Methylene Blue Protects Against Acute Ethanol-Induced Oxidative Stress and Organ Damage

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ABSTRACT

Ethanol (EtOH) intake is an important global health problem which affects many organs such as the brain, liver and stomach. The aim of the study was to examine the effect of the redox dye methylene blue (MethyB) on oxidative stress and histologic damage to the liver, gastric mucosa, and brain induced high dose ethanol (EtOH). Male rats were treated with EtOH (2 ml/rat, 96%) via intragastric route (for two consecutive days). MethyB (20 or 40 mg/kg, intraperitoneally) was given immediately after EtOH administration. The control group received saline. Rats were euthanized three hours after the last treatment. Brain and liver levels of malondialdehyde (MDA), reduced glutathione (GSH), and paraoxonase-1 (PON-1) as well as brain 5-lipoxygenase (5-LOX) and butyrylcholinesterase (BChE) were determined. Histopathological assessment of brain, liver and gastric damage was done. Results indicated that compared to saline treated animals, EtOH caused significant increase in MDA, along with decreased GSH and PON-1 activity in brain and liver. Additionally, it significantly increased 5-LOX and decreased brain BChE activity. The EtOH group showed the presence of dead and red neurons, and damage of glial cells. The liver exhibited vacuolar degeneration, apoptotic hepatocytes and foci of necrosis. The gastric mucosa showed areas of tissue damage, mucosal atrophy, and loss of normal architecture of glandular cells. The EtOH induced biochemical and histopathological alterations were alleviated after treatment with MethyB at a dose-dependent manner. These results demonstrate that MethyB is able to protect against from acute effects of EtOH on brain, liver and gastric tissue via an antioxidant action. MethyB might be of value in reducing tissue injury in acute EtOH intoxication.

1. Introduction

Ethanol (EtOH) intake either acute or chronic constitutes an important global health problem with significant economic and social consequences and could be the gate for more serious health hazards eg., accidents, increased susceptibility to infection or exacerbation of underlying pathology (Vonghia et al., 2008). The intake of excessive amounts of EtOH is associated with health problems that involve many organs such as the brain, liver and stomach. Chronic intake results in volume reduction in cortical and subcortical brain regions (Sullivan and Pfefferbaum, 2005) while

animal studies indicate that bing intake or alcohol intoxication causes neuronal death (Obernier et al., 2003), cytotoxic edema and changes in cerebral metabolites (Liu et al., 2014). EtOH at high doses has profound effect on transmitter release suppressing evoked dopamine release in caudate-putamen in ambulatory rats and in striatal slices in vitro (Budygin et al., 2001). It also reduces local cerebral glucose utilization and thus impairs functional brain activity (Grunwald et al., 1993). Alcoholic liver disease is also the major cause of liver disease in Western countries. The spectrum of liver disease ranges from steatosis, steatohepatitis and liver cirrhosis with its complications of liver failure and ascitis, necessitating liver transplantation (Louvet and Mathurin, 2015). In man, EtOH causes gastric mucosal injury, increases gastric mucosal permeability (Gordon et al., 1995). Endoscopic gastric hemorrhagic damage in body and antrum is seen thirty min after 40% ethanol administration in healthy volunteers (Iaquinto et al., 2003). The intake of alcohol, even in modest amounts, carries the risk of major upper gastrointestinal bleeding and increases the propensity of bleeding associated with regular use of NSAIDs and/or aspirin (Strate et al., 2016).

Oxidative stress is strongly implicated in the pathogenesis of EtOH cellular injury (Comporti et al., 2010; Ceni et al., 2014). The liver is the major site for EtOH metabolism and the organ most affected by EtOH-induced oxidative stress. EtOH is oxidized and transformed into acetaldehyde and then acetate in hepatocytes by the enzymes alcohol dehydrogenase and aldehyde dehydrogenase with coenzyme nicotinamide adenine dinucleotide (NAD+) as a cofactor (Lieber, 2004; Comporti et al., 2010). These reactions result in accumulation of NADH and its subsequent reoxidation to NAD+ by the mitochondrial electron transfer chain with an increase in the formation of reactive oxygen species (ROS). The latter are also released by the activity of the microsomal ethanol oxidizing system and its key enzyme cytochrome, P4502E1 (CYP2E1). Moreover, acetaldehyde, the major metabolite of ethanol, because of its electrophilic nature, can bind and form covalent chemical adducts with proteins, lipids and DNA. The increase in the NADH/NAD ratio also favors the synthesis of fatty acids and deposition of triglycerides in the liver, the principal site for EtOH metabolism (Ceni et al., 2014; Louvet and Mathurin, 2015). It has also been shown that the metabolism of ethanol in primary human neurons by alcohol dehydrogenase or cytochrome P450-2E1 (CYP2E1) generates ROS and nitric oxide due to activation of NADPH/xanthine oxidase and nitric oxide synthase by acetaldehyde accompanied by increased lipid peroxidation and reduced neuronal viability (Haorah et al., 2008). In brain tissue, EtOH oxidation occurs both in the cortex and the subcortex (Wang et al., 2013).

Methylthionine chloride (methylene blue; MethyB) is a synthetic redox dye with important clinical applications such as treatment of cyanide poisoning and methemoglobinaemia (Clifton, 2003; Bradberry, 2003). Other indications are vasoplegic shock (Manghelli et al., 2015) and encephalopathy caused by the chemotherapeutic agent ifosfamide (Turner et al., 2003). MethyB came into attention in the last years in view of animal studies suggesting a neuroprotective effect. MethyB reduced lesion volume, improved motor scores and decreased the number of dark stained Nissl cells and Fluoro-Jade-positive cells in rats with mild traumatic brain injury (Talley Watts et al., 2014). It also reduced soluble Aβ levels and rescued early cognitive deficits in 3xTg-AD mice model of Alzheimer's disease (Medina et al., 2011) and delayed motor deficits in R6/2 Huntington's disease mice model (Sontag et al., 2012). MethyB protected against nigrostriatal damage caused by rotenone (Abdel-Salam et al., 2014), and brain damage following acute toluene (Abdel-Salam et al., 2016a) or the insecticide malathion (Abdel-Salam et al., 2016b) toxicity in the rat via antioxidant action. Moreover, the dye displayed hepatic and gastric protective properties, protecting liver damage caused by bile duct ligation (Aksu et al., 2010), the herbicide paraquat (Chen et al., 2015), preventing vacuolar degeneration and inflammatory cell infiltration in the liver of lipopolysaccharide-treated rats (Abdel-Salam et al., 2018) and protecting against gastric mucosal damage caused by acidified Na+-taurocholate (Abdel-Salam et al., 2019) or the non-steroidal anti-inflammatory drug indomethacin (Morsy et al., 2019). In this study, the effect of MethyB on oxidative stress and histologic damage to the liver, gastric mucosa, and brain induced high dose EtOH was investigated.

2. Material and Methods

2.1. Animals

Male Sprague-Dawley strain rats (150-160 g: National Research Centre, Cairo) were used in the experiments. Rats were housed under a standard 12-h light/dark cycle and had free access to food and water. The experiments were conducted in accordance with the ethical guidelines for care, use and handling laboratory animals by the Ethics Committee of the NRC and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.2. Experimental design

Rats were randomly allocated into four equal groups (6 rats each). Rats were given EtOH (2 ml/rat, 96%) via intragastric route and immediately thereafter treated with either saline or MethyB at doses of 20 or 40 mg/kg for two consecutive days. The control group received a comparable volume of saline. Rats were euthanized three hours after the last treatment by cervical decapitation under light ether anaesthesia. The brain and liver of each rat were then 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.

2.3. Biochemical analyses

2.3.1. Determination of lipid peroxidation

Malondialdehyde (MDA), an end product of lipid peroxidation was measured according to the method described by Nair and Turne (1984). Thiobarbituric acid reactive substances (TBAS) react with thiobarbituric acid forming TBA-MDA adduct and the absorbance is read at 532 nm using spectrophotometer.

2.3.2. Determination of reduced glutathione

Reduced glutathione (GSH) was determined in homogenates according to Ellman (1959). Briefly, DTNB (5,5'-dithiobis (2-nitrobenzoic acid) or Ellman´s reagent is reduced by the free sulfhydryl group on GSH molecule to generate 5-thio-2-nitrobenzoic acid which has yellow color and can be determined by reading absorbance at 412 nm.

2.3.3. Determination of paraoxonase-1

The arylesterase activity of PON-1 was determined by a colorimetric method using phenyl acetate as a substrate. In this assay, PON-1 catalyzes the cleavage of phenyl acetate resulting in phenol formation. The rate of formation of phenol was measured by monitoring the increase in absorbance at 270 nm at 25°C. The working mix consisted of 20 mM Tris/HCl buffer, pH 8.0, containing 1 mM $CaCl_2$ and 4 mM phenyl acetate as the substrate. Samples diluted 1:3 in buffer were added to the above mix and the changes in absorbance were recorded following a 20 s lag time. One unit of arylesterase activity is equal to 1 µmole of phenol formed per min. The PON-1 activity is expressed in kU/l, based on the extinction coefficient of phenol of 1310 $M^{-1}cm^{-1}$. Blank samples containing water were used to correct for the spontaneous hydrolysis of phenyl acetate (Eckerson et al., 1983).

2.3.4 Determination of 5-lipoxygenase

5-lipoxygenase was determined using a double-antibody sandwich enzyme-linked immunosorbent assay (Rat (5-LO/LOX) ELISA kit) from Shanghai Sunred Biological Technology Co., Ltd, Jufengyuan Road, Baoshan District, Shanghai.

2.3.5. Determination of cholinesterase activity

Butyrylcholinesterase (BChE) activity was measured with 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 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) to produced a yellow chromophore which then could be quantified using spectrophotometer (Ellman et al., 1961).

2.4. Histopathological studies

The liver, brain and stomach of different groups were removed and fixed in 10% formol saline. 5 μm thick paraffin sections were stained with haematoxlin and eosin (Drury and Wallington, 1980) and investigated by light microscope. Images were examined and photographed under a digital camera (Microscope Digital Camera DP70, Tokyo) and processed using Adobe Photoshop version 8.0.

2.5. Statistical analysis

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

3. Results

3.1. Biochemical results

3.1.1 Effect of MethyB on brain oxidative stress in EtOH-treated rats

EtOH administration caused significantly increased brain MDA level by 29.7% compared with the saline control group (25.0 ± 1.44 vs. 19.28 ± 0.89 nmol/g.tissue). Meanwhile, significant decrease in GSH by 65.1% (1.29 ± 0.08 vs. 3.70 ± 0.25 μmol/g.tissue) was observed in the brain of EtOH-treated rats. Treatment with 20 mg/kg of MethyB had no significant effect on MDA level. The higher dose of 40 mg/kg, however, reduced MDA by 16.8% (20.82 ± 0.47 vs. 25.0 ± 1.44 nmol/g.tissue). MethyB given at 20 and 40 mg/kg significant increase in GSH by 53.5% and 140%, respectively as compared to the EtOH control (1.98 ± 0.08, 3.1 ± 0.15 vs. 1.29 ± 0.07 μmol/g.tissue) (Fig. 1).

3.1.2. Effect of MethyB on brain PON-1 in EtOH-treated rats

Brain PON-1 activity was decreased by 64.7% after EtOH administration as compared with the saline control value (4.19 ±0.15 vs. 11.86 ± 0.54 kU/l). PON-1 activity increased by 33.4% and 76.1% after MethyB at 20 and 40 mg/kg, respectively (5.95 ± 0.31 and 7.38 ± 0.40 vs. 4.19 ±0.15 kU/l) (Fig. 1).

3.1.3. Effect of MethyB on brain 5-LOX in EtOH-treated rats

Significant increase in brain 5-LOX by 64.7% was observed after EtOH administration as compared with the saline control (68.88 ± 1.77 vs. 54.25 ± 0.85 ng/ml). MethyB given at 40 mg/kg resulted in significant decrease in 5-LOX by 13.9% (59.30 ± 1.40 vs. 68.88 ± 1.77 ng/ml) (Fig. 2).

3.1.4. Effect of MethyB on brain BChE in EtOH-treated rats

In EtOH-treated rats, brain BChE showed significant inhibition by 72.0% compared with the saline group (34.1 ± 3.8 vs. 122.1 ± 8.4 U/l). MethyB given at 20 or 40 mg/kg resulted in significant increase in BChE almost to its saline control value (117.3 ± 5.6 and 199.2 ± 4.1 vs. 122.1 ± 8.4 U/l) (Fig. 2).

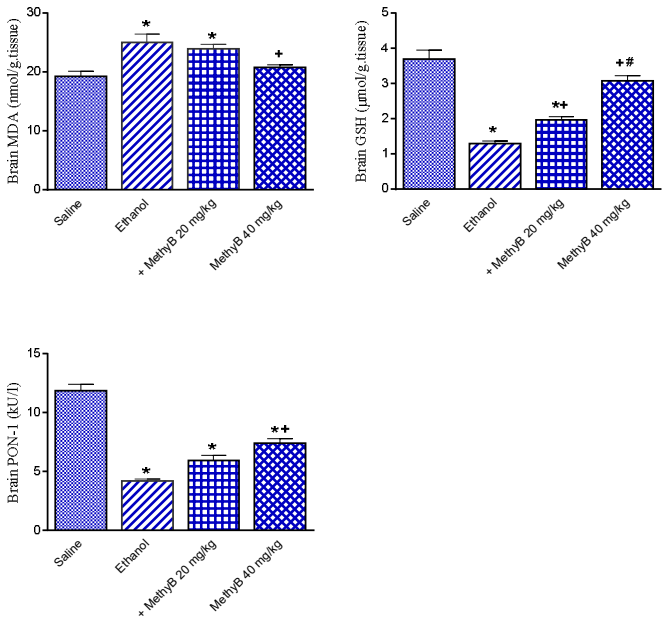

Fig 1. Effects of MethyB on brain malondialdehyde (MDA), reduced glutathione (GSH) and Paraoxonase-1 (PON-1) in EtOH-treated rats
*: p<0.05 vs. saline control group. +: p<0.05 vs. EtOH control. #: p<0.05 vs. MethyB 20 mg/kg.

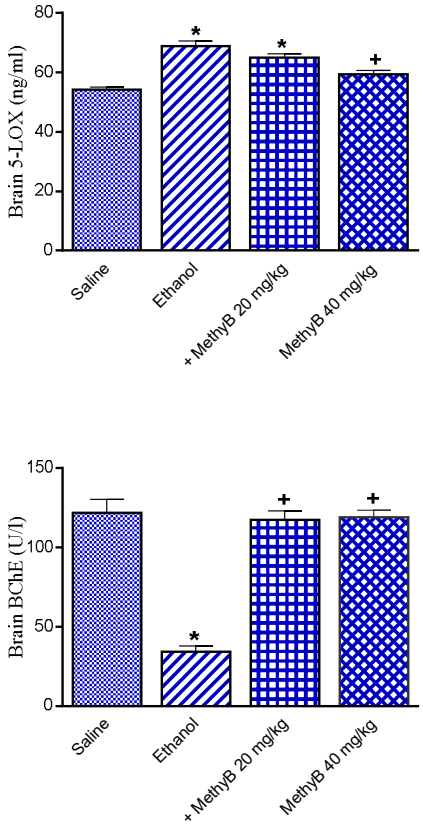

Fig 2. Effects of MethyB on 5-lipoxygenase (5-LOX) and butyrylcholinesterase (BChE) in brain of EtOH-treated rats
*: p<0.05 vs. saline control group. +: p<0.05 vs. EtOH control.

3.1.5. Effect of MethyB on liver oxidative stress in EtOH-treated rats

In rats treated with EtOH there was a significant increase in liver MDA content by 69.7% compared with the saline control group (45.47 ± 1.56 vs. 28.60 ± 1.67 nmol/g.tissue). Meanwhile, significant decrease in GSH by 59.5% (2.41 ± 0.06 vs. 5.95 ± 0.30 µmol/g.tissue) was observed in the liver after EtOH treatment. Treatment with MethyB at 20 or 40 mg/kg resulted in significant decrease in live MDA by 34.4% and 39.5% (29.85 ± 0.70 and 27.52 ± 1.20 vs. 45.47 ± 1.56 nmol/g.tissue). MethyB given at 20 and 40 mg/kg significant increase in GSH by 39.8% and 202.0%, respectively as compared to the EtOH control (3.37 ± 0.38 and 5.11 ± 0.19 vs. 2.41 ± 0.06 µmol/g.tissue) (Fig. 3).

3.1.6. Effect of MethyB on liver PON-1 in EtOH-treated rats

In EtOH-treated rats, PON-1 activity in the liver was significantly decreased by 33.4% compared with the saline group (13.52 ± 0.58 vs. 20.30 ± 0.69 kU/l). MethyB given at 40 mg/kg resulted in significant increase in PON-1 activity to its saline control value (Fig. 3).

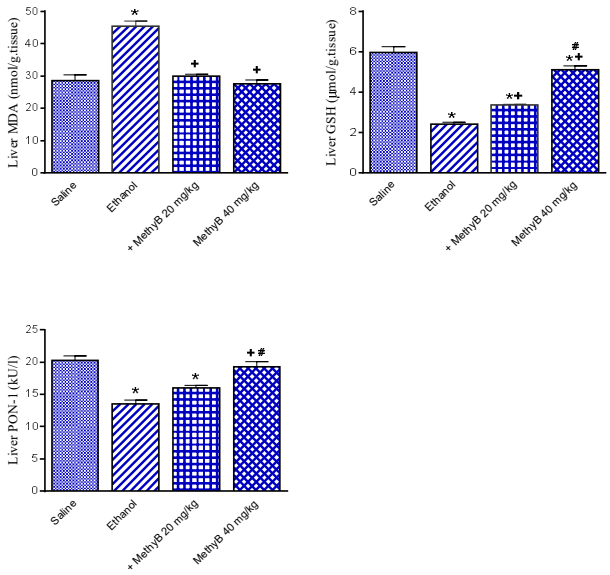

Fig 3. Effects of MethyB on liver malondialdehyde (MDA), reduced glutathione (GSH) and Paraoxonase-1 (PON-1) in EtOH-treated rats

*: p<0.05 vs. saline control group. +: p<0.05 vs. EtOH control. #: p<0.05 vs. MethyB 20 mg/kg.

3.2. Histopathological results

3.2.1. Brain histopathological changes

Microscopic examination for cerebral cortex sections from normal rat shows the normal cortical morphology and neuron structure (Fig. 4A). Ethanol induced lethally injured neurons, abnormal neurons in the form of dead and red neuron, the red coloration is due to pyknosis, apoptosis or degradation of nucleolus, loss of Nissl bodies and damage of glial cells. The pyramidal cells were with pyknotic nuclei and increase in perivascular space with hemorrhage (Fig. 4B & C). Rats treated with ethanol and MethyB at a dose of 20 mg/kg showed some improvement in pathological changes in the form of some neurons that appeared normal with prominent nucleoli, but some degenerated neurons and red neurons were still present. The neuron is surrounded by connective tissue fiber (Fig. 4D). Sections of cerebral cortex of rats treated with ethanol along with MethyB at dose of 40 mg/kg showed that some cortical neurons appeared normal and others with pyknotic neurons. Neuroglia cells are also seen (Fig. 4E).

3.2.2. Liver histopathological changes

The liver of control rats revealed the normal characteristic hepatic architecture (Fig. 5A). Ethanol induced hazardous effects in the form of vacuolar degeneration in the cytoplasm of hepatocytes. Some cells showed multinucleated cells while others showed apoptosis and foci of necrosis. Dilatation and congestion of portal vein, thickening of wall besides the existence of lymphocytic infiltration .Foci of necrosis and minimal fibrosis around portal vein were seen (Figs. 5B, C & D). Liver sections of rats treated with ethanol along with MethyB at dose of 20 mg/kg showed improvement in pathological changes although dilated, congested portal vein and portal tract and aggregation of lymphocytic infiltration around were present (Fig. 5E). The liver of rats treated with ethanol and MethyB at dose of 40 mg/kg showed more improvement in pathological changes, hepatocytes appeared normal but congestion of central vein was also observed (Fig. 5F).

3.2.3. Histological changes of stomach

Sections of control stomach showed normal architecture; the glandular part of stomach with normal gastric pits and intact tubular epithelium (Fig. 6A). Ethanol induced superficial erosions, vacuolation in the lining epithelium of gastric glands. Area of tissue damage and mucosal atrophy and fibrosis could be seen. There were distorted arrangement and loss of normal architecture of glandular cells in addition to inflammatory cellular infiltrate in mucosa and submucosa (Figs. 6B & C). Sections of the stomach of rats treated with ethanol and MethyB at dose of 20 mg/kg showed some degenerative changes in the form of exfoliation of the focal area of superficial epithelial cells, inflammatory cellular infiltrate and hemorrhage but most gastric glands appeared normal (Fig. 6D). Stomachs of rats treated with ethanol and MethyB at dose of 40 mg/kg showed normal architecture although few inflammatory cells were still present (Fig. 6E).

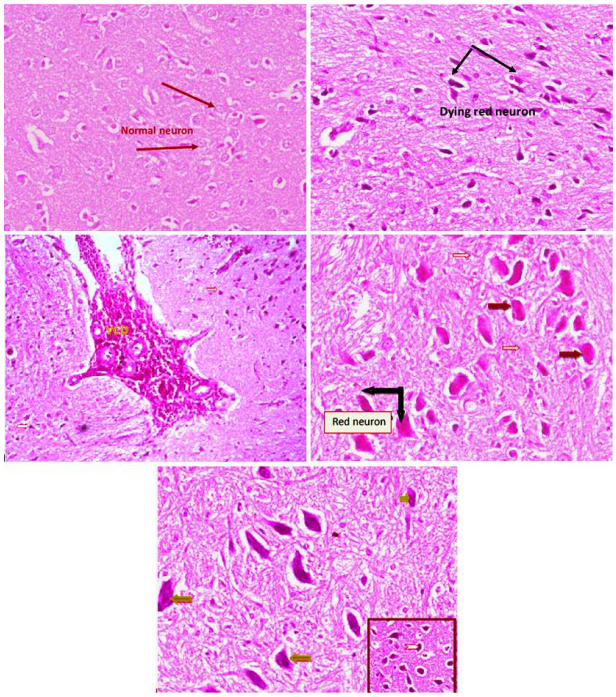

Fig 4. Representative photomicrographs of the cerebral cortex after treatment with**: (A)** Vehicle shows normal histological structure (H & E x 400). **(B)** EtOH only shows most of red neuron are dying (black arrow), vascular proliferation (Hx&E x200). **(C)**: EtOH only shows pyramidal cells with pyknotic nuclei (white arrow) and dilated perivascular space with hemorrhage (VCD) (Hx&E x100). **(D)**: EtOH + MethyB 20 mg/kg shows some neurons appeared normal (red arrow), red neurons still present (black arrow). Neurons are surrounded by connective tissue fiber (white arrow) (Hx&E x400). **(E)**: EtOH + MethyB 40 mg/kg shows some cortical neuron appearing normal (orange arrow) with prominent nucleoli. Pykontic neurons are also seen at the left of figure (white arrow) (Hx&E x400).

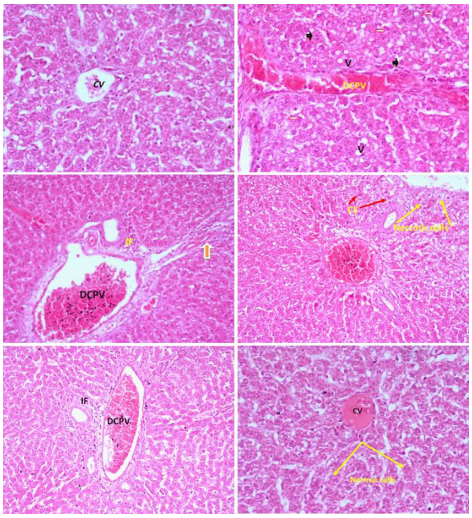

Fig 5. Representative photomicrographs of liver tissue after treatment with**: (A)** Vehicle shows normal histological structure of hepatic lobules and central vein (CV) (Hx & E x 400). **(B)** EtOH only shows vacuolar degeneration(V), dilation and congestion of portal vein (DCPV), some cells with abundant nuclei (black arrow head), and apoptosis of other cells (white arrow) (Hx&Ex200). **(C)** EtOH only shows cellular infiltration around congested portal vein (red arrow), minimal fibrosis (white arrow) and inflammatory cells (IF) (Hx&E x200). **(D)** EtOH only shows foci of necrosis (yellow arrow) and cloudy swelling (red arrow) (Hx&Ex200). **(E)** EtOH + MethyB 20 mg/kg shows restoration of normal architecture although dilated, congested of portal vein (DCPV), portal tract and aggregation of lymphocytic infiltration around (HX&E x200).**(F)** EtOH + MethyB 40 mg/kg shows reduced pathological abnormalities induced by ethanol but congestion of central vein still present (cv) (HX&E x200).

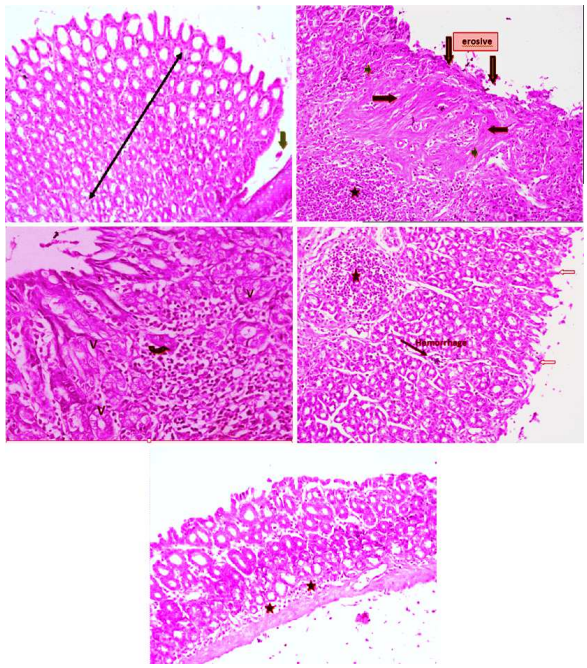

Fig 6. Representative photomicrographs of gastric tissue after treatment with**: (A) Vehicle** shows normal histological structure of gastric gland and gastric pits (H & E X 200). **(B)**: EtOH only shows superficial erosive, disruption of the surface epithelium (black arrow), mucosal atrophy and fibrosis (red arrow), inflammatory cells in mucosa (yellow arrow) and submucosa (star) (Hx&E x200). **(C)** EtOH only (high power) shows vacuolation in the lining epithelium of gastric gland (v), some glands show distorted architecture (black arrow) (Hx&E x400). **(D)** EtOH + MethyB 20 shows exfoliation of the focal area of superficial epithelial cells (white arrow), inflammatory cellular infiltrate (star)and hemorrhage in mucosal layer (arrow), the most of glands appeared normal (Hx&E x200). **(E)** EtOH + MethyB 40 shows restoration of normal architecture but inflammatory cells in submucosal layer are still present (star) (Hx&E x200).

4. Discussion

In this study we have demonstrated that acute oral administration of high dose EtOH for two successive days was capable of inducing brain and liver oxidative stress evidenced by significant increase in lipid peroxidation assessed by measuring MDA (Gutteridge, 1995). There was also depletion of the antioxidant and free radical scavenger reduced glutathione due to its consumption by the increase in free radicals generated by ethanol metabolism and/or binding of glutathione to acetaldehyde. EtOH induced significant increase in brain 5-LOX. The latter catalyzes the oxidation of arachidonic acid and the resultant leukotrienes are potent mediators of inflammation and neurodegeneration (Radmark et al., 2007). Our study also provided the first evidence that EtOH causes significant inhibition of brain and liver paraoxonase-1 (PON-1). This enzyme possesses an esterase and lactonase activities and is involved in xenobiotic metabolism (La Du, 1992) and in protection against oxidative and inflammatory events (Ng et al, 2008). The activity of PON-1 decreases in serum from patients with chronic liver disease (Keskin et al., 2009) and in a number of neurological disorders (Menini and Gugliucci, 2014; Abdel-Salam et al., 2015). We in addition demonstrated that brain butrylcholinesterase (BChE) activity was significantly inhibited by EtOH. Like acetylcholine, BChE is involved in hydrolyzing the neurotransmitter acetylcholine but in addition ester-containing drugs. BChE is widely distributed in the nervous system and there is evidence for a role for the enzyme in cholinergic transmission and Alzheimer's disease (Darvesh et al., 2003; Silman and Sussman, 2005). EtOH-induced oxidative damage was supported by tissue histology which revealed neuronal death, hepatic vacuolar degeneration and necrotic foci as well as gastric mucosal damage. These biochemical and histopathological changes induced by acute EtOH were alleviated by treatment with MethyB at a dose-dependent manner.

Our histological study of the brain tissue in rats treated with EtOH confirmed the development of acute neuronal injury characterized by the presence of dead and red neurons, and damage of glial cells. The pyramidal cells showed pyknotic nuclei and an increase in perivascular space with hemorrhage. Other studies reported shrinkage and loss of cerebellar Purkinje cells (Ramezani et al., 2012). Using in vivo magnetic resonance imaging and spectroscopy, Zahr et al. (2010) reported tissue shrinkage after binge alcohol in rats. We also showed that the administration of absolute EtOH caused liver vacuolar and cloudy degeneration, apoptosis of hepatocytes, foci of necrosis, dilation and congestion of portal vein and infiltration of inflammatory cells. The results are in agreement with those of Bolkent et al. (2006) who reported the presence of vacuolar degeneration in hepatocytes of zones 2 and 3, mild dilation of liver sinusoids and hyperemia and mononuclear cell infiltrations in rats given absolute ethanol. Other researchers found microvacuolar steatosis and necrotic cell death (Souli et al., 2013), distorted hepatic architecture, fatty changes, and apoptotic hepatocytes (Elshennawy et al., 2015). That EtOH causes impairs gastric mucosal integrity and injure the gastric mucosa is well known in humans (Agrawal et al., 1986; Knoll et al., 1998; Iaquinto et al. 2003; Strate et al., 2016) and experimental animals (Motawi et al., 2012; Ellithey et al., 2019). Our study shows superficial erosions, disruption of the surface epithelium, distorted glandular architecture, mucosal atrophy and inflammatory cells in mucosa and submucosa. Other studies showed deep ulceration of gastric mucosa, shedding out of most of mucosal thickness following intragastric administration of absolute EtOH for 1h in the rat (Ellithey et al., 2019).

The pathological changes in the brain, liver and stomach due to EtOH in present work may be mediated by the accumulation of free radicals. The metabolism of EtOH is carried out by three enzyme systems that comprise alcohol dehydrogenase, aldehyde dehydrogenase, catalase and the microsomal ethanol oxidizing system. Each of these enzyme systems can generate ROS (Comporti et al., 2010; Osna et al., 2017). Previous studies showed that tissue damage induced by acute EtOH is associated with increased oxidative stress (Aksu et al., 2010; Abdel-Salam et al., 2014). Acute EtOH administration increased lipid peroxidation in rat hippocampus (Gönenç et al., 2005). EtOH given for two days to rat pups resulted in increased lipid peroxidation and decreased glutathione peroxidase (GPx) in cerebellum accompanied by shrinkage of cerebellar Purkinje cells (Ramezani et al., 2012). In primary human neurons in culture, EtOH caused increases in ROS and the lipid peroxidation product 4-hydroxynonenal (Haorah et al., 2008). The brain is particularly sensitive to an increase in ROS because of the high content of polyunsaturated fatty acids and its modest

antioxidants (Haorah et al., 2005). EtOH or acetaldehyde impairs blood brain barrier, and enhances monocyte migration through the barrier (Ji, 2012), decreases glucose utilization (Grunwald et al., 1993), and upregulates proinflammatory cytokines, inducible nitric oxide synthase and cyclo-oxygenase-2 via activation of toll-like recepror-4 and interleukin-1 receptor, thereby, causing brain inflammation and cell death (Blanco and Guerri, 2006). Studies also reported increase in lipid peroxidation and depletion of reduced glutathione in liver tissue (Bolkent et al., 2006) and gastric tissue (Ellithey et al., 2019) following acute EtOH administration in rats. EtOH induced gastric mucosal injury, however, primarily, involves damage to the microvasculature where endothelial injury and increased vascular permeability precede the development of gastric lesions (Szabo et al., 1985; Tarnawski and Hollander, 1985). Studies showed that EtOH-induced histologic injury develops within 10 min following microvascular stasis (Whittle et al., 1985). Intragastric instillation of EtOH resulted in immediate and marked vasoconstriction of submucosal venules, followed by congestion of submucosal capillaries and the development of gross mucosal lesions (Yonei and Guth, 1991). Moreover, there is evidence for neutrophil-mediated endothelial cell injury in the pathogenesis of EtOH -induced gastric mucosal injury (Kvietys et al., 1990). The pro-inflammatory cytokines TNF-α and IL-1β were also increased in the gastric mucosa of rats given 96% ethanol, indicative of an inflammatory process being involved in EtOH-induced gastric mucosal damage (Ellithey et al., 2019).

Our present study indicates that MethyB was capable of alleviating the brain, liver and gastric injury caused by acute EtOH. This protective effect of MethyB was demonstrated at the levels of biochemical and histological changes. In both brain and liver tissues, co-treatment with MethyB caused alleviated in a dose-dependent manner the increase in MDA, the depletion of reduced glutathione and the inhibition of PON-1 induced by EtOH. The effect of MethyB was more marked in the liver where the higher dose restored MDA, reduced glutathione and PON-1 to their saline control values. MethyB at the doses used in this study prevented the inhibition of BChE and the increase in 5-LOX induced by EtOH. Several studies indicated that MethB exerts protective effects in experimental models of cerebral (Talley Watts et al., 2014; Abdel-Salam et al., 2016a,b), spinal cord (Lin et al., 2017), and hepatic injury (Aksu et al., 2010; Chen et al., 2015). These protective effects of MethyB can be attributed to an antioxidant and cell respiration-enhancing action (Rojas et al, 2012). Under physiological conditions, MethyB undergoes a catalytic redox cycle between its blue oxidized form and colorless uncharged reduced (leucoMethyB) by nicotinamide adenine dinucleotide phosphate (NADPH) or thioredoxin. LeucoMB is then spontaneously reoxidized by O_2 (Schirmer et al., 2011). MethyB cycles between its oxidized and reduced forms in the mitochondrial electron transport chain, and inhibits superoxide and hydroxyl radical formation, thereby protecting the mitochondria from oxidative damage (Kelner et al., 1988; Atamna et al., 2008; Tretter et al., 2014). MethyB has been shown to; (i) protect against oxidative and nitrosative stress in experimental brain and liver injury (Abdel-Salam et al., 2014; Aksu et al., 2010); (ii) reduce oedema and inflammatory gene (IL-1β, TNF-α) expression in the hippocampus following traumatic brain injury (Fenn et al., 2015) (iii) attenuate the increase in serum NF-κB, thereby, reducing brain cytokine expression and the inflammatory response in toluene intoxicated rats (Abdel-Salam et al., 2016a); (iv) decrease TNF-α and caspase-3 and increase the antiapoptotic marker Bcl-2 (B-cell lymphoma 2) in the striatum of rotenone-treated rats (Abdel-Salam et al., 2014); (v) decease the activation of NLRP3 inflammasome and ROS on microglia after spinal cord injury in the rat (Lin et al., 2017); (vi) increase cerebral blood flow in different brain regions (Lin et al., 2012); (vii) decrease glial cell activation in malathion-intoxicated rats (Abdel-Salam et al., 2016b). It is suggested that a combination of an antioxidant and anti-inflammatory actions have accounted for the protective effects of MethyB in the present study.

In summary, the present study demonstrates for the first time that the redox dye MethyB co-administered with high dose EtOH exerts protective effects against the deleterious effects of EtOH in brain, liver and gastric tissues. Thus, MethyB might prove of value in reducing tissue injury in acute EtOH intoxication.

Conflicts of interest

The authors declare that there are no potential conflicts of interest.

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