

THE ROLE OF PARA-AMINOSALICYLIC ACID (PAS) AND N-(2-HYDROXYETHYL) ETHYLENEDIAMINE TRIACETIC ACID (HEDTA) IN ALLEVIATING THE OXIDATIVE CHANGES INDUCED BY MANGANESE NEUROTOXICITY IN ALBINO RATS

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ABSTRACT

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This study was conducted to explore the capability of PAS (Para-aminosalicylic acid) and HEDTA [N-(2-hydroxyethyl) ethylenediamine triacetic acid] either alone or in combination in reducing some oxidative changes in different brain regions (cerebral cortex, cerebellum and medulla oblongata) in rats exposed to manganese. Seventy five male weanling rats (PND 21) were divided into two groups, group (A) served as (-ve) control group (C1) and group (B) received manganese chloride tetrahydrate (MnCl₂ · 4 H₂O) via drinking water for 60 days in a concentration of 5 mg MnCl₂/ ml of H₂O. Twenty four hours after cessation of Mn exposure, group B was divided into 5 subgroups. Rats of group B1 were killed directly after cessation of Mn exposure and served as +ve control group. Rats of group B2 received saline solution 0.9% intraperitoneally (i/p) for 4 weeks served as withdrawal group. Rats of group B3 received 200 mg PAS / Kg b.w. S/C daily for 5 days/week for 4 weeks, group B4 rats were received 50 mg HEDTA / Kg b.w. i/p daily for 5 days/week for 4 weeks and group B5 received mixture of both PAS and HEDTA in the same manner and concentration as in group B3 and B4. Animal groups treated with each chelating agent separately, recovered the neurotoxicity and oxidative stress induced by Mn. 4H₂O and that indicated by significant improvement in superoxide dismutase (SOD), catalase, AChE and glutathione peroxidase activity as well as marked decrease in TBARS and nitric oxide production as compared to Mn treated group. Withdrawal group showed no improvement in most of the previous parameters which may be attributed to the irreversible damage of Mn to the brain tissues.

Keywords: PAS, HEDTA, Manganese neurotoxicity, chelation, oxidative changes.

INTRODUCTION

Environmental pollution is a one of the most deleterious agents to the biological life. Industrialization offered additional hazards to the environment surrounding man and animals (Antoniou *et al.*, 1995). Because of a wide distribution of the heavy metals throughout the earth crust, in air, soil, and water, as well as the remarkable environmental pollution, these heavy metals represent global problems that are a growing threat to the environment (Alloway, 1995). Some metals have bio-importance as trace elements and are essential for all living organisms such as manganese (Mn), iron, copper and zinc but the toxic effects of many of them are of great concern and constitute major contaminants such as lead, cadmium and mercury (Duruibe *et al.*, 2007).

Mn is an essential for biological tissues and necessary for normal functioning of a variety of

physiological processes. Mn is also an important cofactor for a variety of enzymes, including the antioxidant enzyme SOD in brain, as well as enzymes involved in neurotransmitter synthesis and metabolism (Erikson *et al.*, 2005 and 2007). Despite its essentiality, Mn has been known to be a neurotoxicant (ATSDR, 2000 and Baldwin *et al.*, 2008). The primary source of Mn intoxication in humans is due to occupational exposure in miners, smelters, welders and workers in battery factories (Bowler *et al.*, 2006; Jiang *et al.*, 2006 and Elder *et al.*, 2006). Several countries including USA and Canada have replaced lead in gasoline with Mn-containing antiknock compound methyle cyclopentadienyle manganese tricarbonyl (MMT), the combustion of MMT in the automobile with the expected increase in ambient Mn level has raised concern about the health risks associated with environmental exposure to Mn (Frumken and Solomon, 1997). Health risks of exposure to Mn have been associated with organic Mn-containing

pesticides as Mn-ethylene-bis-dithiocarbamates (Thibault *et al.*, 2002), inorganic Mn dust or vapor among steel manufacturing workers or welders (Wang *et al.*, 1989 and Elder *et al.*, 2006), or a cocaine-based drug called Bazooka, which is contaminated with Mn carbamates (Roth and Garrick, 2003 and Dobson *et al.*, 2004). Chronic exposure to Mn can cause a neurodegenerative disease named manganism, display an extrapyramidal syndrome in a pattern similar to, but not identical to idiopathic Parkinson's disease, including tremor, bradykinesia and gait difficulties. Patients can also display neuropsychological difficulties that include memory loss, apathy and even psychosis (Olanow, 2004; Aschner *et al.*, 2007; Crossgrove and Zheng, 2004). Exposure to Mn either through air or diet induces severe disorders in the CNS, extensive neural damage, reproductive and immune systems dysfunction, nephritis, testicular damage, pancreatitis and hepatic damage (Webster and Valois, 1987; Keen and Zidenberg-Cherr, 1990).

Many chelating agents as cyclohexane-diamine-tetraacetic acid (CDTA), ethyleneglycol-bis-(beta-aminoethylether)-N, N-tetraacetic acid (EGTA), HEDTA, isonicotinyl hydrazine (INH), L-dopa, sodium 4,5-dihydroxy-1,3-benzenedisulphonate (Tiron) and PAS have a role on the excretion and tissue distribution of Mn (Sánchez *et al.*, 1995 and Lan *et al.*, 2011). Many studies used poly amino carboxylic acid compounds as Para-aminosalicylic acid (PAS) in treatment of acute and short term Mn exposure (Tandon *et al.*, 1975 and Tandon, 1978). HEDTA which was used to treat Mn poisoned rabbits and mice (Khandelwal *et al.*, 1980 and Sanchez *et al.*, 1995). PAS effectiveness against Mn toxicity may be explained by two putative mechanisms. First, Mn^{3+} can form a stable complex with hard donor atoms such as oxygen donors in PAS structure. In contrast, the Mn^{2+} cation has a lower charge density and thus prefers relatively softer donors such as nitrogen, which is also present in PAS structure (Liu and Hider, 2002). Second, from the chemistry point of view, the salicylate moiety in PAS structure possesses an anti-inflammatory effect and recent studies have suggested that sodium salicylic acid, may have neuroprotective benefit, because the inflammatory processes have been shown to play a role in the pathogenesis of neurodegenerative diseases such as Alzheimer disease (Asanuma *et al.*, 2004; Rothstein *et al.*, 2005) and therefore, chemicals such as PAS may facilitate regulation of neurotransmitters, suppress nitric oxide (NO) and protect against oxidative stress in neurons and neuroglia. Acute and short-term neurotoxic effects of manganese are blocked by adding PAS or EDTA. PAS could mobilize Mn from the livers and testis of Mn-intoxicated rats, and enhance the fecal excretion of manganese in Mn-intoxicated rabbits. Clinical studies demonstrated that PAS treatments were successful in alleviating symptoms in patients with chronic Mn poisoning. In addition that PAS treatment

reversed many of the clinical symptoms in a woman with severe chronic Manganism (Jiang *et al.*, 2006 and Michael *et al.*, 2010). PAS treatment reduced Mn levels in brain as well as prolactin levels in the serum concomitant with an increase in brain glutathione levels compared to rats exposed to Mn alone. These results suggest that PAS can effectively attenuate Mn neurotoxicity (Marreilha *et al.*, 2010). The aim of the current study was (1) to evaluate the efficacy of two chelating agents (PAS and HEDTA) in treatment of Mn toxicity (2) to investigate whether PAS and HEDTA treatment could either alone or in combination to alleviate oxidative changes induced by manganese toxicity in rats.

MATERIALS and METHODS

1- Chemicals: Manganese chloride tetrahydrate ($MnCl_2 \cdot 4 H_2O$), Para-aminosalicylic acid (4- amino-2-hydroxybenzoic acid ($C_7H_7NO_3$)) (PAS) and N-(2-hydroxyethyl) ethylenediamine triacetic acid ($C_{10}H_{15}N_2O_7Na_3$) (HEDTA) were obtained from Sigma chemical Co., Cairo. SOD, Catalase, Glutathione peroxidase, Acetyl cholinesterase, Lipid peroxide and Nitric oxide kits were obtained from Biodiagnostic Co., Cairo.

2- Animals: Seventy five male weanling albino rats (PND 21) 7-8 weeks old with body weight 200 ± 10 g were used in this study. All animals were maintained under good ventilation, standard hygienic conditions with free access to tap water and standard pellet diet for one week before starting the experiment.

3- Experimental protocol: Seventy five male rats were divided into group A [10 rats, received only distilled water for 60 days and served as (-ve) control group (C1)] and group B [65 rats, received Mn in the form of Mn chloride tetrahydrate (5 mg $MnCl_2$ / ml) in drinking water for 60 days according to Zheng *et al.* (2009). Twenty four hours after cessation of Mn exposure, rats in group B were divided into 5 subgroups. Subgroup B1 includes 5 rats were killed directly after cessation of Mn administration, and served as (+ve) control group. Subgroup B2, includes 15 rats were received saline solution 0.9% intraperitoneally (i/p) for 4 weeks, and served as withdrawal group. Subgroup B3, includes 15 rats were received 200 mg PAS/kg b.w. s/c daily for 5 days/week for 4 weeks. Subgroup B4 includes 15 rats were received 50 mg HEDTA / Kg b.w. i/p daily for 5 days/week for 4 weeks (Flora *et al.*, 2003). Subgroup B5 includes 15 rats were received a mixture from both PAS and HEDTA in the same dose as in group B3 and group B4.

4- Serum and brain biochemical parameters: Blood samples were collected from treated animal groups as well as control group from retro-orbital venous plexus by means of heparinized micro

capillary tubes (Halpern *et al.*, 1951). The brains were obtained and various brain regions (cerebral cortex, cerebellum, medulla oblongata) were dissected and stored at -80°C prior to analysis as described by Zheng *et al.* (1998) and Li *et al.* (2006). The brain samples were used in estimation of biochemical parameters of oxidative stress. Brain regions samples rapidly removed rinsed from blood using distilled water, blotted between two damp filter papers, then weighted. The whole brain was placed in pre-chilled glass tube with calculated volume of cold saline and the tube surrounding by cooling mixture (ice+sodium chloride+acetone) then homogenized by homogenizer. The final homogenate was 10% weight/volume. The homogenate is centrifuged and the supernatants were taken for different biochemical studies. Superoxide dismutase (SOD) was determined according to Marklund and Marklund (1974) and catalase enzyme according to Cohen *et al.* (1970). Lipid peroxide (Malonaldehyde, MDA) was determined as thiobarbituric acid reactive substances (TBARS) (Beuge and Aust, 1978), nitric oxide (Ding *et al.*, 1988) and glutathione peroxidase (Moron *et al.*, 1979). Acetylcholinestrace (AChE) in brain homogenate was assayed colorimetrically using commercial kits (Ellman, 1961).

6- Statistical analysis: The results were expressed as the mean ± SE. all data were analyzed using one way analysis of variances (ANOVA) followed by Duncan TEST using SPSS 11.0 statistical software (SpSS, Inc, Chicago, IL, 2001). ^{abcdef} Means with different superscripts in the same column differ significantly at (p<0.05).

RESULTS

1- SOD, catalase and Glutathione peroxidase enzymes activity concentration in cerebrum, cerebellum and medulla oblongata of rats brain show extremely significant decrease in SOD activity in cerebrum compared to control group in Mn treated group, while animal treated with chelating agents separately showed increase in SOD activity in comparable with Mn treated group. SOD activity was decreased in group treated with (PAS + HEDTA) group as well as in withdrawal group as compared to control and MnCl₂. 4H₂O treated group. The results

were in cerebellum as the same in cerebrum region. In medulla oblongata, PAS treated group showed significant increase in SOD activity compared with Mn treated group but there was a little value of significance between HEDTA and Mn treated groups (Table 1 and Figure 1). Also the observed results showed that Mn produced significant depletion in catalase enzyme activity in cerebrum in Mn treated group compared with control group.

The treated rats exhibited a significant increase in catalase activity as compared to Mn treated group and the data of PAS treated group was closely related to control group while in cerebellum, the results showed significance decrease in catalase activity in Mn treated rats compared with control one. Treated groups with each chelating agent separately increased catalase activity and the results were nearly similar to control group. In mixture treated group as well as in withdrawal group significant decrease in catalase activity was also evident and no response was shown. In medulla oblongata, all treated groups showed increase in enzyme activity than MnCl₂.4H₂O treated group but results of mixture treated group as well as withdrawal group were not closely related to control one, but showed some elevation in enzyme activity than that of Mn treated one (Table 1, and Figure, 1).

There was significance decrease in glutathione peroxidase level in cerebrum of Mn treated group in comparable with control group. All other treated groups as well as withdrawal one recovered the effect of MnCl₂.4H₂O and increased the enzyme activity and the observed data of PAS treated group was nearly related to control one. In cerebellum, the data revealed that treated rats with PAS showed elevation in glutathione peroxidase activity than that of Mn treated animals, while in HEDTA the value of enzyme was fare from control value. Also, in mixture group and withdrawal ones, the results showed failure of improvement of enzyme activity. In medulla oblongata, there was significance decrease in glutathione peroxidase level in Mn treated group in comparable with control as well as other treated groups. However, other treated group improved the glutathione peroxidase level but there was extreme significance decrease in enzyme level in withdrawal group as compared to others (Table 1 and Figure, 1).

Table 1: Effect of PAS and HEDTA treatment on Superoxide dismutase, catalase and Glutathione peroxidase in Cerebrum, Cerebellum and Medulla oblongata of rat brain

Groups	Superoxide dismutase (U/g)			Catalase (U/g)			Glutathione peroxidase (Um/ml)		
	Cerebrum	Cerebellum	Medulla oblongata	Cerebrum	Cerebellum	Medulla oblongata	Cerebrum	Cerebellum	Medulla oblongata
Group A (C1) (-ve control)	0.500 ± 0.005 ^a	1.376 ± 0.006 ^a	3.526 ± 0.005 ^a	0.589 ± 0.007 ^a	2.843 ± 0.047 ^a	5.296 ± 0.074 ^a	64.844 ± 0.079 ^a	84.352 ± 0.314 ^a	68.178 ± 0.112 ^a
Group B1 (Mn)	0.257 ± 0.003 ^e	0.892 ± 0.007 ^d	1.189 ± 0.005 ^d	0.328 ± 0.007 ^e	1.290 ± 0.027 ^d	1.775 ± 0.017 ^f	45.391 ± 0.225 ^f	57.420 ± 0.110 ^d	50.250 ± 0.066 ^d
	I(48.6)	I(35.2)	I(66.3)	I(44.3)	I(54.6)	I(66.5)	I(29.9)	I(31.9)	I(26.3)
Group B2 (PAS)	0.338 ± 0.111 ^b	1.353 ± 0.012 ^b	2.188 ± 0.003 ^b	0.559 ± 0.014 ^b	2.478 ± 0.038 ^c	4.501 ± 0.011 ^b	62.683 ± 0.016 ^b	71.329 ± 0.178 ^b	64.905 ± 0.078 ^b
	I(32.4)	I(1.7)	I(37.9)	I(5.1)	I(12.8)	I(15.0)	I(3.3)	I(15.4)	I(4.8)
Group B3 (HEDETA)	0.328 ± 0.057 ^b	1.326 ± 0.005 ^c	1.758 ± 0.006 ^c	0.493 ± 0.006 ^c	2.595 ± 0.005 ^b	4.001 ± 0.020 ^c	51.876 ± 0.041 ^c	58.360 ± 0.315 ^c	60.980 ± 0.080 ^c
	I(34.4)	I(3.6)	I(50.1)	I(16.3)	I(8.7)	I(24.5)	I(19.9)	I(30.8)	I(10.6)
Group B4 (PAS+ HEDETA)	0.171 ± 0.003 ^d	0.338 ± 0.006 ^c	0.674 ± 0.004 ^f	0.499 ± 0.002 ^c	0.954 ± 0.016 ^c	2.947 ± 0.067 ^d	56.198 ± 0.146 ^d	47.552 ± 0.055 ^e	61.146 ± 0.088 ^c
	I(65.8)	I(75.4)	I(80.9)	I(15.3)	I(66.4)	I(44.4)	I(13.3)	I(43.6)	I(10.3)
Group B5 (Withdrawal)	0.171 ± 0.004 ^d	0.175 ± 0.004 ^f	0.749 ± 0.015 ^c	0.412 ± 0.006 ^d	0.883 ± 0.006 ^c	2.478 ± 0.010 ^c	58.359 ± 0.189 ^c	32.422 ± 0.237 ^f	38.904 ± 0.026 ^e
	I(65.8)	I(87.3)	I(78.8)	I(30.1)	I(68.9)	I(53.2)	I(10.0)	I(61.6)	I(42.9)

- Data expressed as mean ± S.E. (n= 5samples).
 - ^{a-f} Means with different superscripts in the same column differ significantly at (p<0.05).
 - S or I %: Means stimulation or inhibition when compared with control group.

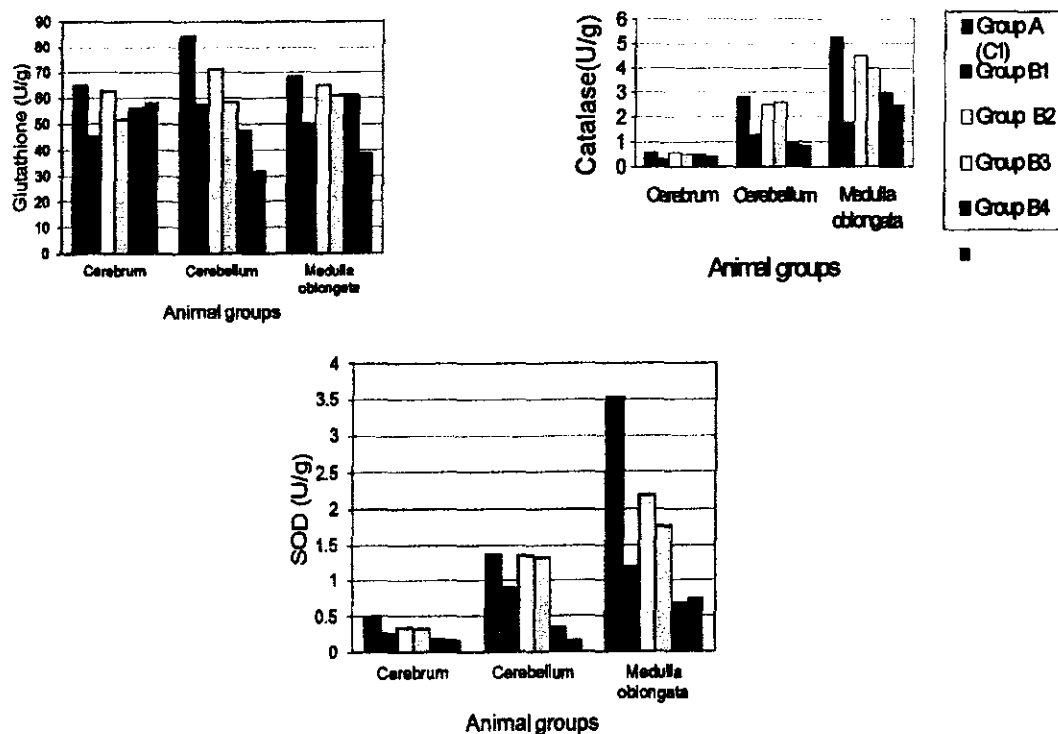


Figure 1: Effect of PAS and HEDTA treatment on catalase, Superoxide dismutase and Glutathione peroxidase in Cerebrum, Cerebellum and Medulla oblongata of rats.

2- Effect of PAS and HEDTA treatment on Lipid peroxides, NO and AChE in cerebrum, cerebellum and medulla oblongata of rat's brain. In cerebrum; the results revealed that Mn treated group showed significance decrease in AChE activity than control group. Other treated groups produced increase in acetyl cholinesterase activity as compared to Mn treated group but there was a little significance between the group exposed to mixture of chelating agents and Mn treated group. In cerebellum, Mn treated group showed significance decrease in enzyme activity as compared to control one. The observed data of all other treated groups indicated that the enzyme activity was improved in comparable with Mn treated group while withdrawal group showed decrease in enzyme activity than others. In medulla oblongata, there was a significance difference between Mn treated group and control group that indicated by decrease of acetyl cholinesterase activity in Mn treated group. the activity of cholinesterase of PAS treated group showed some improvement than that Mn treated group and increased the enzyme activity. HEDTA treated group showed no significant difference in comparable with Mn treated group. However; there was no significance between mixture; withdrawal groups and control group (Table 2 and Figure, 2).

Lipid peroxidation in cerebrum was augmented in Mn treated group which indicated by elevation in TBARS level as compared to control group. Other treated groups showed significance decrease in lipid peroxidation in comparable with Mn treated one. There was a little value of significant difference between Withdrawal and Mn treated groups. In cerebellum, lipid peroxidation was similar as in cerebrum but there was significant elevation in lipid peroxidation in withdrawal group as compared to Mn treated group and control one. In medulla oblongata, the data was similar as in cerebrum but group exposed to mixture of chelating agents showed no significant recovered effect against Mn induced lipid peroxide formation. Mixture and withdrawal groups failed to recover the significance elevation in lipid peroxidation concentration. Regarding the estimation of NO concentration in cerebrum, cerebellum and medulla oblongata of the experimental rats, revealed that exposure to Mn induced significant elevation in nitric oxide concentration in comparison with control group. In chelating agents treated groups, the results denote a marked improvement in NO represented by significant decrease. The same observation was appeared in case of treated group with mixture of chelating agents and in withdrawal group (Table 2 and Figure, 2).

Table 2: Effect of PAS and HEDTA treatment on lipid peroxides, nitric oxide and acetyl cholinesterase in cerebrum, cerebellum and medulla oblongata of rat brain.

Groups	Lipid peroxides Um/g tissue			Nitric oxide Umol/L			Acetyly cholinesterase			U/L
	Cerebrum	Cerebellum	Medulla oblongata	Cerebrum	Cerebellum	Medulla oblongata	Cerebrum	Cerebellum	Medulla oblongata	
Group A (C1) (-ve control)	0.516 ± 0.003 ^f	0.626 ± 0.005 ^d	0.317 ± 0.004 ^f	25.724 ± 0.035 ^e	25.923 ± 0.029 ^d	28.778 ± 0.022 ^c	37.809 ± 0.215 ^a	32.453 ± 0.035 ^b	27.409 ± 0.061 ^a	
Group B1 (Mn)	1.293 ± 0.004 ^a	1.094 ± 0.005 ^b	0.765 ± 0.009 ^c	41.391 ± 0.150 ^a	40.315 ± 0.038 ^a	56.056 ± 0.047 ^a	26.123 ± 0.113 ^c	25.923 ± 0.083 ^c	24.666 ± 0.040 ^c	
	S (150.6)	S (74.8)	S (141.3)	S (60.9)	S (55.6)	S (94.8)	I (30.9)	I (20.1)	I (10.0)	
Group B2 (PAS)	0.645 ± 0.004 ^c	0.501 ± 0.006 ^c	0.412 ± 0.006 ^c	23.254 ± 0.043 ^d	21.058 ± 0.147 ^f	23.401 ± 0.040 ^e	33.743 ± 0.191 ^b	31.006 ± 0.043 ^c	26.669 ± 0.026 ^b	
	S (25.0)	I (19.9)	S (29.9)	I (9.6)	I (18.8)	I (18.7)	I (10.8)	I (4.5)	I (2.7)	
Group B3 (HEDTA)	0.782 ± 0.007 ^d	0.637 ± 0.005 ^d	0.533 ±0.004 ^d	27.448 ± 0.041 ^b	24.680 ± 0.196 ^e	27.790 ± 0.135 ^d	31.280 ± 0.026 ^c	29.224 ± 0.071 ^d	24.812 ± 0.247 ^c	
	S (51.6)	S (1.8)	S (68.1)	S (6.7)	I (4.8)	I (3.4)	I (17.3)	I (9.9)	I (9.5)	
Group B4 (PAS+HEDETA)	0.917 ± 0.008 ^c	0.786 ± 0.017 ^c	1.912 ± 0.034 ^a	23.198 ± 0.248 ^d	28.464 ± 0.184 ^c	28.762 ± 0.259 ^c	28.721 ± 0.162 ^d	36.363 ± 0.040 ^a	27.156 ± 0.082 ^a	
	S (77.7)	S (25.6)	S (503.2)	I (9.8)	S (9.8)	I (0.06)	I (24.0)	I (12.1)	I (0.9)	
Group B5 (Withdrawal)	1.173 ± 0.006 ^b	1.555 ± 0.015 ^a	1.505 ± 0.018 ^b	27.295 ± 0.163 ^b	31.184 ± 0.326 ^b	32.683 ± 0.122 ^b	24.803 ± 0.155 ^f	23.735 ± 0.087 ^f	27.261 ± 0.028 ^a	
	S (127.3)	S (148.4)	S (374.8)	S (6.1)	S (20.3)	S (13.6)	I (34.4)	I (26.9)	I (0.5)	

- Data expressed as mean ± S.E. (n= 5samples).

- ^{a-f} Means with different superscripts in the same column differ significantly at (p<0.05).

-S or I %: Means stimulation or inhibition when compared with control group.

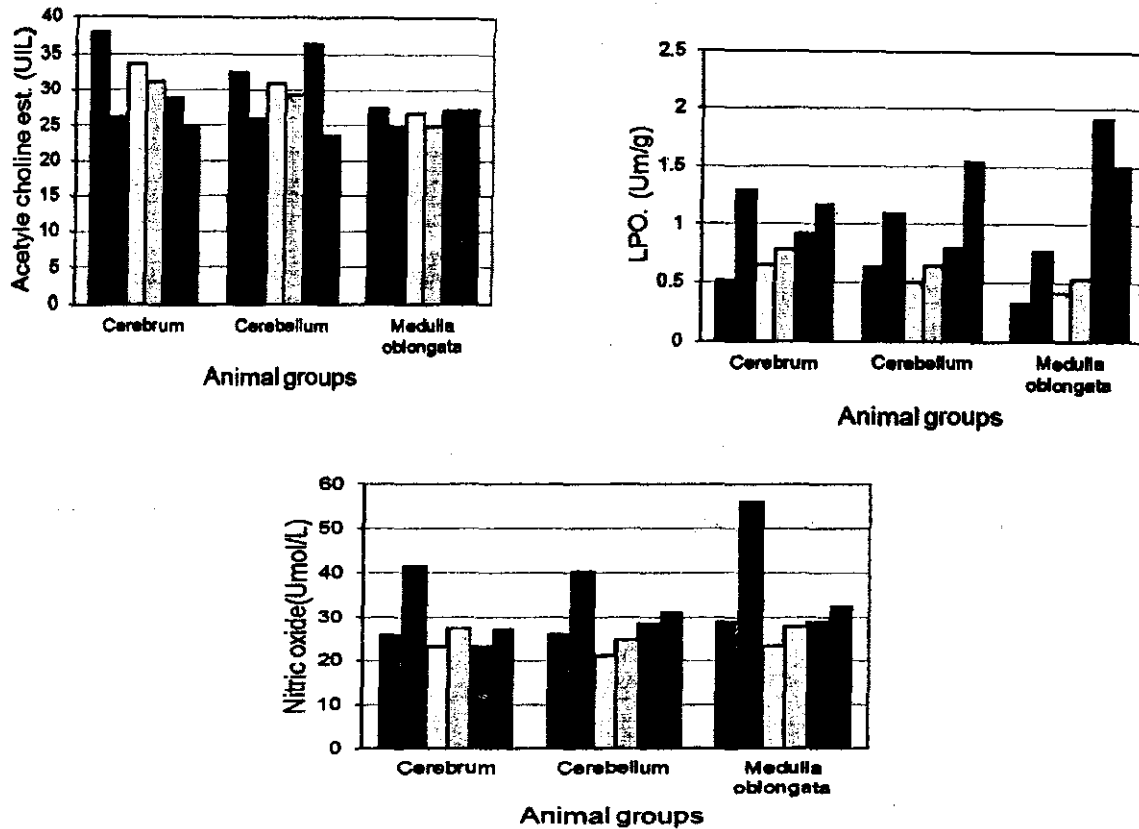


Figure 2: Effect of PAS and HEDTA treatment on lipid peroxides, nitric oxide and acetyl cholinesterase in cerebrum, cerebellum and medulla oblongata of rat brain

DISCUSSION

Anti-oxidants and free radicals: Brain, in general, is highly susceptible to oxidative damage because it has a high rate of oxidative metabolism, high concentrations of poly unsaturated fatty acids and low to moderate levels of antioxidant enzymes. Marked and significant alterations in oxidative stress and free radical formation, which represented by inhibition in activities of SOD, catalase, glutathione peroxidase, acetyl cholinesterase enzyme and elevation in the production of lipid peroxidation (LPO) and nitric oxide concentration. Different Studies have demonstrated that manganese is capable of inducing oxidative stress and free radical formation (Chen and Liao, 2002). Mn increases NO production in cultured astrocytes (Hazell and Norenberg, 1998 and Spranger *et al.*, 1998).

Current evidence indicates that manganese taken up subsequently binds to the inner mitochondrial membrane which is also the location of the electron transport system of the cell, a site for production of oxygen free radicals. Such superoxide species have the ability to oxidize Mn^{2+} to Mn^{3+} ; a possible key event in developing cytotoxicity in brain following exposure to Mn (Archibald and Tyree, 1987). There

is evidence that GSH plays an important role in the detoxication of ROS in brain and so GSH brain variations are associated with the loss of neurons during the progression of neurodegenerative diseases (Sun and Chen, 1998).

Another proposed biomarker of oxidative stress, malondialdehyde (MDA), one of the most frequently used indicators of lipid peroxidation. ROS degrade polyunsaturated lipids, forming MDA which is reactive and potentially mutagenic. A positive correlation between the concentrations of MDA in Mn exposed workers and the Mn level in plasma, suggesting that MDA can be used as an index of lipid peroxidation induced by Mn exposure (Yin *et al.*, 1996). In the present study, the results revealed a significant increase in TBARS as well as increases nitric oxide production which consider a further biomarker of oxidative stress. These results are in the harmony with that recorded by Hazell and Norenberg (1998); Spranger *et al.* (1998) and Diem and Stephen (2004).

Oxidative stress has been implicated as a contributing mechanism by which Mn can be toxic to cells (Aschner, 1997). A potential mechanism for Mn-induced oxidative stress is via the oxidation of dopamine-rich regions, especially in basal ganglia

(Newland, 1999). Another possibility is that sequestration of Mn in mitochondria interfere with proper respiration, thereby leading to excessive production of reactive oxygen species (ROS). Weber *et al.* (2002) reported that one of the proposed mechanisms for Mn-induced neurotoxicity is a cascade of oxidative damage potentiated by the synergism of excess Mn and high concentration of iron and dopamine in affected brain regions. It has been theorized that elevated concentration of Mn might significantly accelerate the oxidation of dopamine and other catecholamines and concurrently amplify the formation of ROS (Sloot *et al.*, 1996). Also it was presented in vitro evidence that divalent Mn increases dopamine autooxidation and thus may induce oxidative damage (Donaldson *et al.*, 1980). It has been demonstrated that divalent Mn catalyzes fenton-like reactions that generate hydroxyl radical and trigger proteolytic degradation and protein turnover (Wedler, 1993). Ali *et al.* (1995) demonstrated dose-related increases in ROS production in rat caudate nucleus after in vivo Mn exposure.

It has been reported that the main mechanism of Mn-induced neurotoxicity is via ROS generation (Aschner *et al.*, 2007 and Zhang *et al.*, 2004). One of the proposed mechanisms is by oxidation of divalent form of Mn^{2+} to trivalent form Mn^{3+} , which is significantly more reactive. Mn^{3+} is able to catalyze DA oxidation, leading to the formation of leucoaminochromes, which are toxic to cells (Diaz-Veliz *et al.*, 2004). Gunter *et al.* (2006) suggested that the production of Mn in the trivalent oxidation state is not of toxic significance and the divalent form is indeed responsible for oxidative damage. It has been found that Mn^{2+} can inhibit mitochondrial respiratory chain complexes, causing decreased ATP production and leading to increased rate of production of oxygen radicals (Boveris and Chance, 1973).

Chelation therapy: In the present study, administration of PAS and/or HEDTA induced significant recovery against the neurotoxic effect of Mn. This alleviating effect was marked and observed when used separately and failed when used in a mixture. The protective effect of two chelating agents was represented by recovery in the activities of antioxidant enzymes and cholinesterase and limitation and reduction in lipid peroxide and nitric oxide as stress factors. Several investigators mentioned that chelation therapy was used against Mn induced alterations in biomarkers of oxidative injury, mitochondrial dysfunction, neuroinflammation and neurodegenerative process (Rui, 2010 and Michael *et al.*, 2010). In general, chelating agents have several carboxylate groups linked to a number of tertiary nitrogen atoms. The functional groups form a stable and water-soluble complex with a donor atom such as metals. The metal cation is centered in the complex, while being

coordinately bound either to nitrogen or to oxygen atom as the anchoring site. Unlike regular ligands, chelating agents can form multiple coordination bonds to a single metal ion (Mika *et al.*, 2011). PAS is a potential therapeutic manganese-chelating agent. Early studies had showed that PAS could mobilize Mn from the livers and testis of manganese-intoxicated rats (Tandon *et al.*, 1975), and enhance the fecal excretion of manganese in manganese-intoxicated rabbits (Tandon, 1978). High and prolonged PAS treatments could reduce body fluid and tissue levels of manganese-exposed Sprague-Dawley rats and that PAS was likely acting as a chelating agent to mobilize and remove tissue manganese (Zheng *et al.*, 2009), in the same time treatment of Mn-exposed rats with subcutaneous injections of 200 mg PAS/kg effectively reduces Mn concentrations in blood, CSF, brain tissues and major organs examined (Rui, 2010). PAS may penetrate the blood-brain barrier more readily than EDTA (Jiang *et al.*, 2007). PAS acid effectiveness against Mn toxicity may be explained by two putative mechanisms. First, Mn^{3+} can form a stable complex with hard donor atoms such as oxygen donors in PAS structure. In contrast, the Mn^{2+} cation has a lower charge density and thus prefers relatively softer donors such as nitrogen, which is also present in PAS structure (Liu and Hider, 2002). Finally, PAS treatment effectively removed Mn from the CSF, a major component of brain extracellular fluids as well as from choroid plexus. The CSF is primarily produced by choroid plexus in brain ventricles. Mn is known to be transported across this tissue and into the CSF (Michalke *et al.*, 2007 and Zheng *et al.*, 2003). Results also suggest HEDTA to be effective in reducing some of the biochemical variables indicative of oxidative stress. This could be attributed to the chelating properties of HEDTA as two amino and three carboxyl groups in HEDTA might be acting as possible binding sites for metal leading to the decreased availability to generate ROS (Mathur *et al.*, 1993). So we can concluded that PAS was clinically successful in the treatment against neurotoxicity evoked by Mn, while the use of HEDTA as a chelating agent against Mn neurotoxicity was not marked as in case of PAS, Also use of mixture of the two tested chelating agents was not successful in relieve against Mn neurotoxicity as in case of separately used of each. The withdrawal group showed no improvement in biochemical parameters of oxidative stress and this may be attributed to persistent neurotoxic damage induced by Mn and consequently not returned to the control limit and this indicate that interference by chelating agents may necessary to overcome the neurotoxic effect of Mn.

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دور حمض البارامينو سالسليك والهيبتا في تخفيف التغيرات الناجمة عن الأكسدة نتيجة التسمم العصبي بالمنجنيز في الفئران البيضاء

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يعتبر المنجنيز من أهم الملوثات الرئيسية التي تسبب مخاطر صحية خطيرة على كلا من الإنسان والحيوان. وبالتالي فإن سمية المنجنيز ومعالمتها بشكل واضح لا تزال مشكلة هامة في دائرة الضوء والمجتمع. ولذلك كان الهدف من هذه الدراسة هو تقييم كفاءة بعض المخليبات (حمض البارامينو سالسليك PAS والهيبتا HEDTA) في علاج التسمم العصبي بكلوريد المنجنيز. وفي هذه الدراسة تم استخدام ٧٥ من ذكور الفئران البيضاء بعد عمر الفطام قسمت إلى مجموعتين، المجموعة الأولى تتكون من ١٠ فئران لا تتعرض لأي معالجة (مجموعة ضابطة) أما المجموعة الثانية تتكون من ٦٥ فأر ويتم إعطاؤها كلوريد المنجنيز مذاب في مياه الشرب عند تركيز (٥ ملجم/ملي) لمدة ٦٠ يوماً. وبعد ٢٤ ساعة من التوقف عن إعطاء كلوريد المنجنيز تم تقسيم هذه المجموعة إلى ٥ مجموعات فرعية، مجموعة تم ذبحها مباشرة بعد التوقف عن التعرض لكلوريد المنجنيز وهي تمثل مجموعة ضابطة ايجابية للمجاميع الأخرى وهي تشمل ٥ فئران. أما الأربع مجاميع الأخرى فإنها تتكون كل منها من ١٥ فأر ومنها مجموعة تلقت محلول ملحي ٠.٩٪ داخل الغشاء البيريتوني وهذه المجموعة حفظت لمعرفة أثر المنجنيز بعد التوقف عن إعطاؤه لمدة شهر ومجموعة تم إعطاؤها حمض البارامينو سالسليك PAS ٢٠٠ ملجم/كجم من وزن الجسم تحت الجلد ٥ أيام/الأسبوع لمدة شهر ومجموعة تلقت الهيبتا HEDTA ٥٠ ملجم/كجم من وزن الجسم داخل الغشاء البيريتوني ٥ أيام/الأسبوع لمدة شهر وأخرى تم تعرضها لخليط من (حمض البارامينو سالسليك PAS والهيبتا HEDTA) ٥ أيام/الأسبوع لمدة شهر. وبعد ٢٤ ساعة من التوقف عن التعرض للمعالجة (بعد شهر) تم تخدير الفئران وأخذت عينات من الدم وفصل الرأس واخذ المخ من الجمجمة وتم تقسيمه إلى ثلاثة أجزاء (قشرة المخ ، المخيخ ، النخاع) لدراسة التغيرات البيوكيميائية (المشتقات الحرة وكماتر الأكسدة). وقد أظهرت النتائج أن لمركبات حمض البارامينو سالسليك PAS والهيبتا HEDTA أثر واضح في تخفيف التسمم بالمنجنيز على أجزاء المخ نتيجة التغلب على تأثير المشتقات الحرة الناجمة عن التسمم بالمنجنيز وكذلك إعادة كماتر الأكسدة إلى مستواها الطبيعي.