DRUG DISCOVERY

Effect of propranolol, bisoprolol and terbutaline in acute brain injury induced by malathion in the rat

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ABSTRACT

The pharmacological management of acute malathion poisoning entails besides the use of the specific antidotes atropine and oximes, the administration of other drugs for controlling associated cardiac and respiratory problems. In this study, we aimed to investigate the effect of the non-selective β-adrenoceptor blocker propranolol, the selective β_1-adrenoceptor antagonist bisoprolol as well as the bronchodilator drug and the selective β_2-adrenoceptor agonist terbutaline, either alone or in combination with atropine on brain oxidative stress and neuronal damage caused by acute malathion administration. Rats were treated with malathion at 150 mg/kg by intraperitoneal (i.p.) injection for two days either alone or combined with propranolol, bisoprolol or turbutaline all at the dose of 1 mg/kg, i.p. Brain damage was assessed by measuring the levels of the oxidative stress biomarkers malondialdehyde, reduced glutathione and nitric oxide in brain tissue and by brain histopathology. Results indicated significant increase in the brain lipid peroxidation marker malondialdehyde and nitric oxide as well as depletion of reduced glutathione in brain of malathion only- treated rats. In the cerebral cortex, focal homogenous deeply eosinophilic plaques, gliosis, neuronal apoptosis, necrosis, perineuronal vacuolation and spongiform degeneration were observed after exposure to malathion. The biochemical changes of malathion were decreased by atropine and β-blockers in addition to marked amelioration of the malathion-induced histopthaological effects. The administration of terbutaline to malathion-treated rats had no significant effects on brain oxidative stress. The extent of histological brain damage (neurodegeneration) was not reduced after terbutaline. The administration of atropine was able to ameliorate the neuropathological changes in brain of rats treated with malathion and β-adrenergic antagonists but not in case of terbutaline. Collectively, the present results indicate that β-blockade may decrease the neurodegeneration in brain of malathion-treated rats. The results also suggest a role for β-adrenoceptor stimulation in the development of the cerebral damage by malathion.

Keywords: Beta adrenoceptors, propranolol, bisoprolol, terbutaline, neuronal injury, malathion toxicity

1. INTRODUCTION

Acute poisoning with organophosphorus insecticides represents a global and important health problem. These compounds are widely used in the household, farms, agriculture with high risk of human exposure. Intoxication may follow inhalation or skin contact with the insecticides (Jokanovic and Kosanovic, 2010; Chowdhary et al., 2014). Malathion (*O*, O-dimethyl-S-1, 2-bis ethoxy carbonyl ethyl phosphorodithionate) and other organophosphates induce their toxicity by irreversibly inhibiting the enzyme acetylcholinesterase (AChE) in cholinergic nerve synapses (Mileson et al., 1998). The enzyme is present in brain, skeletal muscle end-plate and erythrocyte membrane. In cholinergic nerve synapses, AChE acts by hydrolyzing the neurotransmitter acetylcholine (ACh) and thus terminates its action at the synapse. Inhibition of AChE therefore results in the accumulation of ACh with excessive stimulation of ACh receptors in synapses of the autonomic ganglia, central nervous system and neuromuscular junctions (Silman and Sussman, 2005). These patients present with symptoms of increased cholinergic activity being manifested by increased salivation, bronchospasm and increased bronchial secretions, bradycardia or tachycardia, hypertension, fasciculations, muscle weakness or paralysis, headache, confusion, coma and even death from respiratory failure (Simpson and Schuman, 2002; Jokanovic, 2009). There are also delayed consequences of exposure to organophosphate, mainly with affection of the peripheral and central nervous system e.g., polyneuropathy, myelopathy, cognitive deficits and memory impairment, mood disorders and ataxia (Michotte et al., 1989; Chuang et al., 2002; Lotti and Moretto, 2005; Harrison and Ross, 2016).

The classic antidote for the treatment of acute poisoning with organophosphates is atropine, a muscarinic receptor antagonist aimed to block the effect of increased ACh at the muscarinic cholinergic synapses as well as oximes e.g., obidoxime or pralidoxime which reactivate AChE by removal of the phosphate group (Eddleston et al., 2008; Jokanovic, 2009). Symptomatic treatment of cardiac or respiratory symptoms may require the administration of other drugs such as β-adrenergic blockers or bronchodilators eg., those acting on β_2-adrenoceptors like terbutaline or salbutamol.

The aim of the present study was therefore to examine the effect of the β-adrenoceptor antagonists propranolol and bisoprolol as well as the selective β_2-adrenoceptor agonist terbutaline either alone or combined with atropine on the development of oxidative stress and neuronal damage in brain of rats with acute malathion intoxication.

2. MATERIALS AND METHODS

Animals

Experiments were performed using male Sprague–Dawley strain rats weighing 160–170 g of body weight from the colony of the animal house of the institute. Animals were housed under standardized conditions and had free access to standard rat food and water. The study procedures were performed according to the regulations by the Institute Ethics Committee and followed the Guide for Care and Use of Laboratory Animals by the US National Institutes of Health (Publication No. 85-23, revised 1996).

Drugs and chemicals

Malathion was purchased from Naser Chemical Company (Egypt). Other drugs used were propranolol hydrochloride (AstraZeneka, Egypt), terbutaline sulphate (Sedko Pharmaceutical Co., Egypt) and bisoprolol fumarate (Merk Serono, Egypt). The remaining chemicals and reagents were of analytical grade and obtained from Sigma-Aldrich (St. Louis, MO, USA).

Experimental groups

Rats were randomly divided into eight groups with 6 rats in each group.

Group 1 treated with saline (0.2 ml/rat, i.p).
Group 2 treated with malathion at the dose of 150 mg/kg, i.p. and served as positive control.
Group 3 treated with malathion at the dose of 150 mg/kg, i.p. + propranolol (1 mg/kg, i.p.).
Group 4 treated with malathion at the dose of 150 mg/kg, i.p. + propranolol (1 mg/kg, i.p.) and atropine (1 mg/kg, i.p.).
Group 5 treated with malathion at the dose of 150 mg/kg, i.p. + bisoprolol (1 mg/kg, i.p.).
Group 6 treated with malathion at the dose of 150 mg/kg, i.p. + bisoprolol (1 mg/kg, i.p.) and atropine (1 mg/kg, i.p.).
Group 7 treated with malathion at the dose of 150 mg/kg, i.p. + terbutaline (1 mg/kg, i.p.).
Group 8 treated with malathion at the dose of 150 mg/kg, i.p. + terbutaline (1 mg/kg, i.p.) and atropine (1 mg/kg, i.p.).

Treatments were given for two successive days and rats then euthanized by decapitation under ether anesthesia for tissue collection. Their brains were rapidly dissected out on an ice-cold plate. One half of the brain, the right, was washed with ice-cold phosphate-buffered saline (pH 7.4), weighed and stored at –80°C. The brain tissue was homogenized in 0.1 M phosphate-buffered

saline (pH 7.4) to give a final concentration of 10% for use in the biochemical study. The other half of the brain was kept in 10% formol saline for histopathological processing.

Biochemical analysis

Oxidative stress biomarkers were determined in brain homogenates by measurement of malondialdehyde, reduced glutathione and nitric oxide. Malondialdehyde, an end product of lipid peroxidation was assayed by determining thiobarbituric reactive substances (TBAS) using the method by Nair and Turner, (1984) 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. 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 generating 5-thio-2-nitrobenzoic acid. The latter has yellow color and can be determined by reading absorbance at 412 nm (Ellman, 1959). Nitric oxide estimated as nitrate/nitrite was determined by the use of Griess reagent. 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 (Archer, 1993).

Histopathology

Five µm thick paraffin sections were stained with haematoxlin and eosin (Drury and Walligton, 1980) and examined by light microscopy (Olympus Cx 41 with DP12 Olympous digital camera. Olympous optical Co. Ltd, Tokyo. Japan).

Statistical analysis

Data in the study were presented as mean ± SEM. Statistical significance was assessed using one-way ANOVA with Duncan's multiple range test post hoc test. Graphpad Prism software, version 6 (GraphPad Prism Software Inc., San Diego, CA, USA) was used. A probability value of less than 0.05 was considered as statistically significant.

3. RESULTS

Biochemistry results

Rats treated with only malathion had significant increases in brain malondialdehyde (MDA) by 65.7% (29.5 ± 1.4 *vs.* 17.8 ± 0.9 nmol/g.tissue) and nitric oxide by 102.2% (45.1 ± 3.1 *vs.* 22.3 ± 1.7 µmol/g. tissue). On the other hand, brain reduced glutathione (GSH) showed a significant decrease by 56.5% (1.35 ± 0.07 *vs.* 3.1 ± 0.16 µmol/g. tissue) compared with the saline control. Rats treated with malathion/propranolol or malathion/bisoprolol given alone or with atropine exhibited significantly lower MDA and nitric oxide values and higher GSH values compared with the malathion control group. In contrast, treatment with terbutaline either alone or combined with atropine had no significant effect on MDA, nitric oxide or GSH in brain of malathione intoxicated rats (Figure 1 & Table 1).

Table 1 Effect of propranolol, bisoprolol or terbutaline on brain malondialdehyde, reduced glutathione and nitric oxide in rats treated with malathion.

	MDA (nmol/g.tissue)	GSH (µmol/g.tissue)	NO (µmol/g.tissue)
Saline	17.8 ± 0.9	3. 1 ± 0.16	22.3 ± 1.7
Malathion	29.5 ± 1.4*	1.35 ± 0.07*	45.1 ± 3.1*
+ Propranolol	23.1 ± 1.1^{*+}	1.93 ± 0.08^{*+}	33.6 ± 1.2^{*+}
+ Propranolol + atropine	22.0 ± 1.6^{*+}	2.0 ± 0.11^{*+}	32.1 ± 1.82^{*+}
+ Bisoprolol	21.4 ± 1.0^{*+}	2.15 ± 0.10*	36.2 ± 1.3^{*+}
+ Bisoprolol + atropine	21.0 ± 1.3^{*+}	2.33 ± 0.15^{*+}	33.0 ± 1.5^{*+}
+ Terbutaline	34.8 ± 1.9$^{*\#}$	1.20 ± 0.06$^{*\#}$	52.2 ± 3.0$^{*\#}$
+ Terbutaline + atropine	31.6 ± 1.7$^{*\#}$	1.53 ± 0.04$^{*\#}$	47.1 ± 2.6$^{*\#}$

$^{*}p < 0.05$ *vs.* saline $^{+}p < 0.05$ *vs.* malathion control. $^{\#}p < 0.05$ *vs.* propranolol or bisoprolol groups

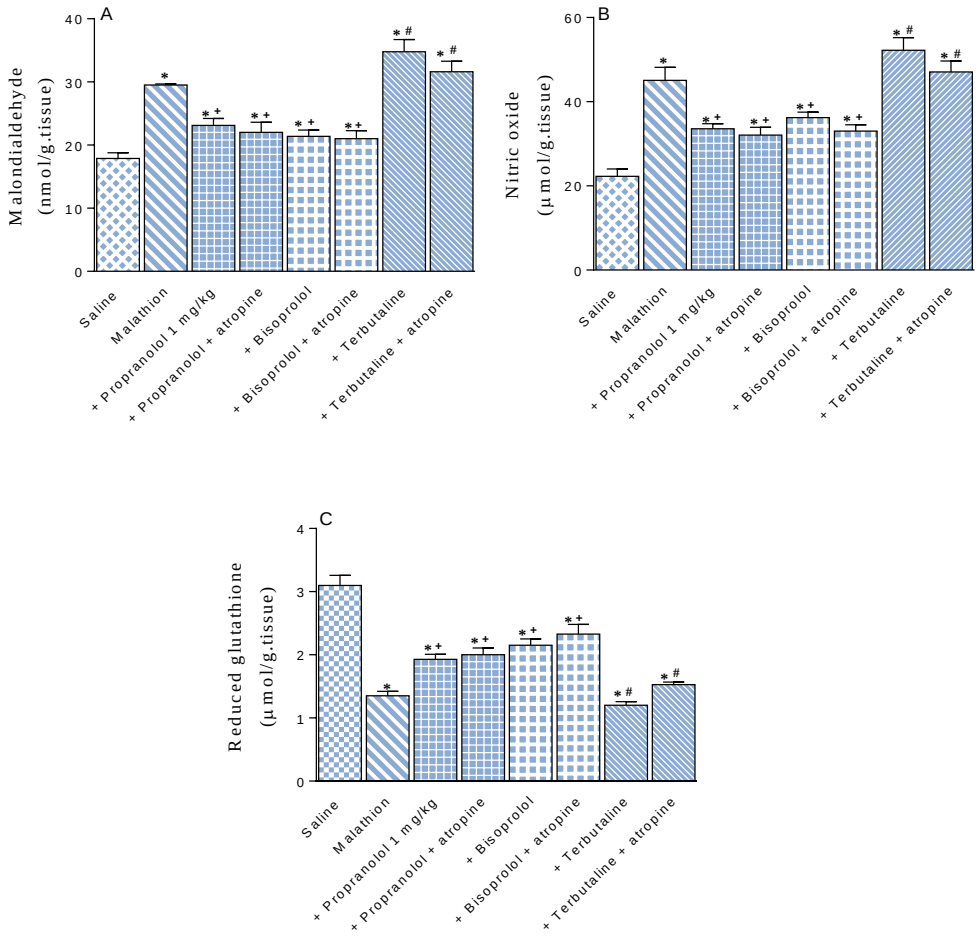

Figure 1 Effect of propranolol, bisoprolol or terbutaline on brain oxidative stress in malathion intoxicated rats. *p < 0.05 *vs.* saline +p < 0.05 *vs.* malathion control. #p < 0.05 *vs.* propranolol or bisoprolol groups

Histopathological results

Microscopic examination of brain tissue from vehicle treated rats showed the normal histologic appearance of cerebral cortex, with an inner most granular layer, composed of many small cells, the central layer or Purkinje cell layer, formed of large flask-shaped cells and an outermost molecular layer that contained few small nerve cells as well as many unmyelinated nerve fibers (Figure 2A & B). Sections from malathion-treated rats showed multiple focal and homogenous deeply eosinophilic plaques of varying sizes and shapes as well as gliosis in the cerebral cortex. The neurons showed intracytoplasmic proliferation of filaments forming visible neurofibrillary tangles and area of neuronal necrosis was seen. There was evidence of pyknotic nuclei and chromatin condensation. Perineuronal vacuolation, spongiform degeneration. Some apoptotic neurons were observed (Figure 2 C, D & E).

In rats that received malathion and the non-selective β adrenoceptor blocker propranolol, most neuronal cells appeared normal but some pathological changes were present in the form of neurofibrillary tangles and thrombotic vessels (vessels with membrane bound vacuoles) (Figure 3A). The cortex of rats treated with malathion together with propranolol and the anticholinergic drug atropine showed some improvements in the histopathological changes, where some neurons appeared normal and there was disappearance of amyloid plaques and neurofibrillary tangles, although some degenerated neurons and perineuronal vacuolations were observed (Figure 3B). Concerning rats treated with malathion and the selective β_1 adrenoceptor antagonist bisoprolol, brain sections showed more improvement of the histopathological changes, in which most neurons appeared normal, although congestion of blood vessels was still present (Figure 3C). Sections from rats treated with malathion and bisoprolol and atropine showed the nearly normal morphological appearance of the cerebral cortex, although congested blood vessel was observed (Figure 3D).

On the other hand, sections from the cortex of rats treated with malathion and terbutaline exhibited some pathological change in the form of deformity of the normal architecture of cerebral cortex. The pyramidal cells were very small in size. Evidence of vacuolar degeneration was commonly observed in neurons, karyorrhexis in other neurons and congested cerebral blood vessels

were also seen (Figure 4A & B). The sections from rats treated with malathion, terbutaline and atropine exhibited some improvement in histological changes in that some neurons appeared normal but others were degenerated (karyorrhexis). Apoptotic neurons were still present as well as gliosis. Vacuolar degeneration was clearly observed (Figure 4C).

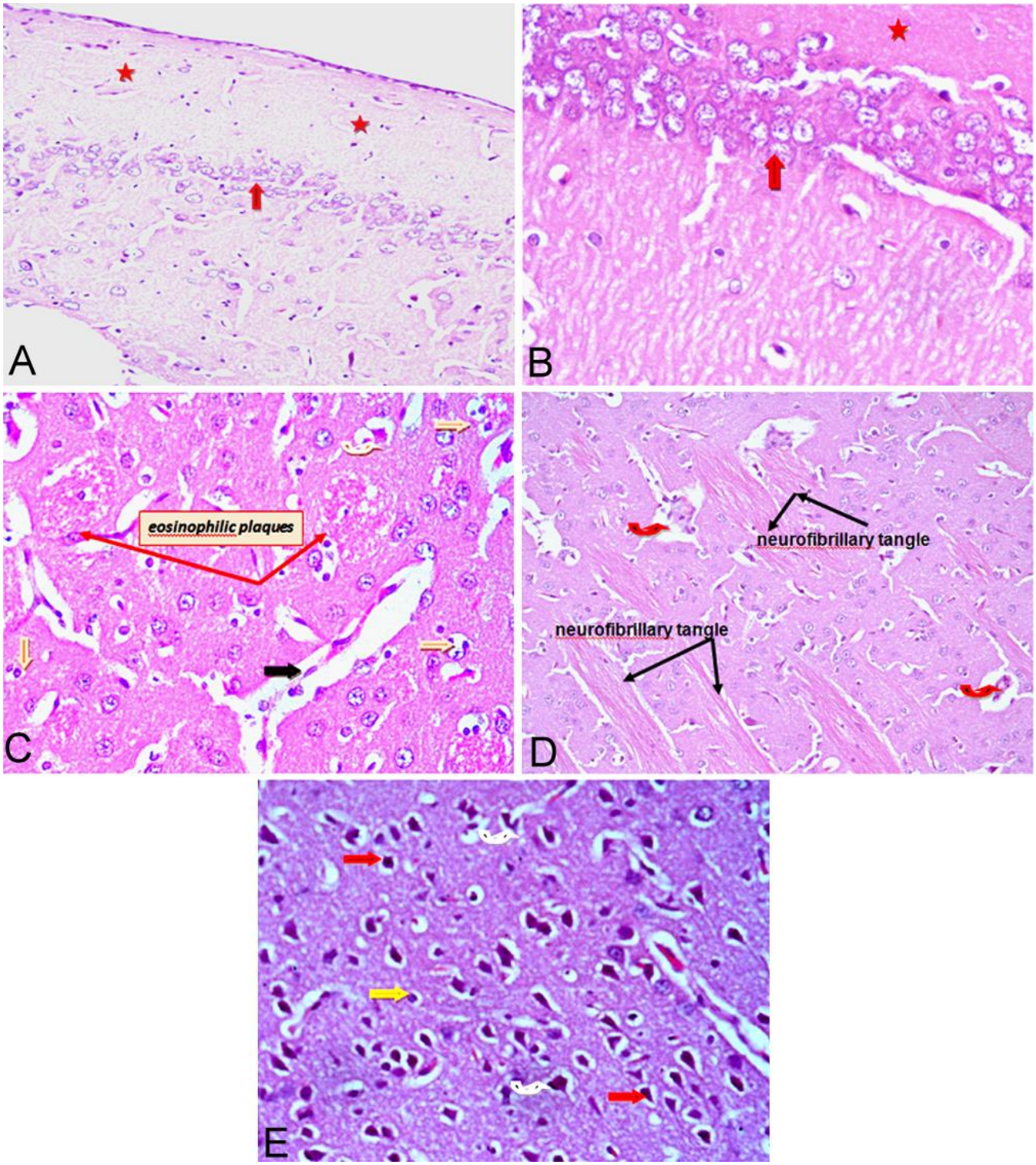

Figure 2 Representative photomicrographs of sections of brain tissue from (A) Vehicle control rat showing the normal histology of the cerebral cortex (Hx & E x200). (B) High power field of the pervious figure (HX & E x400). (C) Malathion only-treated rat showing many homogenous and deeply eosinophilic plaques of variable sizes and shapes (red arrow), congested blood vessel (black arrow) and gliosis (light orange arrow), karyorrhexis of some neurons (white arrow) (Hx & E x400). (D) Malathion only (another filed) showing neurofibrilly tangle (black arrow), neuronal necrosis areas (red arrow) (Hx & E x200). (E) Malathion only (another filed) showing some neurons appeared degenerated (yellow arrow), others apoptotic (white arrow). Perineuronal vacuolation and dark neurons (pyknotic) were observed (red arrow) (Hx & E x200).

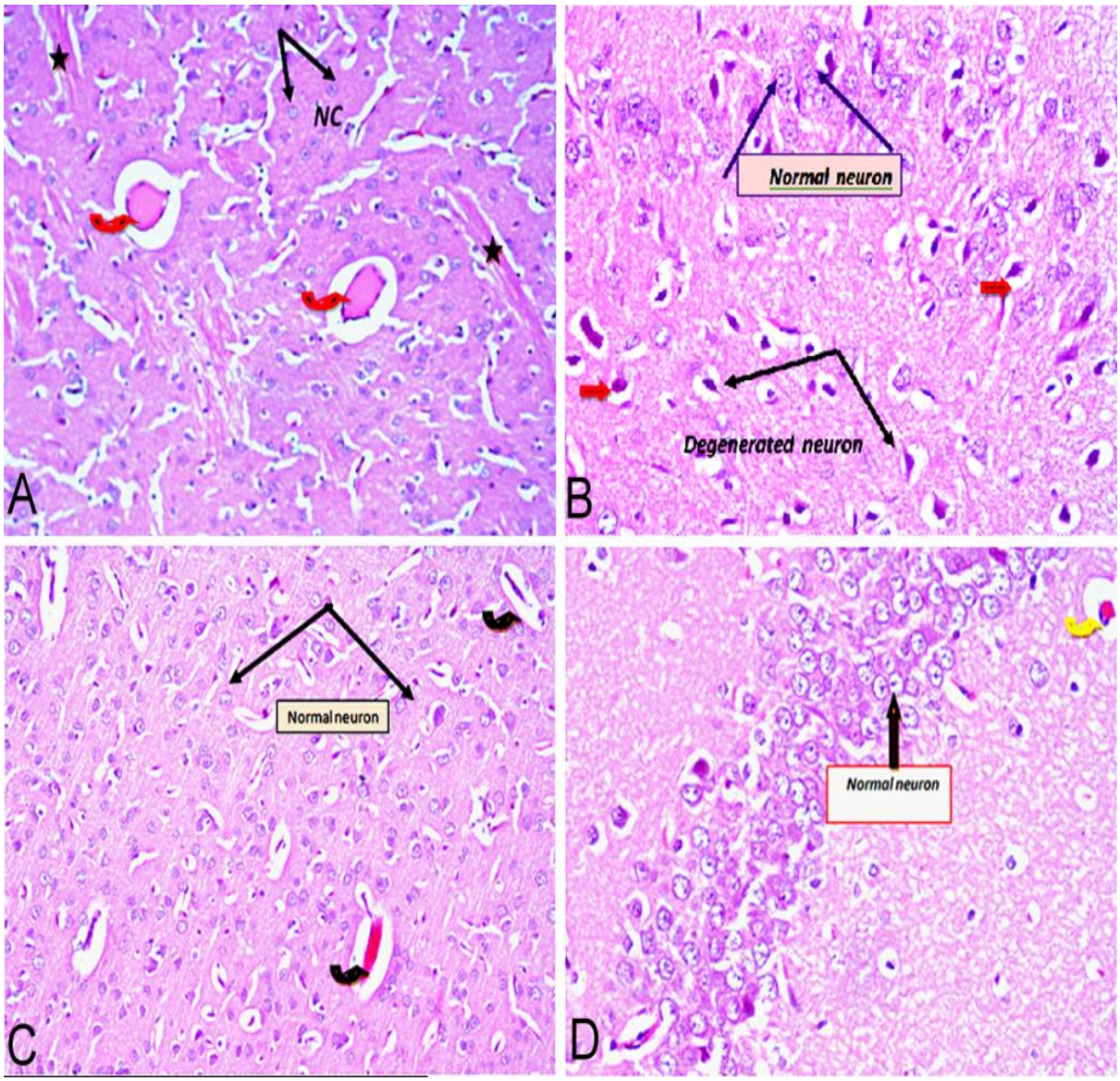

Figure 3 Representative photomicrographs of sections from the brain of rats after treatment with (A) Malathion and propranolol showing most neural cells appeared normal (NC), neurofibrilly tangle (star), thrombotic vessels show a vessel with membrane bound vacuoles (red arrow) (Hx & E x200). (B) Malathion, propranolol and atropine showing some neurons appeared normal and prominent nuclei others are degenerated, perineuronal vacuolation was observed (red arrow) (Hx & E x400). (C) Malathion and bisoprolol showing most of neurons appeared normal shape although congested cerebral blood vessel still present (black arrow) (Hx &E x100). (D) Malathion, bisoprolol and atropine showing nearly normal of morphological appearances of cerebral cortex and normal thickness of granular layer but cerebral blood vessel was observed (yellow arrow) (Hx & E x400).

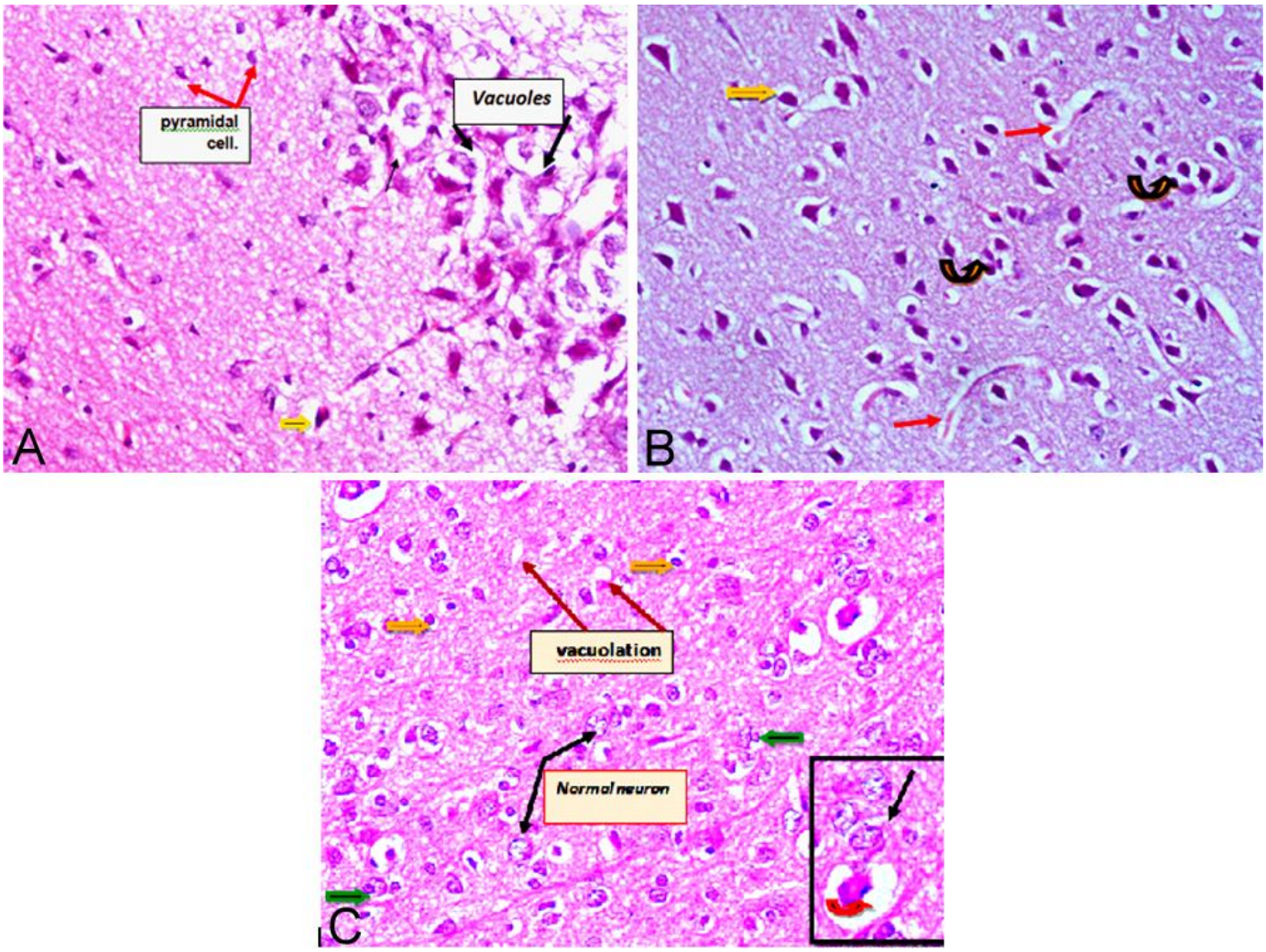

Figure 4 Representative photomicrographs of brain sections from rats after treatment with (A) Malathion and terbutaline showing spongiform degeneration and deformity of the normal structure, vacuoles due to cytoplasmic destruction (black arrow), very small pyramidal cells (red arrow) and some apoptotic neurons (yellow arrow) (Hx & E x400). (B) Malathion and terbutaline (another filed) showing vacuolar degeneration is commonly observed in neurons (orange arrow), other are karyorrhexis (black arrow) and congested cerebral blood vessel (red arrow) (Hx & E x200). (C) Malathion, terbutaline and atropine showing some neurons appeared normal (black arrow), others karyorrhexis (green arrow), some apoptotic neurons (red arrow), gliosis (orange arrow), perineuronal vacuolation was observed (dark red arrow) (Hx & Ex200).

4. DISCUSSION

In the present study we investigated the effect of drugs acting on β-adrenergic receptors on the development of brain oxidative stress and neuronal degeneration in rats intoxicated with the organophosphorus insecticide malathion. These drugs are likely to be used in the pharmacological management of acute intoxication with organophosphorus insecticides. Our findings indicated that the concurrent administration of β-adrenoceptor antagonists propranolol and bisoprolol conferred neuroprotection against malathion neurotoxicity, reducing oxidative stress and histopathological changes. In contrast, evidence of neurodegeneration was present after the administration of the β_2-adrenoceptor agonist terbutaline.

Malathion is a commonly used organophosphate insecticide worldwide and exposure to this agent has been associated with both acute and chronic central nervous system toxicities (Jokanovic and Kosanovic, 2010). We showed that acute exposure to malathion results in severe neurodegenerative changes in the rat brain in the form of deeply eosinophilic plaques, neuronal apoptosis, necrosis intracytoplasmic neurofibrillary tangles and gliosis. These findings are in agreement with our earlier observations (Abdel-Salam et al., 2018a). Rats treated with malathion also showed marked atrophy, proliferation of glial cells and microglial nodules (Abdel-Salam et al., 2018b). The mechanism that underlies the neurotoxic effects of malathion is largely thought to involve free radical-mediated oxidative neuronal injury. Rats exposed to malathion have shown an increase in brain lipid peroxides accompanied with decreased reduced glutathione content (Brocardo et al., 2005; Delgado et al., 2006; Abdel-Salam et al.,

2016, 2018a, b), total antioxidant capacity and reduced activities of the antioxidant enzymes glutathione reductase (Trevisan et al., 2008), glutathione peroxidase and paraoxonase-1 (Abdel-Salam et al., 2016).

In human erythrocytes in vitro, malathion increased lipid peroxidation and decreased the activities superoxide dismutase, glutathione peroxidase and catalase enzymes (Durak et al., 2009). These findings are indicative of increased formation of oxidant free radicals and the consequent consumption of cellular antioxidants. In support of this notion are studies which showed that malathion increased the production of superoxide anion ($O_2^{\cdot-}$) by malathion (Rieger et al., 2017). Malathion is also capable of increasing nitric oxide generation, likely via an increase in inducible nitric oxide synthase expression (Abdel-Salam et al., 2016, 2017) from activated glial cells (Abdel-Salam et al., 2018c). The excess nitric oxide can then react with $O_2^{\cdot-}$ generating peroxynitrite (ONOO-) or with O_2 resulting in nitrogen oxides, capable of oxidizing cellular biomolecules and causing mitochondrial damage and subsequent energy failure (Moncada and Bolanos, 2006; Pacher et al., 2007). In this context, studies have shown the development of mitochondria dysfunction by malathion and other organophosphates as well (Delgado et al., 2006; Kaur et al., 2007; Karami-Mohajeri et al., 2014).

The effect of drugs acting on adrenergic receptors in the modulation of neuronal damage is a focus of research (Amory et al., 2002; Tran et al., 2008; Michalovicz et al., 2021). β-adrenoceptors are expressed by fibrous and protoplasmic astrocytes, the major population of glial cells in brain (Salm and McCarthy, 1989) and thus may be involved in the modulation of their actions. Thus, propranolol was shown to decrease the hypertrophy of astrocytes (glial fibrillary acidic protein immunostaining) in the ventral horn of injured spinal cord and to decrease β-adrenoceptor density in the glial scar region (Sutin and Griffith, 1993). In vitro, Arai et al., (2003) showed that adrenergic antagonists as well as agonists eg., salbutamol and isoproterenol were able to suppress the release of lactic acid dehydrogenase and nitric oxide from retinal neurons following stimulation with lipopolysaccharide. There is evidence, however, suggestive of a neuroprotective effect for β-adrenoceptor antagonists in traumatic or toxic models of brain injury (Michalovicz et al., 2021; Monai et al., 2019) as well as in patients with head injury (Inaba et al., 2008) or after cardiac surgery (Amory et al., 2002). Thus, β-adrenoceptor antagonists timolol, betaxolol, nipradilol were reported to protect rat fetal ganglion cells in culture from hypoxic injury, resulting in an increase in their viability (Chen et al., 2004).

In addition, the selective β_1-adrenoceptor antagonists esmolol or landiolol were shown to decrease infarct size and neurologic squeal in rats with transient occlusion of their middle cerebral artery (Goyagi et al., 2010). In addition, Michalovicz et al., (2021) reported a neuroprotective effect for propranolol in a model of Gulf war illness in which mice were subjected to sarin gas surrogate, corticosterone and lipopolysaccaride. Tumour necrosis factor alpha (TNF-alpha), interleukin-1β (IL-1β) and IL-6 mRNA expression in brain were inhibited by the β_2-adrenoceptor antagonist. Beta-blocker therapy was also found to decrease serum levels of IL-10 and TNF-alpha in patients with dilated cardiomyopathy (Ohtsuka et al., 2001). Moreover, cardiac surgery patients who received β_2-blockade perioperatively showed decreased incidence of neurologic complications eg., stroke or transient iscaemic attack following surgery (Amory et al., 2002). β_2-adrenoceptor blockade may thus confer neuroprotection by decreasing proinflammatory cytokines levels. A vasodilator effect for blockade of β_1-adrenergic receptors may also be involved in their protective effect during ischaemia/reperfusion brain injury (Asano et al., 2020).

Our present findings lend support to the notion that blockade of β-adrenoceptors may confer neuroprotection in brain of malathion-treated rats. In contrast, the stimulation of β_2-adrenoceptors failed to prevent malathion neurotoxicity. Other researchers found that terbutaline which can cross blood brain barrier induced neuronal injury in neonatal rats, decreasing pyramidal cells in cerebral cortex and Purkinji cells in cerebellum besides causing marked gliosis (Rhodes et al., 2004). In vitro, β_2-adrenergic agonists were reported to enhance IL-8 release by human monocytes stimulated with lipopolysaccharide (Kavelaars et al., 1997). This effect may provide a plausible explanation for the failure of terbutaline to reduce the malathion-induced neuronal damage.

5. CONCLUSIONS

The results presented in this study suggest a neuroprotective effect for the β-adrenoceptor antagonists propranolol and bisoprolol in malathion-induced acute brain injury. The use of the selective β_2-adrenoceptor agonist terbutaline failed to reduce brain damage. Results are in favor of β-adrenoceptor blockade in malathion acute neurotoxicity.

Author contribution

OMEAS and AAS conducted the research and biochemical studies, FAM performed the histopathology and its interpretation, OMEAS wrote and prepared the manuscript, OMEAS and AAS and FAM approved the final version of the manuscript.

Ethical approval

The study procedures were performed according to the regulations by the Institute Ethics Committee and followed the Guide for Care and Use of Laboratory Animals by the US National Institutes of Health (Publication No. 85-23, revised 1996).

Informed consent

Not applicable.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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The study has not received any external funding.

Data and materials availability

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

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