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Neuroprotective Effect of Barley Plant (Hardeum Valgara) Against the Changes in MAO Induced By Lead and Cadmium Administration in Different CNS Regions of Male Guinea Pig

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Abstract

Lead and cadmium are highly toxic metals that can be ingested or inhaled from a variety of industrial and dietary sources. The purpose of this study was to determine the impact of chronic administration of lead and cadmium on the activity of monoamine oxidase (MAO) in different central nervous system regions (CNS) of male guinea pigs and the protective effect of barley (Hardeum valgara) plant . Five groups of animals were used in this experiment ,frist group of animals was subcutaneous injected at dose level (30mg/kg. body weight) lead acetate , the second group was injected with (1mg/kg. body weight) cadmium chloride, third group of animals received a mixed dose of the two heavy metals with the same previous dose , the fourth dose was recived an aqueous solution of barley (5mg/kg body weight) for two weeks followed by a mixed dose of lead and cadmium (30mg/kg. body weight), (1mg/kg.) .Animals were decapitated after two,four,six and eight weeks after injection. The activity of monoamine oxidase (MAO) was determined in the cerebral cortex, caudate putamen, thalamus., hypothalamus, superior colliculus, inferior colliculus , cerebellum, pons, medulla and spinal cord in male guinea pig CNS. Lead and cadmium induced a general changes in the level of the membrane bound enzyme (MAO) in most CNS regions and in turn affecting both cholinergic and adrenergic neurotransmitters. These results suggest that lead and cadmium may exerts neurotoxic effect by altering certain membrane bound enzymes and may cause oxidative stress that could lead to neurodegenerative diseases. Supplemented groups with barley exhibited a similar MAO to the control group, suggesting that barly protected the CNS from functional damage resulted from the heavy metales . Accordingly, these results indicate that barley supplementation in amixed dose of lead and cadmium injected pigs normalized MAO in most CNS regions, i.e is lead to a general improvement in MAO activity .

Keywords: Lead, Cadmium, MAO, Barley, CNS, ALP, ASAT, ALAT

Contents

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1. Introduction

An important aspect in the study of mammalian central nervous system (CNS) is measurement of the activity of enzyme associated with the CNS functions . From such enzymes the degrading enzyme MAO which is a monoamine metabolizing enzyme located in the outer mitochondrial membrane of many cells and responsible for oxidation deamination of number of biogenic amines [1].

Although a number of reports have appeared in the literature regarding the neurotoxic effect of lead and cadmium , relatively little is known about the effect on membrane bound enzyme and distribution of both metals in the central nervous system [2] .

Lead and cadmium are the most studied neurotoxins and its effects on the nervous system are well documented [3]. Lead (Pb) is considered to be a multi-target toxicant, Pb-acetate induced toxic manifestation in blood, liver, kidney, brain and heart of Wistar rats [4].

Now it is widely accepted that even small quantities of lead and cadmium are harmful to human and other organisms [5] . Lead poisoning is most commonly caused by occupational exposure [6]. Neurotoxicity of lead and other heavy metals known to have multiple mechanism of actions but these mechanisms are still not clearly understood. According to Silbert5 Hynjm and Rgeld [7] one of these mechanisms may be neurodevelopmental toxicity which includes interference with cell adhesion molecule, resulting in the misfiring of the CNS during early development and destruction of the blood –brain barrier which lead to permanent dysfunction ,edema loss of neurons and gliosis [8] **.**The other possible mechanism could be neuropharmacological toxicity that might involve interaction between the studied heavy metals and calcium or zinc, this may result in interference with neurotransmission at the synapse.Lead and cadmium has the capacity to induce synergistic toxicity in astrocytes that may compromise the BBB and may cause behavioral dysfunction in developing rats [9]. Cadmium exposure and accumulation in the body start at young age, one of the heavy metals, is an important environmental po`llutant and a potent toxicant to organism. It poses a severe threat to the growth of the organism, and also has been recognized as a human carcinogen [10]. Also oxidative stress has been proposed as a possible mechanism involved in Cd toxicity. Prolonged exposuretocadmiumcausestoxic effectdue to the accumulationover timein a variety oftissues, such askidney, liver and central nervous system(CNS),andperipheralsystemsof neurons. Cd can be uptaken from the nasal mucosa or olfactory pathways into the peripheral and central neurons; for the latter and can increase the blood brain barrier (BBB) permeability [11]. Exposure routes in children are mainly via food, environmental tobacco smoke and house dust. Excretion from the body is limited. Cadmium accumulation in the kidney is responsible for effects such as nephrotoxicity and osteoporosis which are observed at adult age [12]. Cadmium exposure through inhalation is also associated with lung cancer in adulthood. Although transfer to the neonate through the placenta and through breast milk is limited, teratogenic and developmental effects were observed in experimental animals [13].

In spite of several publications addressing the action of lead and cadmium on neurotransmitter systems and the associated enzymes, such as monoamine oxidase (MAO), which is a mitochondrial enzyme responsible for the oxidative deamination of a variety of biogenic amines [14] many of these studies are highly variable and lack confirmation [15]. The effect of lead and cadmium depends on various aspects, such as exposure level, duration of exposure, the species of used animals and the developmental stage at which the animals were exposed. [16]. The alterations in functioning of catecholamine could lead to brain dysfunction involving over activation of biogenic amine system [17].This is in agreement with Baksi and Hughes [18] who studied the effect of high lead level doses on the activity of catecholamine, nor epinephrine or dopamine and amino acid precursor, tyrosine.Cadmium may also induced cellular damage and lipid peroxidation in rat brain which lead to destruction in most brain enzymes including MAO [19]. The present work aimed to study the alteration in MAO activity in different CNS regions of adult male guinea pigs injected with lead acetate and cadmium chloride with the study of barley plant as diet benefactor against the influence of heavy metals.

2. Materials and Methods

2.1. Experimental Animals

Adult males guinea pigs weighing (600-800g) were purchased from King Abd El Aziz University Research Institute were used in this study . They were housed under normal environmental conditions of temperature (22±2ºC), relative humidity (50±10) ,and 12 photoperiod (12hr. dark and light cycle) . Animals were allowed free excess of food and water. Guinea pigs were divided randomly into five groups with five animals in each group. The first group served as control i.e was injected with saline solution .Animals in the second group were subcutaneous injected with lead acetate (30mg/kg body weight) parallel with the third group which injected with cadmium chloride (1mg/kg body weight.) , fourth group was injected with mixed dose of lead and cadmium. and the last group was feed with aquas solution of barley (5 ml/kg body weight) by gastric tube for two weeks ,followed by injection with mixed dose of lead acetate (30mg/kg body weight) and cadmium chloride (1mg/kg body weight) . The injection with hevy metals was dialy and the decapitation was after 2,4,6 and 8 weeks of injection (lead acetate and cadmium chloride was purchased from Aldrich Chemical co). Approved by the Committee of King Abd El Aziz University for care and use of laboratory animals.

2.2. Tissue Samples

Animals were decapitated, the brain and spinal cord of each animal were immediately removed and the brain was dissected in dry ice into the following regions: cerebral cortex, caudate nucleus - putamen, Thalamus, hypothalamus, superior colliculus, inferior colliculus, cerebellum, pons and medulla.

2.3. Extraction

Tissue samples were weighed and homogenized in 0.2M. Monoamine oxidase and phosphate buffer pH 7.4 was assayed by the method of Krajl [20] as described by Olcese and Vlaming [21] Aliquots of 0.1 ml of Homogeneoustissue were added to 0.25 ml of substrate (1Mm kynuramine) and 1.4 ml of the phosphate buffer 0.2M

(Ph 7.4). The samples were placed for 1 hr .at 37ºC in a shaker bath .Tissue blank for each samples were subjected to the same treatment , but the substrate was added after the incubation period .The reaction was interrupted by the addition of 0.25ml of a 10%ZnSo4 solution followed by 0.25ml of 1N NaOH. After boiling for 2min. The tubes were centrifuged for 10 min.at 3000 r.p.m.The supernatant was then decanted into another tube to which 2ml of 1N

NaOH were added. The fluorescent products ,4-hydroxyquinoline (4-HQ) was measured in a spectrophotofluorometer ,at *315nm* (excitation) and 380(emission).Standard curve of 4-HQ were run in parallel with each assay.Monoamine oxidase activity is expressed as µM4-HQ/hr./g.fresh tissue .

Statistical analysis has been carried out by student t-test to compare between paired groups ,results are expressed as mean ±SD and the statistical test was considered significant at $p<0.05$ and $p< 0.01$levels [22].

3. Results

The changes in MAO activity in various CNS regions of guinea pigs following subcutaneous injection with lead acetate (30 mg./ kg) ,cadmium chloride(1mg/kg),mixed dose of lead and cadmium ,barley plant and mixed dose are presented in (figs 1-10)

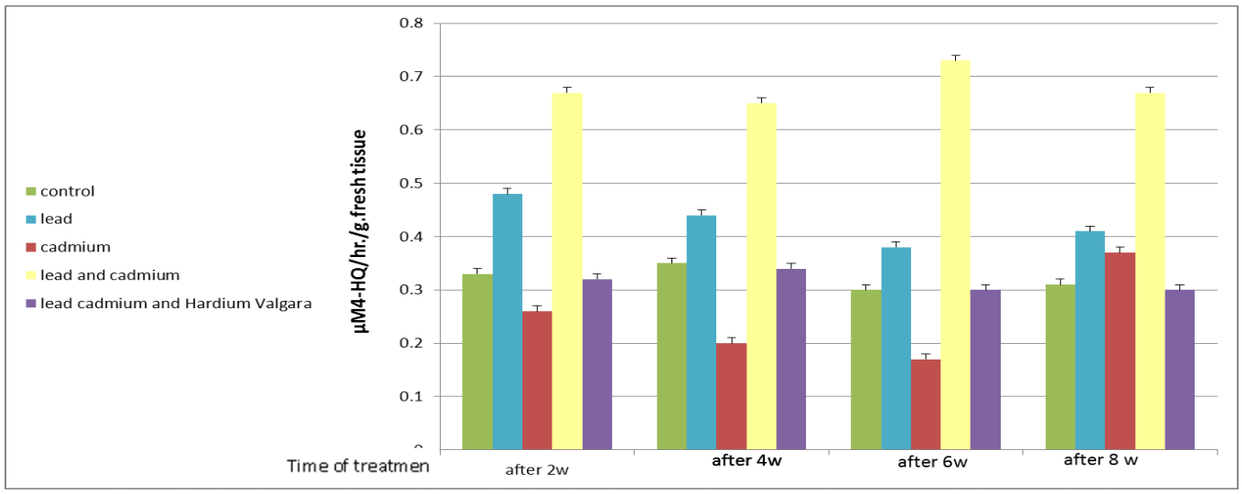

Fig-1. Effect of lead ,cadmium and mixed dose of lead and cadmium on the activity of MAO in cerebral cortex of male guinea pig and treatment of the mixed dose with *Hardium valgara*

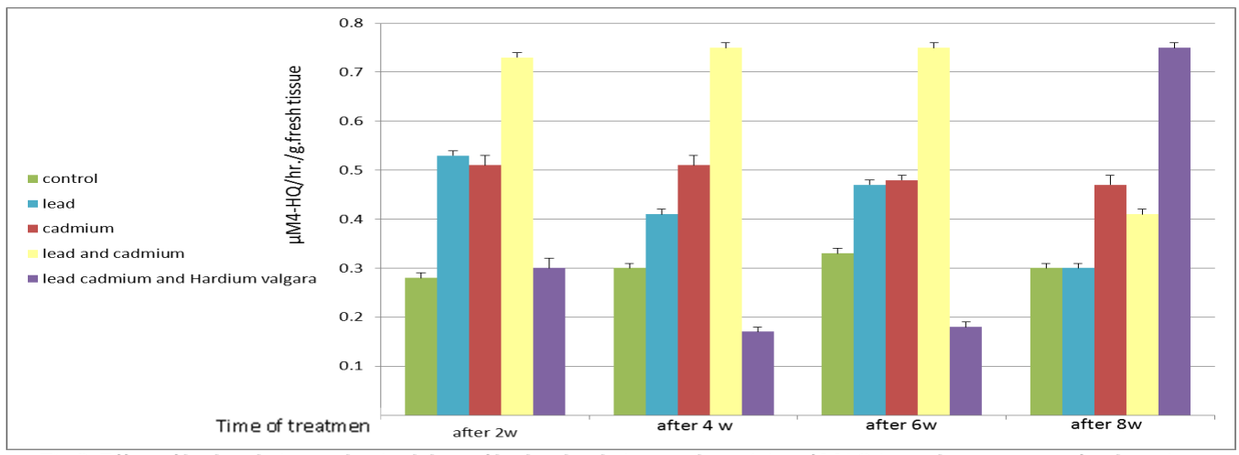

Fig-2. Effect of lead ,cadmium and mixed dose of lead and cadmium on the activity of MAO in caudate-putamen of male guinea pig and treatment of the mixed dose with *Hardium valgara*

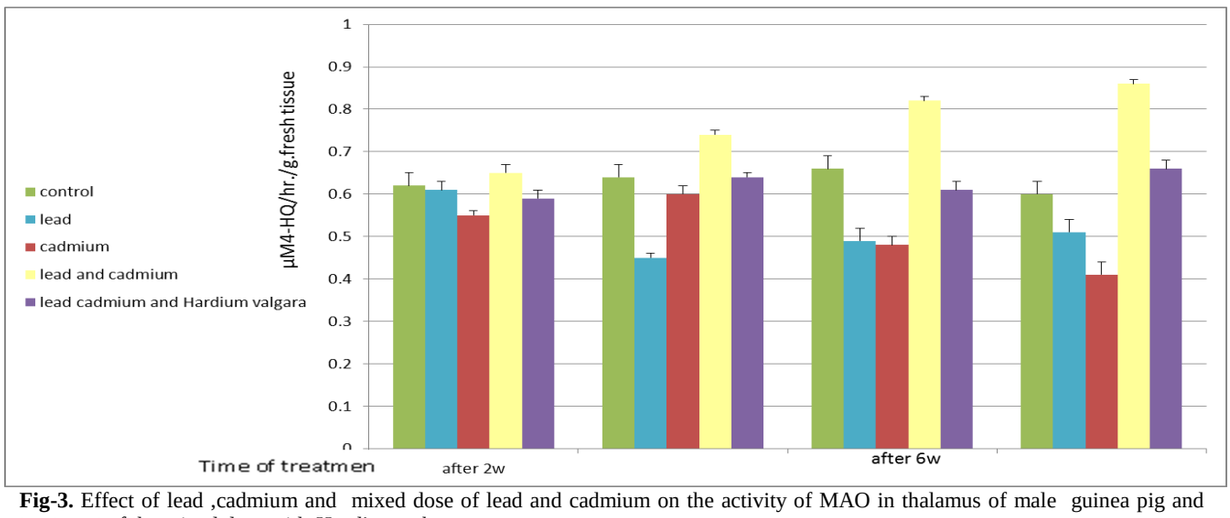

Fig-3. Effect of lead ,cadmium and mixed dose of lead and cadmium on the activity of MAO in thalamus of male guinea pig and treatment of the mixed dose with *Hardium valgara*

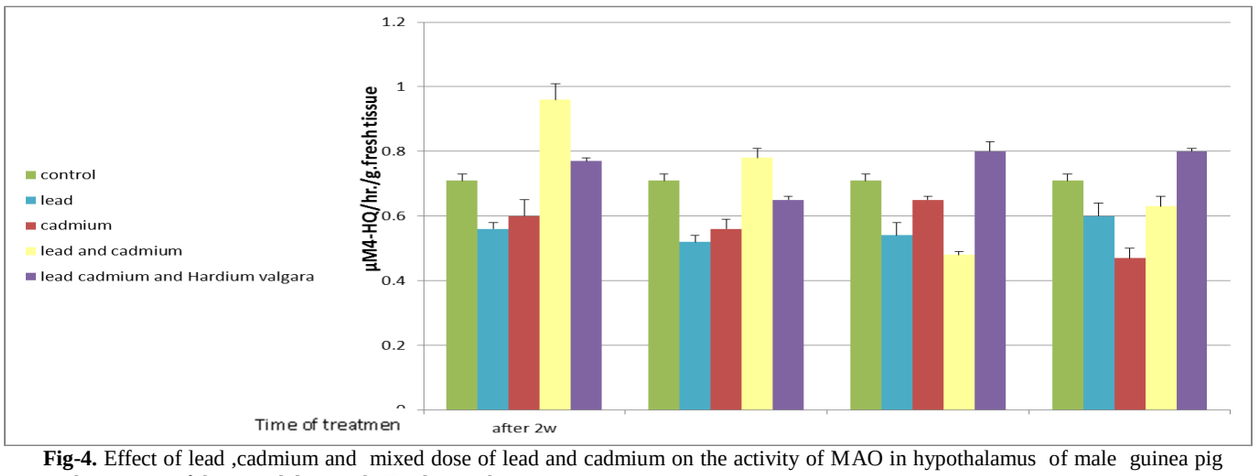

Fig-4. Effect of lead ,cadmium and mixed dose of lead and cadmium on the activity of MAO in hypothalamus of male guinea pig and treatment of the mixed dose with *Hardium valgara*

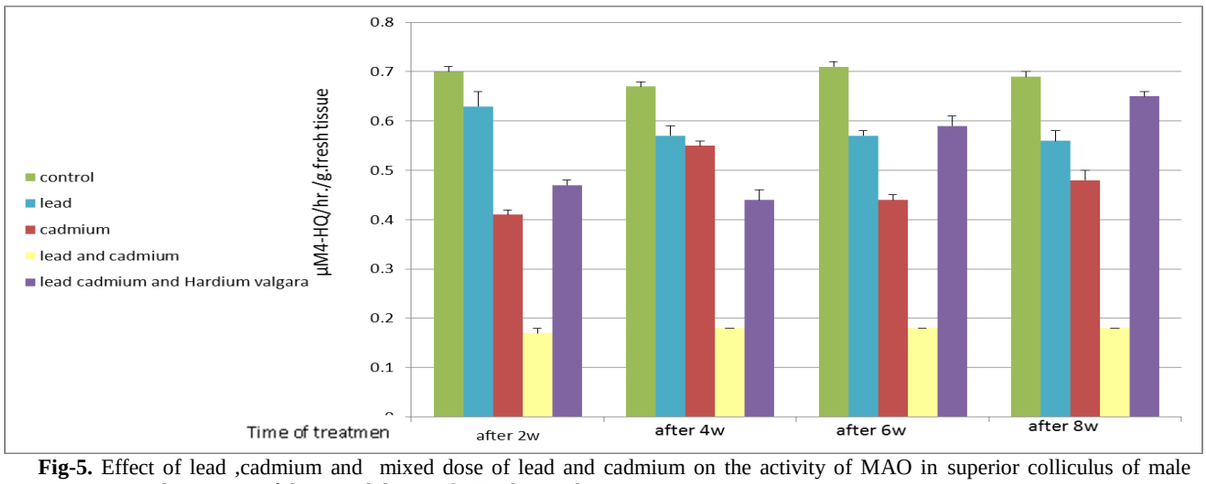

Fig-5. Effect of lead ,cadmium and mixed dose of lead and cadmium on the activity of MAO in superior colliculus of male guinea pig and treatment of the mixed dose with *Hardium valgara*

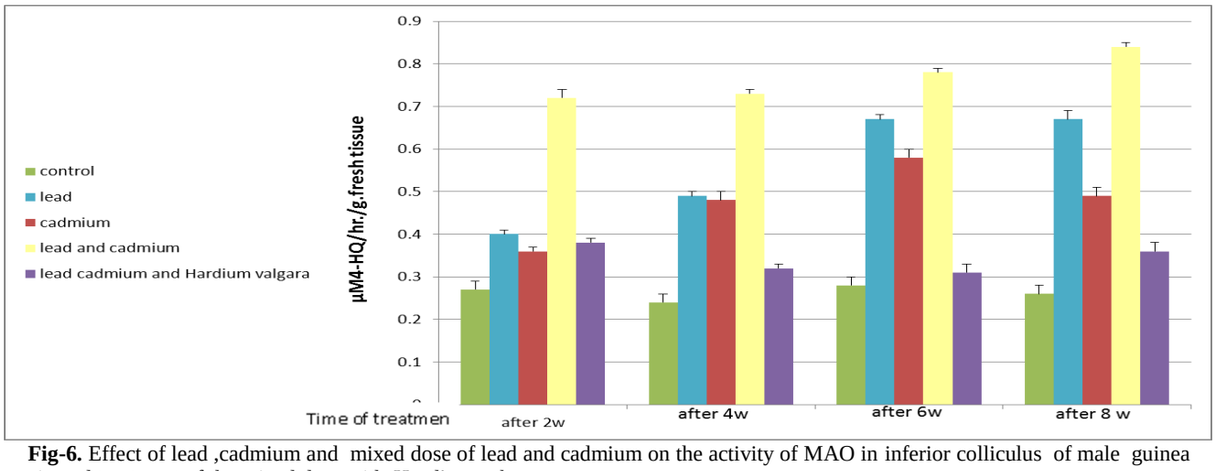

Fig-6. Effect of lead ,cadmium and mixed dose of lead and cadmium on the activity of MAO in inferior colliculus of male guinea pig and treatment of the mixed dose with *Hardium valgara*

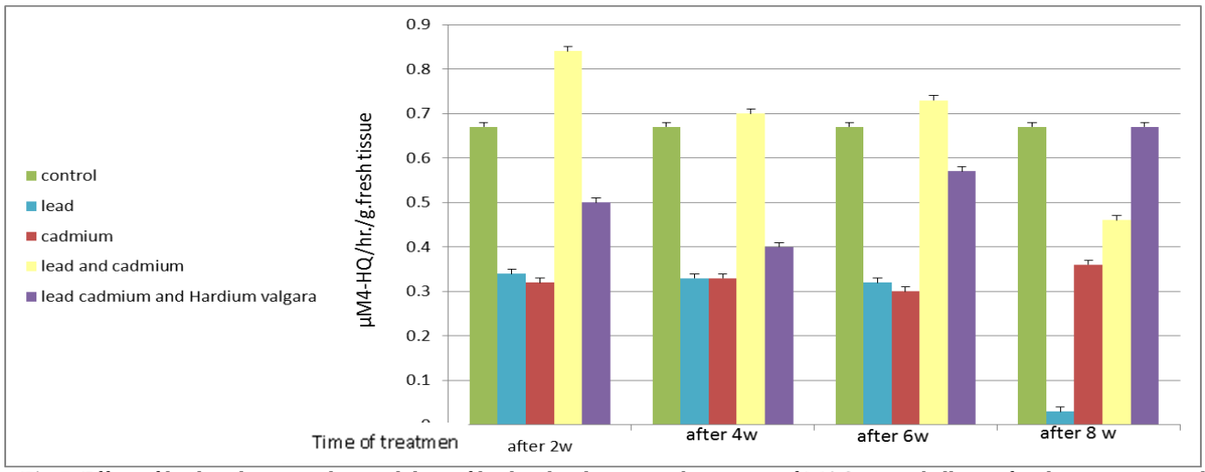

Fig-7. Effect of lead ,cadmium and mixed dose of lead and cadmium on the activity of MAO in cerebellum of male guinea pig and treatment of the mixed dose with *Hardium valgara*

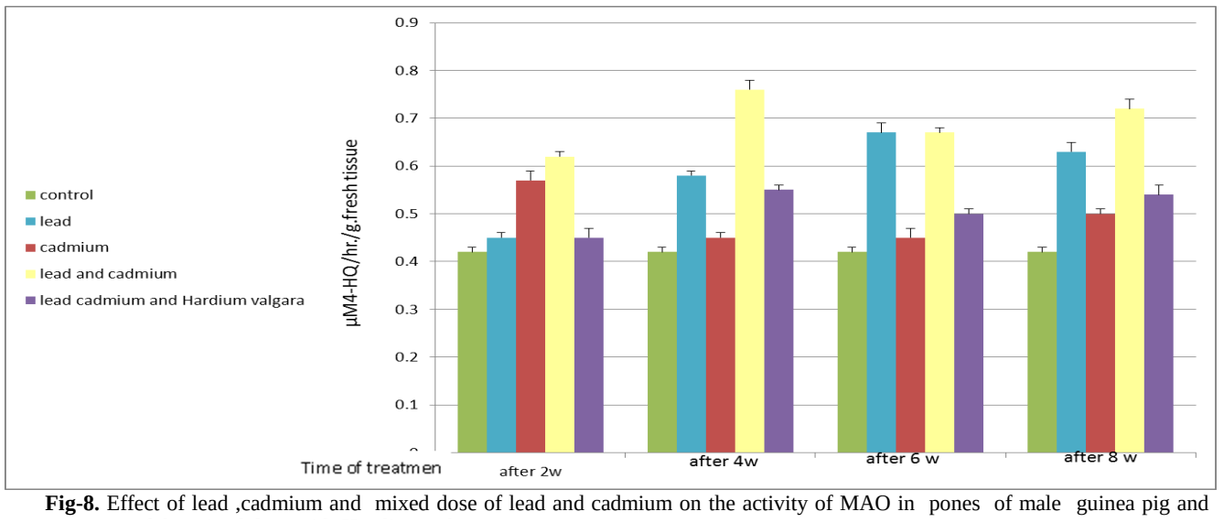

Fig-8. Effect of lead ,cadmium and mixed dose of lead and cadmium on the activity of MAO in pones of male guinea pig and treatment of the mixed dose with *Hardium valgara*

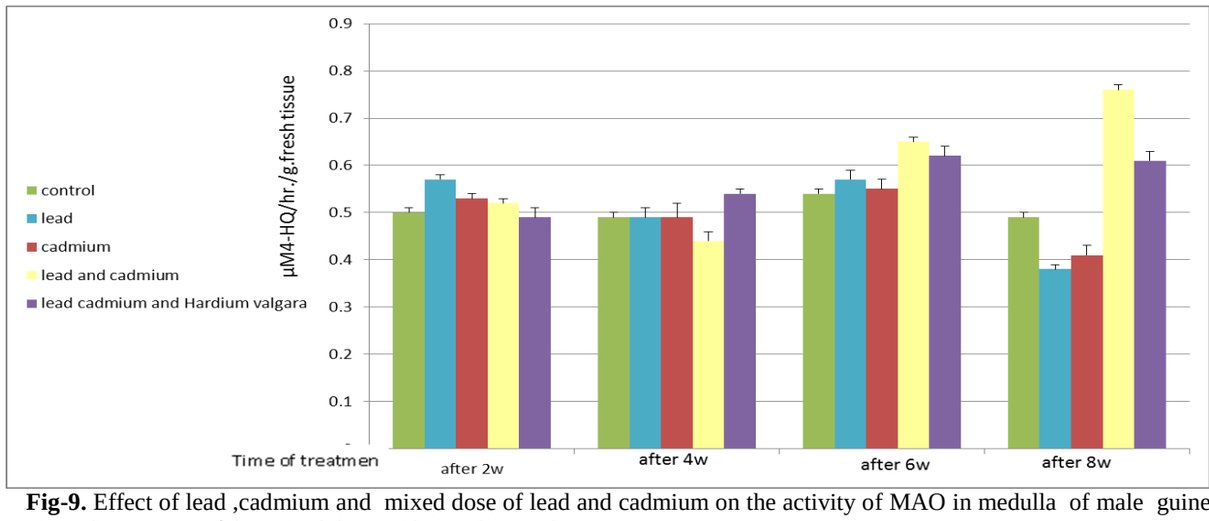

Fig-9. Effect of lead ,cadmium and mixed dose of lead and cadmium on the activity of MAO in medulla of male guinea pig and treatment of the mixed dose with *Hardium valgara*

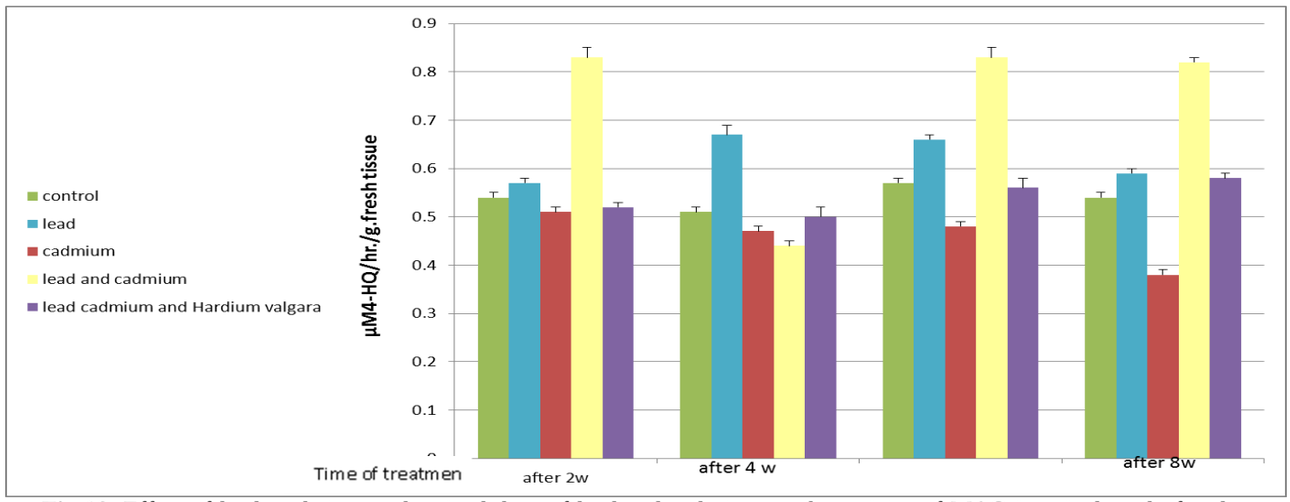

Fig-10. Effect of lead ,cadmium and mixed dose of lead and cadmium on the activity of MAO in spinal cord of male guinea pig and treatment of the mixed dose with *Hardium Valgara*

The present results in (Fig. 1) showed that the subcutaneous injection with lead and mixed dose provoked an outstanding increase in the MAO activity in cerebral cortex while cadmium induced general decrease, However , the enzyme activity of the caudate putamen (fig. 2) exhibited a marked increase after all times of injection , in the thalamus and hypothalamus (fig.3 and 4) the decrease was also noticed . In (Fig. 5) MAO was affected by lead and cadmium administration, activity of this enzyme was decreased when compared to the controls .However a very highly decline occurred in the most time interval. In (fig6)The activity of MAO of guinea pig inferior colliculus was significantly increased in all times of experimental groups when compared with the control. A significant decrease in MAO activity occurred also in cerebellum in all times of experiments (fig. 7).Variable changes was occurred in pons,medulla and spinal cord (fig.8,9 and 10), improvement was noticed in all groups which received barley plant.

4. Discussion

Among heavy metal exposures, lead and cadmium exposure is one of the most common exposures that can lead to significant neuropsychological and functional decline in humans. MAO have the ability to limit the action of many important neurotransmitters, oxidatively deaminate neurotransmitter and xenobiotic amines thereby setting up the

basis of rapid repetitive response [23]. MAO plays an important role in catabolizing the neuroactive amines [24]. The general decrease of the activity of these enzyme observed after the treatment with lead and cadmium suggested that lead and cadmium is able to reach the CNS and impair its function .Lead and cadmium has been found to be a causative agents of various sorts of disorders, including neurological, nephrological, immunological, cardiac, motor, reproductive and even genetic [25]. Specific transporters take up metals at the apical surface and export them at the basolateral surface, and are involved in their intracellular distribution [26].

Teresa, et al. [27] determined the level of acid phosphatase, alkaline phosphatase, catalase, acetylecholinesterase and ATPase after lead and cadmium administration and suggested that both metals exert an additive effect, either competing for the same inhibitory binding sites or increasing oxidative damage in the CNS.

The data of the present work showed that, lead and cadmium administration experimented provoked variable changes in MAO in the CNS regions of guinea pigs, which may be due to that lead resulted alterations in membrane bound enzymes, alteration in the level of neurotransmitters receptors which in turn affects on membrane bound enzymes and these data agreement with Reddy, et al. [28]. The results of the present study showed that lead-injection altered the aminergic system with a decrease in mitochondrial MAO activity and this converging agreement with studies of Zhu, et al. [29]. Another evidence suggested that lead can also enter the neuronal intracellular environment, and may activates the calcium mediated synaptic vesicle release mechanisms [30]**.**

Karadas, et al. [31] were found the ability of lead and or cadmium to penetrate and damage the blood-brain barrier very rapidly, leading to a dysfunction of the blood-brain barrier system .

Gnerre., et al. [32] and Swaran, et al. [33] were studiednumbers of chemical compounds which considered as a potent inhibiter of MAO activity in vertebrate tissues and they were found that all experimental animals were depressed under lead injection which may be due decreased of the neurotransmitters and this agreement with Leret, et al. [34] which examined the effects of early simultaneous exposure to low level of lead and cadmium on anxiety – like behavior in the rat and on monoamine levels in different CNS regions and showed an increased in indices of anxiety and change in behavior which related to alteration in serotoninergic and dopaminergic receptors.

In the present study, a significant decrease in MAO activity was noticed in most CNS regions. The decrease in both MAO in CNS regions may be due to cellular damage induced by cadmium and this assumed by studies of Alfano, et al. [35]. The high affinity of lead for sulfhydral groups in enzymes may led to decrease in MAO and catacholamines. This may notbe a direct inhibitory action of lead but a consequence of the inhibitory effects on cholinergic system [28]. Lead also crosses blood brain barrier in developing animals ,disrupts the main structural components of blood-brain barrier by injuring the brain glial cells. Also it competes with calcium for common binding sites and is incorporated into calcium transport systems in nervous system, where it is important for neurotransmitter release and regulation [36] .A significant depletion of MAO observed in the present study also reported by Saxena and Flora [37]. According to Shaffi [38] the alterations in the activity of MAO may produce marked changes in the CNS function as a responses to the contamination of the brain with the metal in different manner. The general decrease of the activity of enzymes observed in the experimented animals after treatment with lead and cadmium suggest that these metals able to reach the CNS and impair its function by neurochemical changes such as increasing oxidative damage in the CNS [39, 40]. Experiments carried out by Sabbioni and Marafanta [41] with fractional liver mitochondria from rats injected with a tracer dose of lead revealed that most of the radioactivity was associated with inner membrane mitochondria and itsfractions **.** MAO, which is also considered to be interneuronal enzymes, located largely in the outer membrane of mitochondria or in the exoplasm .

The specific localization of MAO and the accumulation of lead in the mitochondria could act as an inhibitor of the enzyme [42] . Lead may led to alterations in membrane fluidity, which in turn result inactivation of membrane bound enzymes, changed ion permeability and alteration in the levels of neurotransmitter receptors, which in turn may result into alteration of membrane function [43] and Govinder and Prahald [44]. The brain is also considered as a sensitive organ prone to oxidative damage because of its low levels of protective enzymes to eliminate free radicals resulted from lead intoxication ,may led to brain damage and change in the activity of the related enzymes [45]. An impairment of the cholinergic function was described in lead and cadmium treated animals ,including alterations in neuorotransmitters, Suszkiw [46].

The results obtained in the present work suggest that the decrease of degrading enzyme activities as in the case of MAO following lead and cadmium administration in thalamus andhypothalamus area may be led to intoxication and neurobehavioral changes such as cognitive and attention deficit as well as hyperactivity ,which is commonly observed both in lead and cadmium intoxication and perturbed nonaminergic neurotransmission . Sanah , et al. [47] explain the decrease of MAO in most CNS regions under the effect of Cadmium administration as ,cadmium have neurotoxic effects, via damages cells of the cerebellar cortices of young rats as well as rabbits. Simultaneous injection of lead and cadmium in a mixed dose increased monoamine oxidase activity all ten parts of rat CNS when compared to treated group. In overall, it has been found that barley may have a protective-like ability to hinder lead and cadmium toxicity in the central nervous system. Barley grass is rich in vitamins and minerals, has antioxidant properties, and has been shown to reduce neurotoxicity of the heavy metals and also used as a cancer preventive,detoxification of pollutants, protection against solar and other forms of radiation, and boosting energy and immunity [48]. Treatment with barley increases monoamine oxidase activity and contents of all central nervous system regions. When male guinea pig were feed aqueous barley solution dose (5 mg) for two weeks and then treated with a mixture of lead and cadmium occurred a significant improvement of the enzyme monoamine oxidase in each of the cerebral cortex, , caudate nucleus and putamen, thalamus , hypothalamus, superior colliculus , inferior,cerebellum cerebellum, pones and spinal cord when compared to the group exposed to a mixture of lead and cadmium . This results was agree with the study by Bawazir [49] which found that the use of barley (oral) in albino mice has led to an improvement in the level of neurotransmitters (DA, GABA and 5-HT) in different

Table-1. Comparison of ALP, ASAT and ALAT activities (mean±SD) in the serum of rats in the control (G1), in Hg (G2) and in Hg+*ascorbic acid* (G3) after 5 consecutive weeks.

	Group1	**Group 2**	**Group 3**
ALP (IU/L)	63.4 ± 4.6 (a)	40.2± 3.8	52.2 ± 4.2 (d)
ASAT (IU/L)	24.1 ± 2.8 (a)	37.8± 3.4 (c)	32.8± 2.4 (d)
ALAT(IU/L)	31.5± 3.4	28.8 ± 1.6	29.2± 2.2

Letters differ significantly at p<0.05. a: G1 vs G2 c: G2 vs G3; d: G1 vs G2 vs G3

Table-2. Comparison of urea, creatinine and uric acid concentrations (X±SD) in the serum of male rats in the control (G1), in Hg (G2) and in Hg+*ascorbic acid* (G3) after 5 consecutive weeks.

	Group1	**Group 2**	**Group 3**
Urea (g/L)	0.25± 0.06 (a)	0.38± 0.038	0.34 ± 0.04 (d)
Creatinine (mg/L)	9.4± 1.8	10.2 ± 2.4	10.3 ± 2.6
Uric acid (mg/L)	11.1 ± 2.4	11.3±2.1	10.8± 1.6

Letters differ significantly at p<0.05. a: G1 vs G2; d: G1 vs G2 vs G3

Table-3. Comparison of triglycerides, total cholesterol, HDL- and LDL- Cholesterol concentrations (X±SD) in the serum of male rats in the control **(G1), in Hg (G2) and in Hg+*ascorbic acid* (G3) after 5 consecutive weeks.**

	Group1	**Group 2**	**Group 3**
Triglycerides (g/L)	1.14 ± 0.086	1.21 ± 0.082	1.32 ± 0.048
Total cholesterol (g/L)	0.54 ± 0.08	0.51 ± 0.034	0.56 ± 0.0.42
HDL- cholesterol (g/L)	0.22 ± 0.034	0.19± 0.046	0.21 ± 0.022
LDL-cholesterol (g/L)	0.12 ± 0.008	0.14 ± + 0.004	0.12 ± 0.006

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