ROS

RESEARCH ARTICLE

Novel Protective Effects of Brilliant Blue G in Acute Malathion Toxicity

Omar M.E. Abdel-Salam[1], Amany A. Sleem[2], Eman R. Youness[3], Nermeen Shaffie[4], and Abeer Y. Ibrahim[1]

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

Correspondence: omasalam@hotmail.com (O.M.A-S.)

Abdel-Salam OM et al. Reactive Oxygen Species 6(18):414–427, 2018; ©2018 Cell Med Press
http://dx.doi.org/10.20455/ros.2018.873
(Received: July 31, 2018; Revised: August 19, 2018; Accepted: August 20, 2018)

ABSTRACT | We investigated the effect of brilliant blue G on the development of oxidative stress and tissue damage in rats with acute malathion intoxication. Rats received a single dose of malathion (150 mg/kg) intraperitoneally (ip) and then treated with either saline or brilliant blue G (5, 10, or 20 mg/kg, ip). The control group received saline. Rats were euthanized 4 h after the last injection, and the brain and liver were removed for biochemical studies including determination of the lipid peroxidation malondialdehyde (MDA), nitric oxide, reduced glutathione (GSH), paraoxonase-1 (PON-1), butyrylcholinesterase (BChE), and Na^+-K^+ ATPase activities. Histopathological evaluation of the brain and liver tissue was also performed. Results indicated that rats treated with only malathion exhibited significantly increased MDA and nitric oxide in the brain and liver accompanied with significant decline in tissue levels of GSH. In addition, there was significant decline in brain and liver PON-1 activity and a significantly decreased brain BChE and Na^+-K^+ ATPase activities. Brilliant blue G at doses of 5–20 mg/kg caused a dose-dependent decrease in MDA, and at doses of 10–20 mg/kg decreased nitric oxide, and increased GSH in the brain and liver of malathion-treated rats. It also increased PON-1 activity in the brain (at doses of 10–20 mg/kg) and liver (at a dose of 20 mg/kg). Brilliant blue G at doses of 5–20 mg/kg increased BChE activity and at doses of 10–20 mg/kg increased Na^+-K^+-ATPase activity in the brain of malathion-treated rats. In conclusion, treatment with brilliant blue G decreased oxidative stress in the brain and liver and restored BChE and Na^+-K^+ ATPase activities in the brain of malathion-intoxicated rats. The dye afforded protection against malathion-induced neuronal and liver cell injury.

KEYWORDS | Brilliant blue G; Cholinesterase; Hepatotoxicity; Malathion; Na^+-K^+ ATPase; Neurotoxicity; Nitric oxide; Organophosphates; Oxidative stress; Paraoxonase-1

ABBREVIATIONS | AChE, acetylcholinesterase; BChE, butyrylcholinesterase; GPx, glutathione peroxidase; GSH, reduced glutathione; MDA, malondialdehyde; PON-1, paraoxonase-1

CONTENTS

ROS

RESEARCH ARTICLE

1. INTRODUCTION

Humans are frequently exposed to organophosphate insecticides in agriculture, garden, and veterinary processes, as well as in the household [1]. These compounds pose a risk for both acute and long-term toxicity. Organophosphates exert their toxic effects mainly by inhibiting acetylcholinesterase (AChE) activity at the neuronal synapse, motor end-plate, and nerve endings. In the development of acute toxicity, exposure to high concentrations of organophosphates results in the accumulation of acetylcholine at the above sites leading to excessive nicotinic and muscarinic stimulation and the development of such symptoms/signs as bronchospasm, bradycardia, muscle twitches, convulsions, muscle paralysis, respiratory depression, confusion, and even death might ensue [2, 3]. Occupational or accidental exposure can also result in delayed neurological complications including neuropsychiatric symptoms, depression [4], memory decline, ataxia, extrapyramidal symptoms [5, 6], and peripheral sensory neuropathy [7]. Organophosphorus insecticides have also been implicated in neurodegeneration such as that occurring in Parkinson's disease [7, 8] and dementia [9]. In rats, exposure to malathion causes neuronal degeneration in the cerebral cortex and hippocampus, reactive gliosis, and increased expression of glial fibrillary acidic protein (GFAP) [10–12]. These neurotoxic effects of organophosphates involve such pathogenetic mechanisms as oxidative/nitrosative stress [10–15], impaired mitochondrial dynamics, and compromised mitochondrial bioenergetics [16, 17]. Thus, increased lipid peroxidation along with decreased reduced glutathione (GSH) levels and decreased activities of the antioxidant enzymes glutathione reductase and glutathione peroxidase (GPx) and decreased total antioxidant capacity in the brain, liver, and blood have been shown in rats treated with the organophosphate insecticide malathion [10–12, 15, 17, 18]. Malathion also resulted in DNA damage of peripheral blood lymphocytes [10, 19] and reduced superoxide dismutase, catalase, and GPx activities in human erythrocytes [20]. The insecticide in addition increased brain interleukin-1beta (IL-1β) [19] and the expression of the inducible form of nitric oxide synthase

and increased nitric oxide content in the brain and liver of rats [10, 21]. The agent was also shown to inhibit mitochondrial complexes I [16] and IV [14]. Other organophosphates were shown to alter mitochondrial dynamics and to impair axonal transport at concentrations that do not inhibit AChE activity [22].

Brilliant blue G, also known as Coomassie brilliant blue, is used as a food additive and in the staining of proteins in biomedical applications [23]. It is a purinergic P2X7 receptor antagonist. The latter is an integral plasma membrane protein which, when activated by excess adenosine 5′-triphosphate (ATP) released following cell injury, mediates Ca^{2+} influx, release of pro-inflammatory cytokines, increased formation of reactive oxygen metabolites, and cell death [24, 25]. Recently, studies suggested a neuroprotective potential for brilliant blue G in models of neurodegenerative diseases such as Huntington's disease [26], amyotrophic lateral sclerosis [27], Parkinson's disease [28, 29], and in traumatic brain damage [30]. In these studies, the dye was shown to exert inhibitory effects on nitric oxide, nuclear factor kappaB (NF-κB) [27, 29], IL-1β [27], and to decrease the activation of microglia [31]. The aim of the present study was, therefore, to investigate the therapeutic potential of brilliant blue G in malathion-induced neuronal and liver damage.

2. MATERIALS AND METHODS

2.1. Animals

Male Sprague-Dawley rats (180–200 g in body weight) were used in the study. Rats were obtained from the animal house colony of the National Research Centre (Cairo, Egypt). Standard laboratory food and water were provided ad libitum. The study was performed in accordance with animal protocols and the recommendations of the United States National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.2. Drugs and Chemicals

Malathion (commercial grade, 57%) from El-Naser Chemical Co. (Cairo, Egypt) was used. Brilliant blue G was purchased from Sigma-Aldrich (St Louis, MO, USA) and dissolved in isotonic saline solution immediately before use. Other chemicals and reagents were purchased from Sigma-Aldrich. Selection of the doses of brilliant blue G were based on previous studies [25].

2.3. Study Design

Rats were randomly allocated into five equal groups, six rats each. Group 1 (normal control) treated with 0.9% saline intraperitoneally (ip) (0.2 ml/rat). Group 2–5 received malathion (150 mg/kg, ip) followed immediately by ip saline (served as malathion control), brilliant blue G at doses of 5, 10, or 20 mg/kg. Rats were by euthanized 4 h later by decapitation for tissue collection, and the brains and livers then quickly dissected out on an ice-cold plate, washed with ice-cold phosphate-buffered saline (pH 7.4), weighed, and stored at −80C for biochemical assays. The brain was homogenized in 0.1 M phosphate-buffered saline at pH 7.4 to give a final concentration of 20% w/v, and homogenates were used in biochemical assays.

2.4. Biochemical Assays

2.4.1. Determination of Lipid Peroxidation

Lipid peroxidation was measured by determining its product malondialdehyde (MDA) according to the method described by Ruiz-Larrea et al. [32]. In this assay, 2-thiobarbituric acid reacts with MDA at 25°C to yield a red-colored complex which is measured spectrophotometrically at 532 nm.

2.4.2. Determination of GSH

GSH was determined according to the method described by Ellman et al. [33]. Ellman´s reagent [DTNB; 5,5′-dithiobis(2-nitrobenzoic acid)] reacts with the free thiol group of GSH to form 2-nitro-S-mercaptobenzoic acid. The chromophore has a yellow color and is determined spectrophotometrically at 412 nm.

2.4.3. Determination of Nitric Oxide

Nitric oxide was determined indirectly via measuring the amount of nitrite using the Griess reagent. In the assay, nitrate is converted to nitrite by the enzyme nitrate reductase. Nitrite then reacts with the Griess

reagent to form a purple azo compound, and its absorbance is measured at 540 nm with a spectrophotometer [34].

2.4.4. Determination of Paraoxonase-1 (PON-1) Activity

The arylesterase activity of PON-1 was determined in supernatants using phenylacetate as a substrate. In this assay arylesterase hydrolyzes phenylacetate resulting in the formation of phenol. The rate of phenylacetate hydrolysis is measured by monitoring spectrophotometrically the increase in absorbance at 270 nm at 25°C. One unit of arylesterase activity is equivalent to 1 μmol of phenol formed per min. Enzyme activity expressed as kU/l is calculated based on the molar extinction coefficient of 1,310 $M^{-1}cm^{-1}$ for phenol at 270 nm, pH 8.0, and 25°C [35].

2.4.5. Determination of Brain Cholinesterase Activity

Butyrylcholinesterase (BChE) activity was measured in supernatants using a commercially available kit (Ben Biochemical Enterprise, Milan, Italy). BChE catalyzes the hydrolysis of butyrylthiocholine as a substrate into butyrate and thiocholine. The latter reacts with DTNB to produce a yellow chromophore which then could be quantified using a spectrophotometer [36].

2.4.6. Determination of Brain Na^+-K^+ ATPase Activity

Na^+-K^+ ATPase activity was determined using an ELISA kit purchased from Sunlong Biotech Co. (Zhejiang, China).

2.5. Histopathological Studies

Brain and liver samples of all animals were dissected immediately after euthanasia. The specimens were then fixed in 10 % neutral-buffered formalin saline for at least 72 h. All the specimens were washed in tap water for half an hour and then dehydrated in ascending grades of alcohol, cleared in xylene, and embedded in paraffin. Serial sections of 5 μm thick were cut and stained with hematoxylin and eosin for histopathological investigation. Images were examined and photographed under a digital camera (Microscope Digital Camera DP70, Tokyo, Japan), and processed using Adobe Photoshop version 8.0 (San Jose, CA, USA).

2.6. Statistical Analysis

Data are presented as mean ± SEM. One-way analysis of variance (ANOVA) was used for data analysis and post-hoc individual comparisons were performed with Duncan's multiple range test.

3. RESULTS

3.1. Biochemical Results

3.1.1. Brain Oxidative Stress

In rats exposed to malathion, the level of the lipid peroxidation product MDA increased by 112.6% compared with the saline-control group (52.3 ± 2.7 vs. 24.6 ± 1.4 nmol/g tissue). There was also 86.6% increase in nitric oxide in the brain after malathion administration (72.4 ± 4.1 μmol/g tissue), compared with the control value of 38.9 ± 2.2 μmol/g tissue (**Figure 1**). Meanwhile, the concentration of GSH was reduced by 37.6% (2.41 ± 0.10 vs. 3.86 ± 0.17 μmol/g tissue).

Rats treated with malathion and brilliant blue G showed a significant and dose-dependent decrease in brain MDA by 26.9%, 45.9%, and 48.2%, respectively, compared with malathion only control group (38.2 ± 2.9, 28.3 ± 2.1 and 27.1 ± 1.5 vs. 52.3 ± 2.7 nmol/g tissue). There was also a significant decrease in brain nitric oxide by 17.1% and 36.7% with brilliant blue G given at 10 and 20 mg/kg, respectively (60.0 ± 3.0 and 45.8 ± 2.3 vs. 72.4 ± 4.1 μmol/g tissue). Brilliant blue G given at 5 mg/kg had no significant effect on brain GSH concentration, but the higher doses of 10 or 20 mg/kg resulted in 25.3% and 33.6% increments in GSH (3.02 ± 0.12 and 3.22 ± 0.19 vs. 2.41 ± 0.10 μmol/g tissue) (**Figure 1**).

3.1.2. Brain PON-1 Activity

Rats treated with malathion only exhibited a 65.9% decrease in brain PON-1 activity (4.71 ± 0.18 vs. 13.8 ± 1.5 kU/l). A significant increase in PON-1 activity by 30.0%, 62.2%, and 131.4% was observed after brilliant blue G at 5, 10, and 15 mg/kg, respect-

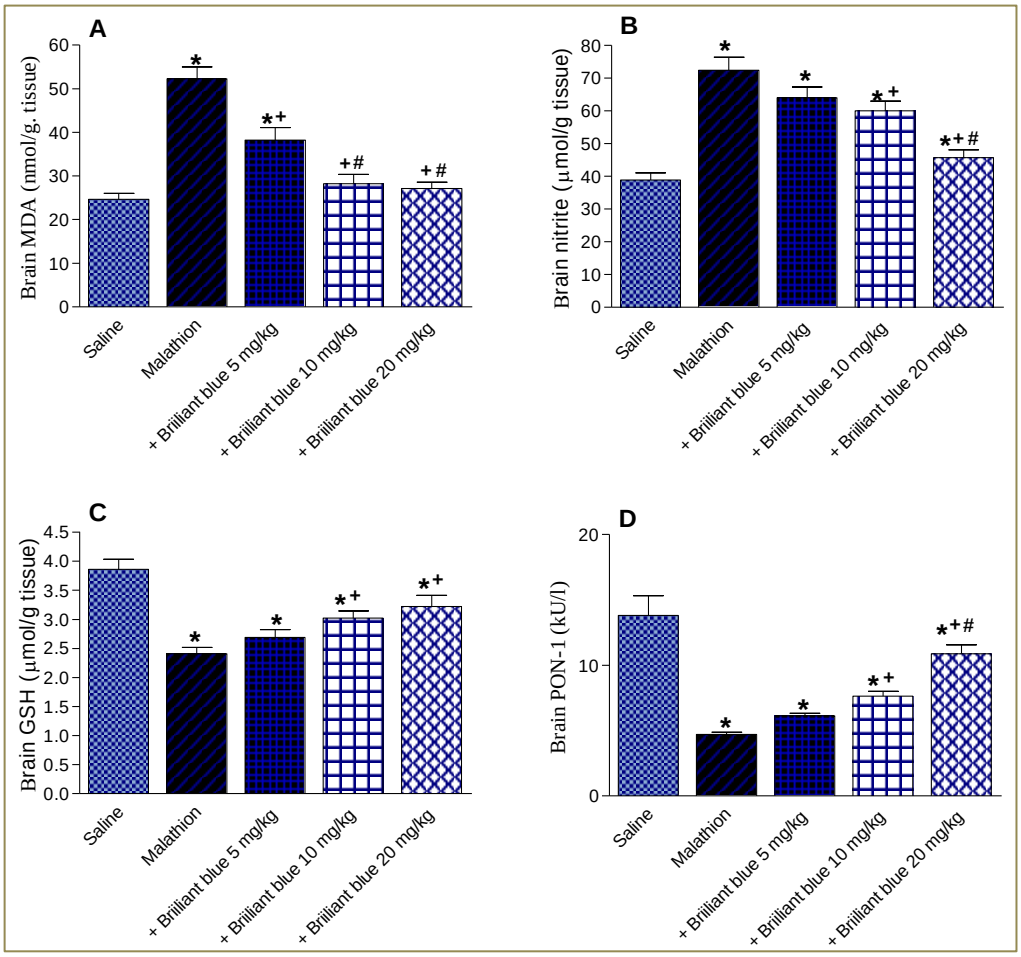

FIGURE 1. Effect of brilliant blue G on malondialdehyde (MDA)(A), nitrite (B), reduced glutathione (GSH) (C), and paraoxonase-1 (PON-1) (D) in the brain of malathion-treated rats. *, $p < 0.05$ vs. corresponding vehicle-treated group; +, $p < 0.05$ vs. malathion only group; #, $p < 0.05$ vs. brilliant blue at 5 or 10 mg/kg group.

ively (6.12 ± 0.20, 7.64 ± 0.36 and 10.9 ± 0.66 vs. 4.71 ± 0.18 kU/l) (**Figure 1**).

3.1.3. Liver Oxidative Stress

In the liver of malathion only-treated rats, MDA values increased by 38.6% compared with the saline group (53.8 ± 3.0 vs. 38.8 ± 2.5 nmol/g tissue). Malathion caused 63.2% increase in nitric oxide (70.2 ± 3.3 µmol/g tissue compared with the control value of 43.0 ± 1.9 µmol/g tissue (**Figure 2**). There was also 29.8% decrease in GSH level (4.83 ± 0.19 vs. 6.88 ± 0.32 µmol/g tissue). Brilliant blue G given at 10 and 20 mg/kg resulted in a significant decrease in MDA by 18.8%, and 22.1%, respectively, compared with malathion control group (43.7 ± 1.4 and 41.9 ± 3.1 vs. 53.8 ± 3.0 nmol/g tissue). There was also a significant decrease in nitric oxide by 23.8%, 37.2%, and 43.9%, respectively (53.5 ± 4.2, 44.1 ± 2.6 and 39.4 ± 2.1 vs. 70.2 ± 3.3 µmol/g tissue). Brilliant blue G given at 10 and 20 mg/kg caused 20.7% and 20.1% increments in GSH, respectively (5.83 ±0.35 and 5.80 ± 0.26 compared with the malathion control value of 4.83 ± 0.19 µmol/g tissue) (**Figure 2**).

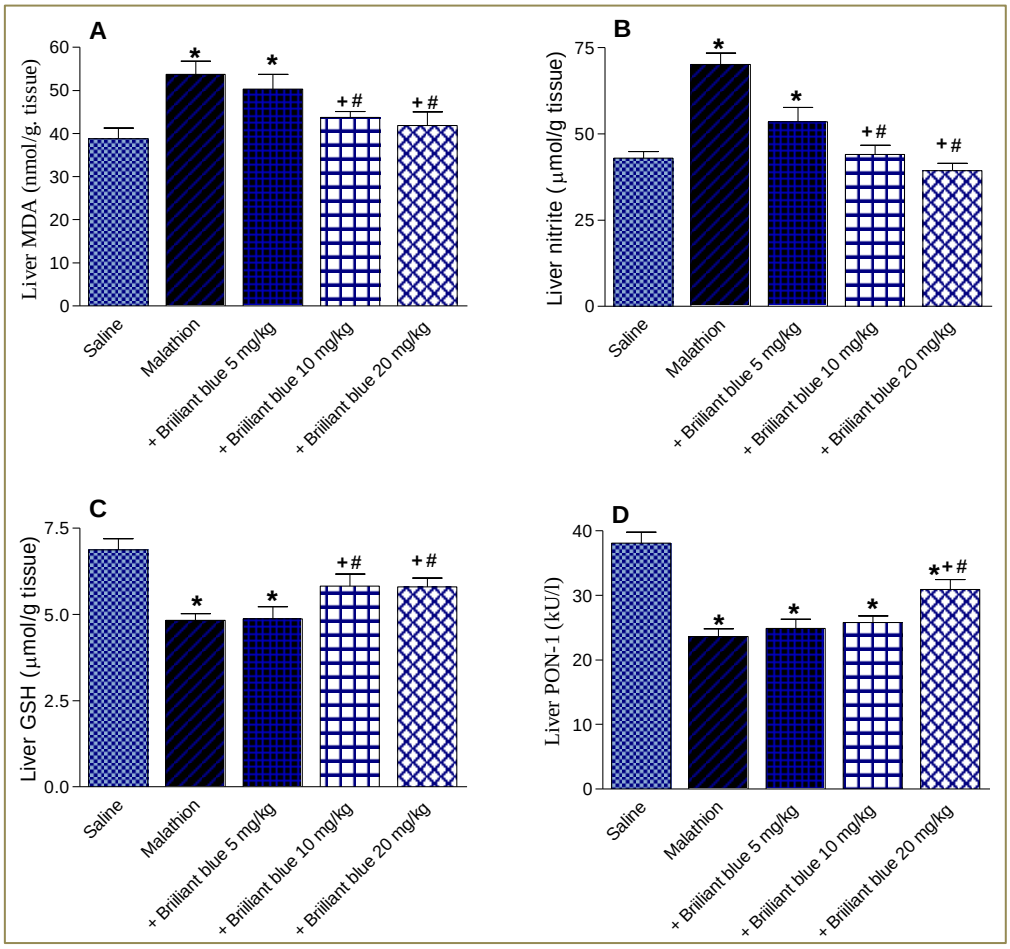

FIGURE 2. Effect of brilliant blue G on malondialdehyde (MDA) (A), nitrite (B), reduced glutathione (GSH) (C), and paraoxonase-1 (PON-1) (D) in the liver of malathion-treated rats. *, $p < 0.05$ vs. corresponding vehicle-treated group; +, $p < 0.05$ vs. malathion only group; #, $p < 0.05$ vs. brilliant blue at 5 or 10 mg/kg group.

3.1.4. Liver PON-1 Activity

A significant decrease in PON-1 activity by 38.1% was found in the liver of rats exposed to malathion (23.6 ± 1.2 vs. 38.1 ± 1.7 kU/l). There was a significant increase in PON-1 activity by 30.9% after brilliant blue G at 20 mg/kg (30.9 ± 1.5 vs. 23.6 ± 1.2 kU/l) (**Figure 2**).

3.1.5. Brain Cholinesterase Activity

A significant decrease in BChE activity by 42.2% was observed in the brain of malathion-intoxicated rats (109.2 ± 6.7 vs. 189.0 ± 11 U/l). Brilliant blue G treatment resulted in 63.7, 72.8, and 109.2% increments in brain BChE activity compared with the malathion control value (178.8 ± 9.4, 188.7 ± 13.9 and 228.5 ± 12.0 vs. 109.2 ± 6.7 U/l) (**Figure 3**).

3.1.6. Brain Na^+-K^+ ATPase Activity

There was a significant reduction in brain α_1-Na^+-K^+ ATPase activity by 35.4% after malathion exposure compared with saline group (0.245 ± 0.017 vs. 0.379 ± 0.015 µg Eq/ml). Rats treated with brilliant blue G at 10 and 20 mg/kg showed 68.9 and 74.7% incre-

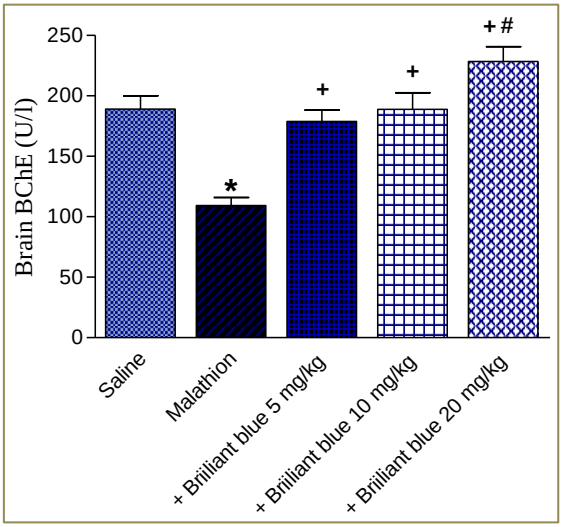

FIGURE 3. Effect of brilliant blue G on brain butyrylcholinesterase (BChE) activity in malathion-treated rats. *, $p < 0.05$ vs. corresponding vehicle-treated group; +, $p < 0.05$ vs. malathion only group; #, $p < 0.05$ vs. brilliant blue at 5 or 10 mg/kg group.

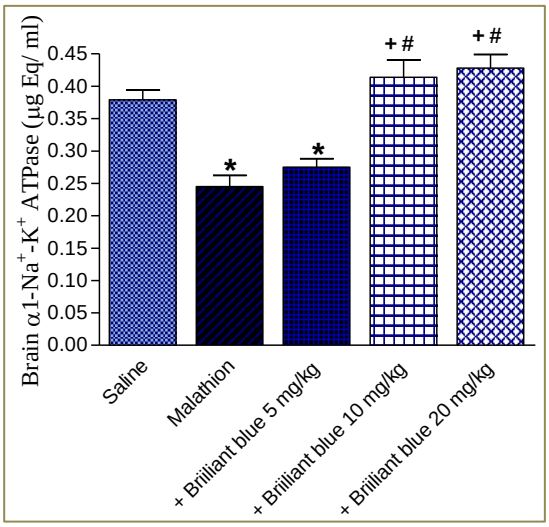

FIGURE 4. Effect of brilliant blue G on brain Na^+-K^+ ATPase activity in malathion-treated rats. *, $p < 0.05$ vs. corresponding vehicle-treated group; +, $p < 0.05$ vs. malathion only group; #, $p < 0.05$ vs. brilliant blue at 5 mg/kg group.

ments in Na^+-K^+ ATPase activity, respectively (0.414 ± 0.027 and 0.428 ± 0.021 vs. 0.245 ± 0.017 µg Eq/ml) (**Figure 4**).

3.2. Histopathological Results

3.2.1. Brain Tissue

Malathion caused marked neuronal signs of degeneration and atrophy in the cerebral cortex, hippocampus, and cerebellum. These changes were not altered by brilliant blue G at 5 mg/kg, but were ameliorated by the higher doses of the dye in a dose-dependent manner (**Figures 5–7**).

3.2.2. Liver Tissue

Rats treated with only malathion exhibited vacuolar degeneration, karyorrhexis, karyolysis, and dilatation of blood sinusoids and main blood vessels. The administration of brilliant blue G at 10 mg/kg resulted in marked amelioration of the malathion-induced pathological changes, while the dose of 20 mg/kg resulted in normalization of liver tissue (**Figure 8**).

4. DISCUSSION

In this study, we examined the ability of the purinergic P2X7 receptor antagonist brilliant blue G to protect against toxicity caused by the organophosphate insecticide malathion. Brilliant blue G was able to alleviate oxidative stress in the brain and liver of malathion-intoxicated rats. The dye was also shown to ameliorate PON-1 inhibition and to restore brain BChE and Na^+-K^+ ATPase activities. These observations clearly suggest a protective action for brilliant blue G against the malathion toxicity. The purinergic P2X7 receptors are ATP-gated ion channels, expressed on astrocytes and microglia. When activated by extracellular ATP released during tissue injury, these cells release proinflammatory cytokines and inflammatory mediators resulting in neuroinflammation. The latter has been implicated in the initiation and/or progression of neuronal loss in a number of neurodegenerative diseases [25]. Several studies indicated a neuroprotective potential for brilliant blue G in rodent models of amyotrophic lateral sclerosis, Parkinson's disease, brain trauma, and spinal cord injury [26–31]. In these studies, the systemic admin-

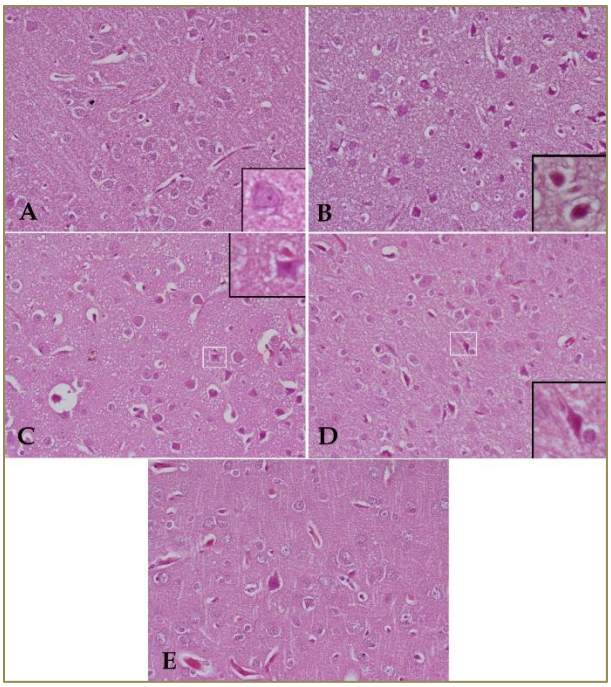

FIGURE 5. Representative photomicrographs of sections of the cerebral cortex from rats in various groups. (A) Vehicle: normal shape of neurons with large vesicular nuclei and well defined nucleoli. (B) Malathion: many neurons with signs of degeneration (acidophilic cytoplasm and deeply-stained nuclei). (C). Malathion + brilliant blue G 5 mg/kg: neurons with signs of degeneration are still observed. (D) Malathion + brilliant blue G 10 mg/kg: most of neurons appear normal, although some neurons with degenerative signs are still observed. (E) Malathion + brilliant blue G 20 mg/kg: only few neurons with degenerative signs while most of the cells are quite normal.

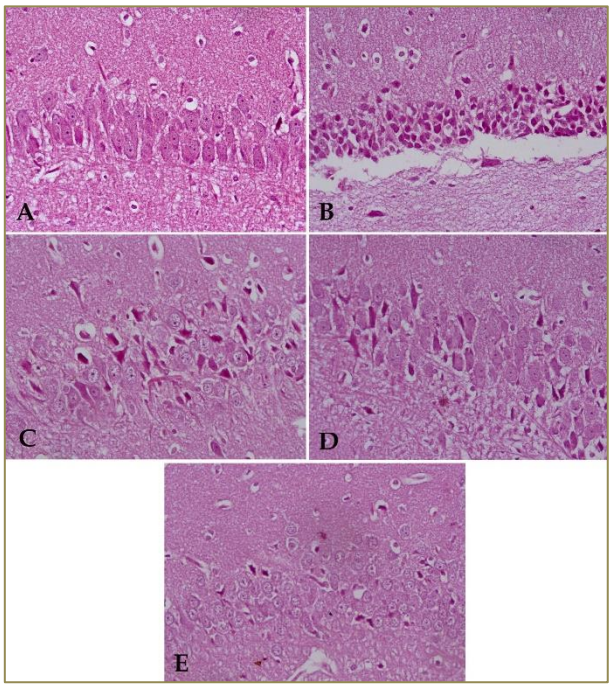

FIGURE 6. Representative photomicrographs of sections of hippocampal area from rats in various groups. (A) Vehicle: normal shape and organization of neurons in a band of cells. (B) Malathion: atrophy of most of neurons that show signs of degeneration (acidophilic cytoplasm and deeply-stained nuclei). (C) Malathion + brilliant blue G 5 mg/kg: disorganization of neurons, with many of them showing degenerative signs. (D) Malathion + brilliant blue G 10 mg/kg: mild amelioration of the malathion damaging effect. Some neurons with signs of degeneration are still observed. (E) Malathion + brilliant blue G 20 mg/kg: only a few neurons with degenerative signs, although disorganization of neurons is still observed.

istration of the dye has been shown to decrease the release/levels of IL-1β, IL-10 [27], nitric oxide [29], NF-κB [27, 29], protein kinase Cγ [30], tumor necrosis factor-α, and microglia activation [31]. Previously, we have demonstrated the ability of brilliant blue G to protect against the rotenone neurotoxicity in the rat brain [29]. The increments in nitric oxide and NF-κB in the brain of rotenone-intoxicated rats were reduced by brilliant blue G which also alleviated the inhibition in PON-1 activity. The pathways by which brilliant blue G modulates neuronal injury are likely to be through inhibition of oxidative stress and neuroinflammation. In addition, the dye exerted an anti-apoptotic action, decreasing caspase-3 immunoreactivity, and rescuing the pigmented dopaminergic neurons in the substantia nigra and GFAP-positive astrocytes [29]. The findings in the present study indicate that brilliant blue G could reduce the malathion-induced oxidative stress and cell injury in both the brain and the liver. This is a potentially important observation in view of the ability of malathion and other organophosphates to cause potentially serious

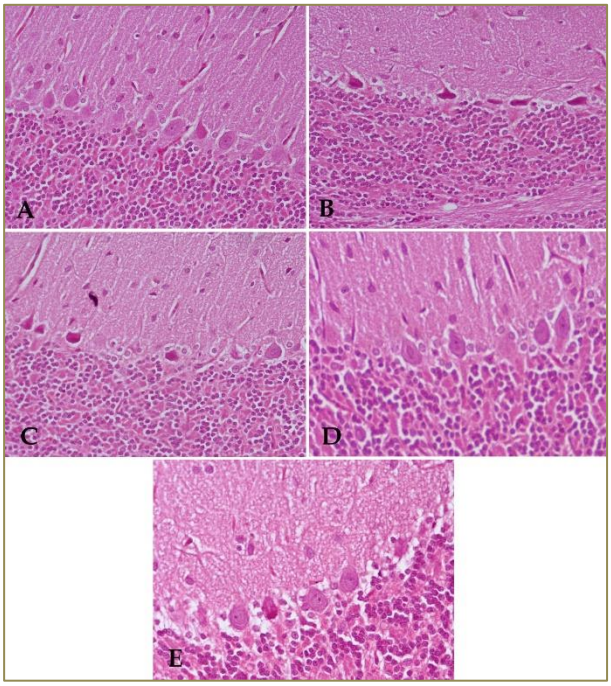

FIGURE 7. Representative photomicrographs of sections of the cerebellum from rats in various groups. (A) Vehicle: normal structure showing Purkinje (large flask-shaped) separating the molecular layer from granular one. (B) Malathion: depletion and atrophy and deep staining of many of Purkinje cells with appearance of a fibrous band in the granular layer. (C) Malathion + brilliant blue G 5 mg/kg: similar results to that of Group B, although no fibrous tissue is noticed in the granular layer. (D) Malathion + brilliant blue G 10 mg/kg: mild amelioration in the shape and structure of Purkinje cells. (E) Malathion + brilliant blue G 20 mg/kg: normalization of most of Purkinje cells.

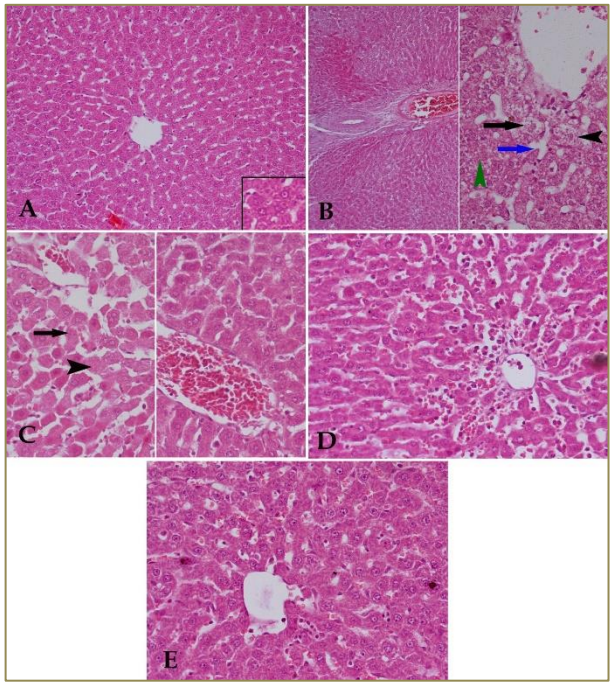

FIGURE 8. Representative photomicrographs of sections of the liver tissue from rats in various groups. (A) Vehicle: normal structure. (B) Malathion: dilatation and congestion of main blood vessels with fibrosis around (in the left part of figure). The right part shows cellular changes in hepatocytes in the form of vacuolar degeneration (black arrow), karyorrhexis (black arrowhead), karyolysis (green arrowhead), and dilatation of blood sinusoids (blue arrow). (C) Malathion + brilliant blue G 5 mg/kg: no fibrosis is detected, although cellular changes such as karyolysis (arrowhead) and karyorhexis (arrow) are still observed. (D) Malathion + brilliant blue G 10 mg/kg: only dilatation and congestion of blood sinusoids are noticed. (E) Malathion + brilliant blue G 20 mg/kg: normalization of the liver tissue.

neurological complications and possibly the development of neurodegeneration, such as Parkinson's disease [8, 37–39].

Our observations are in accordance with previously published studies showing increased lipid peroxidation [10–12, 14, 15, 18], decreased GSH [10–12, 19, 21], reduced activities of the antioxidant enzymes GPx [21], catalase, superoxide dismutase, and glutathione reductase [20], and decreased total antioxidant capacity [10, 21] in the brain and liver of malathion-treated rats. Malathion increased the formation of superoxide anions in hippocampal submitochondrial particles and resulted in inhibition of mitochondrial complexes I and IV activities [14, 16]. These data thus suggest the involvement of free radical-mediated mechanisms and mitochondrial impairment in the malathion-induced tissue damage. In support of this notion are the findings that the neurotoxic actions of malathion (and other organophosphates) could be attenuated through the use of

antioxidants such as grape seed extract [19], vitamin C, and green tea [40]. Moreover, lipid peroxidation and the changes in antioxidant enzymes induced by malathion in rat and human erythrocytes could be reduced by chain breaking antioxidants, including ascorbate and α-tocopherol [13, 20]. The findings in the present study of increased tissue level of the lipid peroxidation end product MDA and the concomitant decrease of the antioxidant and free radical scavenger GSH following malathion treatment support a role for reactive oxygen metabolites in the toxicant-induced neuronal and liver tissue injury. We have also shown that brilliant blue G caused a dose-dependent decrease in MDA, and increased GSH in the brain and liver of malathion-treated rats, suggesting a decrease in the malathion-induced oxidative stress and the consequent tissue damage by the dye.

Our results also show markedly elevated nitric oxide levels in the brain and liver of malathion-intoxicated rats. Neurotoxic and hepatotoxic changes as well as DNA damage of peripheral blood lymphocytes induced by malathion could be attenuated with inhibitors of nitric oxide synthase. This suggested the involvement of increased nitric oxide generation in the malathion-induced tissue damage [21]. Malathion increases endogenous nitric oxide biosynthesis and the expression of the inducible form of nitric oxide synthase (iNOS) in the rat brain and liver [10, 21]. The generation of nitric oxide in excessive amounts could result in oxidative and nitrosative injury. This occurs through the reaction of nitric oxide with molecular oxygen to form a variety of nitrogen oxide species or by reacting with the superoxide forming the reactive peroxynitrite ($ONOO^-$) [41]. In this study, brilliant blue G was shown to decrease nitric oxide concentrations in the brain and liver of malathion-treated rats. This finding suggests that inhibition of nitric oxide synthesis might be one mechanism by which the dye protects against the malathion toxicity.

This study also indicated that exposure to malathion was associated with significant inhibition of PON-1 activity in the brain and liver. This finding is in agreement with our previously published data [10, 11, 19, 21, 42]. The A-esterase PON-1 enzyme hydrolyzes the active metabolites (oxons) of some organophosphate insecticides, such as diazoxan, dichlorvos, and chlorpyrifos oxon [43]. Reduced enzyme activity is found in farm workers after exposure to anticholinergic pesticides and following acute intoxication with organophosphates, indicating PON-1 inhibitory effect for these compounds [44]. The decrease in PON-1 activity was also shown to be associated with a greater inhibition of BChE activity in the plasma of agricultural workers exposed to organophosphates [45]. On the other hand, a decline in the catalytic efficiency of PON-1 enzyme has been shown to determine the susceptibility to organophosphates and the risk for developing Parkinson's disease in subjects exposed to these compounds [43, 46, 47]. Studies also implicated exposure to organophosphates in the development and progression of the motor and cognitive symptoms in Parkinson's disease, especially in subjects with the PON1L55M genotype, the slow metabolizer variant of PON-1 [48]. On the other hand, the administration of exogenous PON-1 to rats and mice could protect against organophosphate toxicity [43, 49]. As shown in the present work, the administration of brilliant blue G to malathion-intoxicated rats resulted in a significant increase in PON-1 enzyme activity. The mechanism that underlies this effect of brilliant blue G is not clear. PON-1 activity could be inhibited by oxidative stress [50] and thus, it is possible that the increase in enzyme activity is due to reduced levels of oxidative stress by the dye.

The neurotoxicity of organophosphate insecticides is largely ascribed to their ability to irreversibly inactivate the enzyme AChE, resulting in excessive accumulation of acetylcholine at the synaptic cleft and motor-end plate [2, 3]. BChE is found in the brain, plasma, smooth muscles, and heart [51]. BChE acts to hydrolyze excess acetylcholine [52] and to protect nerve terminals from increased acetylcholine [53]. BChE activity declines in the brain of rats treated with malathion [10, 11, 19, 21]. In malathion-intoxicated rats, brain BChE activity could be increased by treatment with grape seed extract, an antioxidant or by atropine along with reduced neuronal injury [10]. In the present study, there was a marked increase in BChE activity after treatment of malathion-intoxicated rats with brilliant blue G. Whether this is related to the neuroprotection afforded by the dye is not clear.

Our results also show that malathion resulted in decreased brain Na^+-K^+ ATPase activity. The latter is responsible for maintaining Na^+ and K^+ ion gradients across the cell membrane and the resting membrane potential by an energy-dependent mechanism [54]. A mitochondrial bioenergetic deficit is likely to be in-

volved in the neurotoxic effects of malathion and other organophosphate insecticides [10, 14, 16, 17]. The decrease in the activity of the Na^+-K^+ ATPase in the present study might thus be due to a decrease in ATP production. The administration of brilliant blue G was able to restore Na^+-K^+ ATPase activity in the brain of malathion-treated rats. The study suggests that brilliant blue G might rescue neurons during malathion intoxication by maintaining Na^+-K^+ ATPase activity.

In conclusion, the findings of the present study indicate that the neurotoxic and hepatotoxic alterations caused by malathion could be attenuated with the use of the purinergic P2X7 receptor antagonist brilliant blue G. Such neuro- and hepato-protection is likely to involve a decrease in oxidative/nitrosative stress.

ACKNOWLEDGMENTS

This work was not supported by research grants. The authors declare no conflicts of interest.

REFERENCES

1. Hayes Jr. WJ. In: *Pesticides Studied in Man*. Williams and Wilkins, Baltimore, MD, USA. 1982.
2. Mileson BE, Chambers JE, Chen WL, Dettbarn W, Ehrich M, Eldefrawi AT, et al. Common mechanism of toxicity: a case study of organophosphorus pesticides. *Toxicol Sci* 1998; 41(1):8–20. doi: 10.1006/toxs.1997.2431.
3. Jokanovic M, Kosanovic M. Neurotoxic effects in patients poisoned with organophosphorus pesticides. *Environ Toxicol Pharmacol* 2010; 29(3):195–201. doi: 10.1016/j.etap.2010.01.006.
4. Wesseling C, van Wendel de Joode B, Keifer M, London L, Mergler D, Stallones L. Symptoms of psychological distress and suicidal ideation among banana workers with a history of poisoning by organophosphate or n-methyl carbamate pesticides. *Occup Environ Med* 2010; 67(11):778–84. doi: 10.1136/oem.2009.047266.
5. Salvi RM, Lara DR, Ghisolfi ES, Portela LV, Dias RD, Souza DO. Neuropsychiatric evaluation in subjects chronically exposed to organophosphate pesticides. *Toxicol Sci* 2003; 72(2):267–71. doi: 10.1093/toxsci/kfg034.
6. Detweiler MB. Organophosphate intermediate syndrome with neurological complications of extrapyramidal symptoms in clinical practice. *J Neurosci Rural Pract* 2014; 5(3):298–301. doi: 10.4103/0976-3147.133616.
7. Jamal GA, Hansen S, Pilkington A, Buchanan D, Gillham RA, Abdel-Azis M, et al. A clinical neurological, neurophysiological, and neuropsychological study of sheep farmers and dippers exposed to organophosphate pesticides. *Occup Environ Med* 2002; 59(7):434–41.
8. Elbaz A, Clavel J, Rathouz PJ, Moisan F, Galanaud JP, Delemotte B, et al. Professional exposure to pesticides and Parkinson disease. *Ann Neurol* 2009; 66(4):494–504. doi: 10.1002/ana.21717.
9. Lin JN, Lin CL, Lin MC, Lai CH, Lin HH, Yang CH, et al. Increased risk of dementia in patients with acute organophosphate and carbamate poisoning: a nationwide population-based cohort study. *Medicine (Baltimore)* 2015; 94(29):e1187. doi: 10.1097/MD.0000000000001187.
10. 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.
11. Abdel-Salam OME, Eman R, Youness ER, Esmail RSE, Mohammed NA, Khadrawy YA. Methylene blue as a novel neuroprotectant in acute malathion intoxication. *React Oxyg Species* (Apex) 2016; 1(2):165–77. doi: 10.20455/ros.2016.821.
12. Abdel-Salam OME, Youness ER, Esmail RSE, Mohammed NA, Khadrawy YA, Sleem AA, et al. Protection by neostigmine and atropine against brain and liver injury induced by acute malathion exposure. *J Nanosci Nanotechnol* 2018; 18(1):510–21. doi: 10.1166/jnn.2018.13933.
13. John S, Kale M, Rathore N, Bhatnagar D. Protective effect of vitamin E in dimethoate and malathion induced oxidative stress in rat erythrocytes. *J Nutr Biochem* 2001; 12(9):500–4.
14. Delgado EH, Streck EL, Quevedo JL, Dal-Pizzol F. Mitochondrial respiratory dysfunction and oxidative stress after chronic malathion exposure. *Neurochem Res* 2006; 31(8):1021–5. doi:

10.1007/s11064-006-9111-1.
15. Trevisan R, Uliano-Silva M, Pandolfo P, Franco JL, Brocardo PS, Santos AR, et al. Antioxidant and acetylcholinesterase response to repeated malathion exposure in rat cerebral cortex and hippocampus. *Basic Clin Pharmacol Toxicol* 2008; 102(4):365–9. doi: 10.1111/j.1742-7843.2007.00182.x.
16. Karami-Mohajeri S, Hadian MR, Fouladdel S, Azizi E, Ghahramani MH, Hosseini R, et al. Mechanisms of muscular electrophysiological and mitochondrial dysfunction following exposure to malathion, an organophosphorus pesticide. *Hum Exp Toxicol* 2014; 33(3):251–63. doi: 10.1177/0960327113493300.
17. dos Santos AA, Naime AA, de Oliveira J, Colle D, dos Santos DB, Hort MA, et al. Long-term and low-dose malathion exposure causes cognitive impairment in adult mice: evidence of hippocampal mitochondrial dysfunction, astrogliosis and apoptotic events. *Arch Toxicol* 2016; 90(3):647–60. doi: 10.1007/s00204-015-1466-0.
18. Brocardo PS, Pandolfo P, Takahashi RN, Rodrigues AL, Dafre AL. Antioxidant defenses and lipid peroxidation in the cerebral cortex and hippocampus following acute exposure to malathion and/or zinc chloride. *Toxicology* 2005; 207(2):283–91. doi: 10.1016/j.tox.2004.09.012.
19. Abdel-Salam OME, Galal AF, Hassanane MM, Salem LM, Nada SA, Morsy FA. Grape seed extract alone or combined with atropine in treatment of malathion induced neuro- and genotoxicity. *J Nanosci Nanotechnol* 2018; 18(1):564–75. doi: 10.1166/jnn.2018.13943.
20. Durak D, Uzun FG, Kalender S, Ogutcu A, Uzunhisarcikli M, Kalender Y. Malathion-induced oxidative stress in human erythrocytes and the protective effect of vitamins C and E in vitro. *Environ Toxicol* 2009; 24(3):235–42. doi: 10.1002/tox.20423.
21. Abdel-Salam OME, Youness ER, Mohammed NA, Yassen NN, Khadrawy YA, El-Toukhy SE, et al. Nitric oxide synthase inhibitors protect against brain and liver damage caused by acute malathion intoxication. *Asian Pac J Trop Med* 2017; 10(8):773–86. doi: 10.1016/j.apjtm.2017.07.018.
22. Middlemore-Risher ML, Adam BL, Lambert NA, Terry AV, Jr. Effects of chlorpyrifos and chlorpyrifos-oxon on the dynamics and movement of mitochondria in rat cortical neurons. *J Pharmacol Exp Ther* 2011; 339(2):341–9. doi: 10.1124/jpet.111.184762.
23. Ferreira LG, Faria RX, Ferreira NC, Soares-Bezerra RJ. Brilliant blue dyes in daily food: how could purinergic system be affected? *Int J Food Sci* 2016; 2016:7548498. doi: 10.1155/2016/7548498.
24. Jiang LH, Mackenzie AB, North RA, Surprenant A. Brilliant blue G selectively blocks ATP-gated rat P2X(7) receptors. *Mol Pharmacol* 2000; 58(1):82–8.
25. Sperlagh B, Illes P. P2X7 receptor: an emerging target in central nervous system diseases. *Trends Pharmacol Sci* 2014; 35(10):537–47. doi: 10.1016/j.tips.2014.08.002.
26. Diaz-Hernandez M, Diez-Zaera M, Sanchez-Nogueiro J, Gomez-Villafuertes R, Canals JM, Alberch J, et al. Altered P2X7-receptor level and function in mouse models of Huntington's disease and therapeutic efficacy of antagonist administration. *FASEB J* 2009; 23(6):1893–906. doi: 10.1096/fj.08-122275.
27. Apolloni S, Amadio S, Parisi C, Matteucci A, Potenza RL, Armida M, et al. Spinal cord pathology is ameliorated by P2X7 antagonism in a SOD1-mutant mouse model of amyotrophic lateral sclerosis. *Dis Model Mech* 2014; 7(9):1101–9. doi: 10.1242/dmm.017038.
28. Abdel-Salam OME, Youness ER, Mohammed NA, Yassen NN, Shaffie N, Sleem AA. Brilliant blue G protects against rotenone-induced neuronal damage in the rat brain. *React Oxyg Species* 2017; 4(11):336–50. doi: 10.20455/ros.2017.855.
29. Wang XH, Xie X, Luo XG, Shang H, He ZY. Inhibiting purinergic P2X7 receptors with the antagonist brilliant blue G is neuroprotective in an intranigral lipopolysaccharide animal model of Parkinson's disease. *Mol Med Rep* 2017; 15(2):768–76. doi: 10.3892/mmr.2016.6070.
30. Wang YC, Cui Y, Cui JZ, Sun LQ, Cui CM, Zhang HA, et al. Neuroprotective effects of brilliant blue G on the brain following traumatic brain injury in rats. *Mol Med Rep* 2015; 12(2):2149–54. doi: 10.3892/mmr.2015.3607.
31. Peng W, Cotrina ML, Han X, Yu H, Bekar L, Blum L, et al. Systemic administration of an antagonist of the ATP-sensitive receptor P2X7

improves recovery after spinal cord injury. *Proc Natl Acad Sci USA* 2009; 106(30):12489–93. doi: 10.1073/pnas.0902531106.
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.
33. Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959; 82(1):70–7.
34. Archer S. Measurement of nitric oxide in biological models. *FASEB J* 1993; 7(2):349–60.
35. Eckerson HW, Wyte CM, La Du BN. The human serum paraoxonase/arylesterase polymorphism. *Am J Hum Genet* 1983; 35(6):1126–38.
36. Ellman GL, Courtney KD, Andres V, Jr., Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* 1961; 7:88–95.
37. Semchuk KM, Love EJ, Lee RG. Parkinson's disease and exposure to agricultural work and pesticide chemicals. *Neurology* 1992; 42(7):1328–35.
38. Hancock DB, Martin ER, Mayhew GM, Stajich JM, Jewett R, Stacy MA, et al. Pesticide exposure and risk of Parkinson's disease: a family-based case-control study. *BMC Neurol* 2008; 8:6. doi: 10.1186/1471-2377-8-6.
39. Narayan S, Liew Z, Paul K, Lee PC, Sinsheimer JS, Bronstein JM, et al. Household organophosphorus pesticide use and Parkinson's disease. *Int J Epidemiol* 2013; 42(5):1476–85. doi: 10.1093/ije/dyt170.
40. Elzoghby RR, Hamuoda AF, Abdel-Fatah A, Farouk M. Protective role of vitamin C and green tea extract on malathion-induced hepatotoxicity and nephrotoxicity in rats. *Am J Pharmacol Toxicol* 2014; 9(3):177–88. doi: 10.3844/ajptsp.2014.177-188.
41. Brown GC. Nitric oxide and neuronal death. *Nitric Oxide* 2010; 23(3):153–65. doi: 10.1016/j.niox.2010.06.001.
42. Abdel-Salam OME, Sleem AA, Youness ER, Morsy FA. Preventive effects of cannabis on neurotoxic and hepatotoxic activities of malathion in rat. *Asian Pac J Trop Med* 2018; 11(4):272–9.
43. Costa LG, Li WF, Richter RJ, Shih DM, Lusis A, Furlong CE. The role of paraoxonase (PON1) in the detoxication of organophosphates and its human polymorphism. *Chem Biol Interact* 1999; 119–120:429–38.
44. Hernandez AF, Gil F, Lacasana M, Rodriguez-Barranco M, Gomez-Martin A, Lozano D, et al. Modulation of the endogenous antioxidants paraoxonase-1 and urate by pesticide exposure and genetic variants of xenobiotic-metabolizing enzymes. *Food Chem Toxicol* 2013; 61:164–70. doi: 10.1016/j.fct.2013.05.039.
45. Hofmann JN, Keifer MC, Furlong CE, De Roos AJ, Farin FM, Fenske RA, et al. Serum cholinesterase inhibition in relation to paraoxonase-1 (PON1) status among organophosphate-exposed agricultural pesticide handlers. *Environ Health Perspect* 2009; 117(9):1402–8. doi: 10.1289/ehp.0900682.
46. Manthripragada AD, Costello S, Cockburn MG, Bronstein JM, Ritz B. Paraoxonase 1, agricultural organophosphate exposure, and Parkinson disease. *Epidemiology* 2010; 21(1):87–94. doi: 10.1097/EDE.0b013e3181c15ec6.
47. Lee PC, Rhodes SL, Sinsheimer JS, Bronstein J, Ritz B. Functional paraoxonase 1 variants modify the risk of Parkinson's disease due to organophosphate exposure. *Environ Int* 2013; 56:42–7. doi: 10.1016/j.envint.2013.03.004.
48. Paul KC, Sinsheimer JS, Cockburn M, Bronstein JM, Bordelon Y, Ritz B. Organophosphate pesticides and PON1 L55M in Parkinson's disease progression. *Environ Int* 2017; 107:75–81. doi: 10.1016/j.envint.2017.06.018.
49. Wang NN, Dai H, Yuan L, Han ZK, Sun J, Zhang Z, et al. Study of paraoxonase-1 function on tissue damage of dichlorvos. *Toxicol Lett* 2010; 196(2):125–32. doi: 10.1016/j.toxlet.2010.04.008.
50. Nguyen SD, Sok DE. Oxidative inactivation of paraoxonase1, an antioxidant protein and its effect on antioxidant action. *Free Radic Res* 2003; 37(12):1319–30.
51. Çokuğraş AN. Butyrylcholinesterase:structure and Physiological Importance. *Turk J Biochem* 2003; 28(2):54–61.
52. Duysen EG, Li B, Darvesh S, Lockridge O. Sensitivity of butyrylcholinesterase knockout mice to (–)-huperzine A and donepezil suggests humans with butyrylcholinesterase deficiency may not tolerate these Alzheimer's disease drugs

and indicates butyrylcholinesterase function in neurotransmission. *Toxicology* 2007; 233(1–3):60–9. doi: 10.1016/j.tox.2006.11.069.

53. Girard E, Bernard V, Minic J, Chatonnet A, Krejci E, Molgo J. Butyrylcholinesterase and the control of synaptic responses in acetylcholinesterase knockout mice. *Life Sci* 2007; 80(24–25):2380–5. doi: 10.1016/j.lfs.2007.03.011.
54. Jorgensen PL, Hakansson KO, Karlish SJ. Structure and mechanism of Na,K-ATPase: functional sites and their interactions. *Annu Rev Physiol* 2003; 65:817–49. doi: 10.1146/annurev.physiol.65.092101.142558.