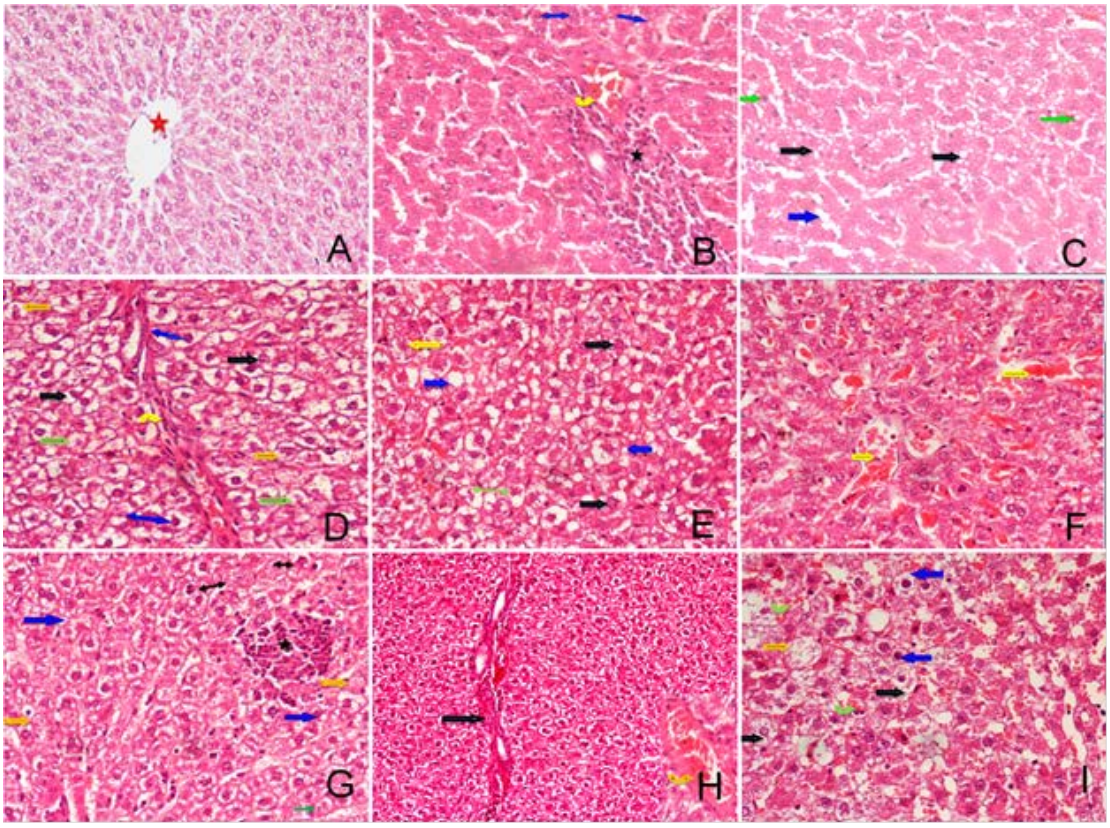

FIGURE 6. Microphotographs of representative sections of the liver of rats in various groups. (A) Saline control showing normal histological structure of hepatic lobules and central vein (star) . (B) Toluene only showing massive inflammatory infiltrate (star) and hemorrhage (yellow arrow), and signs of degeneration in the form of karyorrhexis (blue arrow). (C) Toluene only showing vacuolar degeneration (black arrow) with pyknotic nuclei of some hepatocytes (green arrow). Slight dilatation of blood sinusoids (blue arrow) is observed. (D) Toluene + amiodarone 18 mg/kg showing cellular necrosis in most of hepatocytes in the form of vacuolated cytoplasm (black arrow) with pyknotic (blue arrow), karyorrhectic (orange arrow), and karyolytic (light green arrow) nuclei. (E) Toluene + amiodarone 36 mg/kg showing swollen hepatocytes with accumulation of macrovesicular and microvesicular fat droplets within cytoplasm (blue arrow), karyorrhexis (black arrow) and karyolysis (light green arrow), coagulation necrosis (yellow arrow), and cloudy swelling (white arrow). (F) Toluene + amiodarone 36 mg/kg showing dilated and congested blood sinusoids (arrow). (G) Toluene + chlorpropamide 18 mg/kg showing aggregates of inflammatory infiltrate (star) and minute vacuolar degeneration (blue arrow) with pyknotic nuclei in some cells (black arrow) and karyolysis in other hepatocytes (green arrow). Necrosis appears in some hepatocytes. (H) Toluene + chlorpropamide 18 mg/kg showing congested central blood vessel, thickening of the wall (yellow arrow), and fibrosis (black arrow). (I) Toluene + chlorpropamide 36 mg/kg showing ballooning degeneration of hepatocytes (yellow arrow), and cellular necrosis in most hepatocytes in the form of vacuolated cytoplasm (black arrow) with pyknotic nuclei (blue arrow). Dilated and congested blood sinusoids are seen (green arrow). H&E staining with magnification for A, B, D, E, F, G, and I: of 400×; for C and H: of 200×.

quences [11]. In the present work, we investigated the possible modulation by the K^+ channel blockers chlorpropamide and amiodarone of the oxidative stress, apoptosis, and neuronal damage caused by

toluene. Our results indicated the development of increased oxidative stress in the brain and liver in rats given toluene. There were also decreased brain PON-1 and BChE activities and decreased level of the anti-apoptotic protein Bcl-2. The solvent resulted in marked neurodegeneration in cerebral cortex, degeneration of cerebellar Purkinje cells, and extensive liver tissue damage. The co-administration of either amiodarone or chlorpropamide was associated with an increase in biochemical alterations and exacerbation of the pathological changes caused by the solvent in the brain and liver tissues.

Our findings showed increased levels of MDA in the brain and liver of toluene-treated rats. MDA is one end product of lipid peroxidation, which is indicative of increased generation of ROS and the peroxidation of membrane lipids [30]. There was also a marked decrease in GSH, an important antioxidant and free radical scavenger. GSH is the most abundant non-protein thiol in the cell. Glutathione maintains the redox equilibrium in cells by shuttling between its reduced and oxidized form or glutathione disulfide [31]. GSH directly reacts with hydroxyl radical, peroxyl radical, superoxide, NO, singlet oxygen, and peroxynitrite [31, 32]. It also detoxifies hydroperoxides, lipid peroxides, and peroxynitrite through enzymatic reactions involving glutathione peroxidases and peroxiredoxins [33]. The decrease in GSH during oxidative stress has been linked to the development of neurodegeneration in various disease pathologies [34] and in liver diseases [35]. Our results are thus in agreement with other studies that showed increased lipid peroxidation products and decreased antioxidants such as GSH, superoxide dismutase, and glutathione peroxidase in the rat brain following toluene exposure [36–39]. The increased generation of ROS was demonstrated in crude mitochondrial fractions from the brain of toluene-treated rats [14]. Studies in humans confirmed increased levels of oxidative stress (serum MDA) in workers with paint thinner that contained toluene [40].

In addition, this study indicated marked increases in brain and liver NO levels in toluene-treated rats. Physiological concentrations of NO are important in cell signaling and marinating vasomotor tone [41]. In toxic and inflammatory states, however, NO is generated at high amounts and for long time by activated phagocytes, microglia, and astrocytes. This increase in NO can cause cell death by impairing mitochondrial respiration and causing energy failure [42]. This action of NO is mediated through the formation of reactive nitrogen species, such as peroxynitrite and nitrogen oxides and subsequent oxidative/nitrosative stress. These species are capable of oxidation, nitrosylation, and/or nitration of cell proteins and lipids [41, 42].

The calcium-dependent esterase PON-1 plays an important role in detoxifying some organophosphorus insecticides, nerve agents, and other xenobiotics [43]. The enzyme possesses anti-oxidative and anti-inflammatory properties [44] and a decrease in its activity was observed in a number of neurological disorders [45] and in liver diseases [46], suggesting that PON-1 has a potential role in neuro- and hepatic protection. One of the effects of toluene is the inactivation of PON-1 as shown in this study, which confirms previously published data [39]. Workers exposed to toluene or abusers might therefore have lower than normal activity of the enzyme, and consequently increased vulnerability to other toxicants. In this context, it is worthy to mention that a decreased PON-1 activity determines the susceptibility to develop Parkinson's disease in subjects exposed to organophosphate insecticides [47].

In addition, we demonstrated significant inhibition of brain BChE by toluene which is in agreement with previous studies [38, 39]. Brain acetylcholinesterase (AChE) is also inhibited by toluene in vivo [38] and in vitro [48]. The cholinesterases hydrolyze the neurotransmitter acetylcholine and have a role in neural function and cognition [49]. BChE also functions to protect from the neurotoxicity of natural and synthetic anti-cholinesterase substances [50]. It is suggested that this action of toluene on cholinergic neurotransmission might underlie the cognitive changes and other neurological manifestations seen in abusers of this solvent.

In this study, we investigated the anti-arrhythmic drug amiodarone and the anti-diabetic agent chlorpropamide for their possible modulation of the toluene toxicity. Our findings, however, indicated exacerbation of the toluene neuro- and hepatotoxicity by either drug. The underlying mechanisms may involve an increase in oxidative stress. Either agent caused a decrease in the level of GSH and PON-1 activity along with increased NO, thereby, further compromising the antioxidant side of the redox balance in the cell. These results were unexpected since sulfonylurea members displayed neuroprotective potential in preclinical models of

brain injury as well as in patients having brain infraction, hemorrhage, or contusion [19, 20]. Only in the presence of a coexisting inflammatory response evoked by lipopolysaccharide endotoxin that amiodarone (300 mg/kg) caused an increase in serum alanine aminotransferase [51]. At low concentrations, amiodarone causes mild uncoupling of mitochondrial oxidative phosphorylation, inhibits the Ca^{2+}-induced mitochondrial swelling, and prevents the release of an apoptosis-inducing factor during ischemia-reperfusion. Higher concentrations, however, inhibit the mitochondrial respiration [52]. This could be one factor by which amiodarone exacerbates the toxicant-induced tissue injury. Rats given toluene exhibited increased lipid peroxidation and nitrite levels not only in the brain but also in the serum. In addition, NF-κB level increased while PON-1 decreased in the serum after toluene exposure [39]. Toluene thus induces a state of systemic oxidative stress. Under these conditions, K^+ channel blockade with amiodarone or chlorpropamide exacerbates tissue injury. It is suggested, therefore, that under certain pathological states, blockade of the K^+ channel could be deleterious to tissue integrity. Our findings thus should provide alert as to the use of these drugs in systemic toxic states.

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