THE EFFECT OF N- ACETYLCYSTEINE AND L-METHIONINE ALONE OR COMBINED WITH ASCORBIC ACID AND α-TOCOPHEROL ON OXIDATIVE STRESS INDUCED BY LEAD IN MALE ALBINO RATS

BY

Essam M. Ali, Adel A. Zalata, Adel M. EL-Mansoury, Ibrahim E. Mohamed** and Afaf M. Attia*

Departments of Forensic Medicine and Clinical Toxicology , Biochemistry, Pathology**, Faculty of Medicine, Mansoura University, Egypt*

ABSTRACT

The study was carried on 140 male Albino rats which were divided into 14 groups (10 rats each) . The negative control group was given normal saline by intraperitoneal route. Group I (positive control) was given lead acetate in a dose of 1 mg/kg/day (1/200 of LD_{50}) intraperitonealy daily for 8 weeks. Groups IIa, IIIa, IVa, Va, VIa, VIIa received antioxidant before the daily injection of lead acetate for 8 weeks. Groups IIb, IIIb, IVb, Vb, VIb, VIIb received antioxidant daily in the ninth week i.e. after giving lead acetate in daily injection for 8 weeks. N-acetylcysteine was given by oral gavage in a dose of 800 mg/kg/day. Each of L-methionine, ascorbic acid and α-tocopherol was given by oral gavage in a dose of 100 mg/kg/day. Antioxidants were given as follow; N-acetylcysteine in groups IIa and IIb, L-methionine in groups IIIa and IIIb, combination of N-acetylcysteine, ascorbic acid and α-tocopherol in groups IVa and IVb, combination of L-methionine, ascorbic acid and α-tocopherol in groups Va and Vb , combination of N-acetylcysteine and L-methionine in groups VIa and VIb , combination of N-acetylcysteine , L-methionine, ascorbic acid and α-tocopherol in groups VIIa and VIIb. Rats were sacrificed at the end of experiment by cut throat and blood samples were obtained for determination of Thiobarbituric Acid Reactive Substance (TBARS), glutathione peroxidase, superoxide dismutase, blood lead level, total thiol and total protein concentrations. Brain was immediately excised and prepared for histopathological examination. The results showed that antioxidants significantly decreased all lead induced oxidative stress as well as the blood lead level, but not to the initial values of the control groups. The use of antioxidants combined together before lead exposure showed the best response than other groups. Furthermore, the histopathological changes observed in the brain sections of lead exposed rats which were pretreated with the different antioxidants were found to be less marked than those found in lead exposed rats which post treated with the different antioxidants.

INTRODUCTION

Several studies have reinforced that exposure to lead remains an important hazard worldwide (Waalkes et al., 1995). Exposure to lead, particularly through its wide commercial use, results in a variety of lesions. In addition to disturbances of the central nervous system, impairment of haematopoiesis, and cardiovascular damage, lead has been shown to induce biochemical alterations in the liver and kidney (Tian and Lowrence, 1995). Exposure to lead produces wide range of toxic biochemical effects in man and experimental animals despite mandated reduction in environmental lead; such exposure still poses a public health hazard for children, with devastating effects on CNS development (Oberto et al., 1996).

It is well documented that lead deposition inside the cells is associated with an increased elimination of free radicals (Hsu et al., 1998-a). Gürer and Ercal (2000) have found that lead causes oxidative stress by inducing the generation of reactive oxygen species, reducing the antioxidant defense system via depleting glutathione, inhibiting sulfhydryl-dependent enzymes, and interfering with some essential metals needed for antioxidant enzyme activities.

The potential role for oxidative stress associated with lead poisoning suggests that antioxidants may enhance the efficacy of treatment protocols designed to mitigate lead-induced toxicity (Patra et al., 2001).

Nature has evolved several antioxidants that act in a combined fashion to protect the biological systems against possible oxidant damage effects of heavy metals and noxious stimuli through free radical reaction mediated injury (Hsu et al., 1998-b; Carr and Frei, 2000). These antioxidants include vitamin E (α- tocopherol) which is lipid soluble, non-enzymatic antioxidant that checks the lipid peroxidation through limiting the propagation of chain reaction of lipid peroxidation. It acts as the major lipid soluble antioxidant in cell membrane (Clarkson and Thompson, 2000; Fernandez-Cabezudo et al., 2003). Also, vitamin C (ascorbic acid) scavenges the aqueous reactive oxygen species (ROS) by very rapid electron transfer that thus, inhibits lipid peroxidation. But, the beneficial effect of ascorbic acid on lead concentrations is still inconclusive (Houston and Johnson, 2000).

Another antioxidant is N-acetylcysteine (NAC). It is a thiol-containing antioxidant which has been utilized to mitigate various conditions of oxidative stress. Its antioxidant action is believed to originate from its ability to stimulate glutathione (GSH) synthesis, therefore maintaining intracellular GSH levels and scavenging reactive oxygen species (Yip et al., 1998).

Moreover, L-methionine is a sulfur containing amino acid with non-polar group. After loosing its methyl group, it yields homocysteine which combines with the amino acid serine to yield cystathionine that undergoes breakdown to cysteine which itself, is a component of glutathione. Glutathione is an antioxidant that protects the cells from oxidative damage and plays vital role in detoxification. In addition, the thiol group of methionine may chelate lead from tissues (Lehninger, 1995; Patra et al., 2001).

The aim of the present work is to study the protective effect of N-acetylcysteine and L-methionine alone, combined together or combined with ascorbic acid and α-tocopherol, against oxidative stress produced by chronic lead exposure in male Albino rats. The best protective effect of these antioxidants is evaluated through biochemical parameters and histopathological examination of the brain after giving them alone or in combination prior to or following lead exposure.

MATERIAL & METHODS

Drugs :

Lead acetate trihydrate was supplied from E.Merck Darmstadt, Germany in the form of extra-pure (99.5-100 %) white crystals. Lead acetate solution was prepared by its dissolution in deionized water (1000 mg dissolved in 500 ml deionized water) i.e. each ml contained 2 mg lead acetate. The dose used in this study was 1 mg/kg/day intraperitonealy. This dose is 1/200 of LD50 of the lethal dose of lead acetate in rats (Patra et al., 2001).

Ascorbic acid: "Redoxon effervescent tablets" each tablet contains 1000 mg vitamin C (F.Hoffmann-La Roche Ltd. Basel, Switzerland TURX 3682/01.96). Ascorbic acid solution was freshly prepared by dissolution in distilled water to give a final concentration of 100 mg/ml.The dose used in this study was 100 mg/kg/day (Patra et al., 2001).

α-tocopherol: "Extra- 1000 sedico capsules" each capsule contains 1000 mg natural wheat germ oil (Sedico Pharmaceutical Co. 6-October City- Egypt). Each capsule was evacuated in insuline syringe (1cm, 40 unit).The dose used was 100 mg/kg/day (Patra et al., 2001).

N-acetyl cysteine: "Acetylcysteine sachets", each sachet contains 200 mg acetyl cysteine (Sedico Pharmaceutical Co. 6-October City- Egypt). N-acetylcysteine solution was freshly prepared by dissolution in distilled water to give a final concentration of 80 mg/ml.The dose used was 800 mg/kg /day (Gürer et al., 1998).

L-methionine: "L-methionine pack" contains 995 gram L-methionine (Pharmaceuticals [ADWIA] S.A.E. 10th of Ramadan

city). L-methione solution was freshly prepared by dissolution in distilled water to give a final concentration of 25 mg/1.5 ml. The dose used was 100 mg/kg /day (Patra et al., 2001).

Animals :

A total of 140 male Albino rats were obtained from the Animal House of Faculty of Medicine, Mansoura University. All the chosed rats were males to alleviate the gender effect on the results. Animals were housed in groups at room temperature on a 12 hour light/ dark cycle. Animals fed a mixture containing bread, bran and ground maize as well as thoroughly washed leafy vegetables and free water supply. They were allowed to acclimatize to their new environment for 10 days prior to initiation of lead with or without antioxidants treatment.

Animals were divided into 14 groups (10 rats each). The negative control group was given normal saline by intraperitoneal route. Group I (positive control) was given lead acetate in daily i.p. injection for 8 weeks. Groups IIa, IIIa, IVa, Va, VIa, VIIa received antioxidant before the daily i.p. injection of lead acetate for 8 weeks. Groups IIb, IIIb, IVb, Vb, VIb, VIIb received antioxidant daily in the ninth week i.e. after giving lead acetate in daily i.p. injection for 8 weeks. Antioxidants was given as following; N-acetylcysteine in groups IIa and IIb, L-methionine in groups IIIa and IIIb, combination of N-acetylcysteine, ascorbic acid and α-tocopherol in groups IVa and IVb, combination of L-methionine, ascorbic acid and α-tocopherol in groups Va and Vb , combination of N-acetylcysteine and L-methionine VIa and VIb , combination of N-acetylcysteine and L-methionine, ascorbic acid and α-tocopherol in groups VIIa and VIIb. Rats were sacrificed at the end of experiment by cut throat. Blood samples were collected from all animals before dissection for obtaining the brain.

Methods :

Each blood sample was divided into two parts. A part was heparinized and used for determination of blood lead level by Atomic Absorption Spectrophotometry according to Kotz et al., (1979) and RBCs content of glutathione peroxidase and superoxide dismutase according to Paglia and Valentine (1967). Another part of blood sample was placed in glass centrifuge tubes without anticoagulant for collection of sera for subsequent estimation of Thiobarbituric Acid Reactive Substance (TBARS) concentration according to the method of Walker and Shah (1988), total thiol concentration according to the method of Hu (1994), and total protein concentration according to the method of Josephson and Gyllenswärd (1975).

Brains of sacrificed rats were prepared for histopathological examination by ordi-

nary microscope according to the method of Drury and Wallington (1980).

Statistical analysis:

The statistical analysis was run on IBM compatible personal computer by using the Statistical Package for Social Scientists (SPSS) program for windows version 10.00 (SPSS Inc., Chicago, IL, USA). The following statistical parameters were utilized; arithmetic mean (x) , standard deviation (SD) , Student's t test , Anova t test, Pearson's correlation coefficient for bivaritate correlation, linear regression and Fisher's exact test. The p. value was considered significant if $p < 0.05$.

RESULTS

In the current study the exposure to lead acetate in a dose of 1 mg/ kg of rat's b.w./day by intraperitoneal route (group I) led to a highly significant increase in blood lead level ($p=0.001$), highly significant elevation in serum TBARS level ($p=0.001$) as well as highly significant decrease in total serum protein concentration, total serum thiol concentration, glutathione peroxidase concentration and superoxide dismutase concentration ($p= 0.001$) at the end of 8^{th} week when compared to that of the control group (Tables 1 - 6) indicating the oxidative stress induced by exposure to the lead acetate.

The current results indicated the protective role of (N-acetylcysteine and L-methionine) either singly , combined together or in combination with ascorbic acid and α- tocopherol before or after lead acetate administration in reducing the lipid peroxidation and lead-induced oxidative stress as evidenced by significant decrease in serum TBARS concentration, increase in total serum protein concentration, increase in total serum thiol concentration, increase in glutathione peroxidase concentration, increase in superoxide dismutase concentration, and decrease in blood lead level in comparison to group I (positive control) (Tables 1 - 6).

The comparison between the prophylactic groups (IIa, IIIa, IVa, Va, VIa, VIIa) and the treatment groups (IIb, IIIb, IVb, Vb, VIb, VIIb), showed significant differences in serum TBARS concentration and total serum protein concentration between all groups ($p<0.05$) (Tables 1 and 2) which means that the effect of all antioxidants is more effective if given before than after lead acetate exposure as regards the oxidative stress on lipid and protein.

As regards total serum thiol concentration, there was significant differences ($p<0.05$) between groups (IVa and IVb), (Va and Vb), (VIa and VIb) and (VIIa and VIIb) ($p<0.05$) . While, there was non significant differences between groups (IIa and IIb) and (IIIa and IIIb) (Table 3).

Significant difference in glutathione peroxidase concentration was observed between groups (IVa and IVb), (VIa and VIb) and (VIIa and VIIb) while there was non significant difference between groups (IIa and IIb), (IIIa and IIIb) and (Va and Vb) and this is explain that N-acetylcysteine (group II), L-methionine (group III) and a combination of ascorbic acid, α-tocopherol, L-methionine (group V) have the same better effect on the lead oxidative stress as regards glutathione peroxidase concentration whether they given before or after lead acetate exposure (Table 4).

Furthermore, a non significant difference in superoxide dismutase concentration was observed between groups (IIa and IIb), (IIIa and IIIb), (IVa and IVb) , (Va and Vb), (VIa and VIb) and (VIIa and VIIb) (Table 5).

In addition, there was significant difference in blood lead levels between groups (Va and Vb), (VIa and VIb) and (VIIa and VIIb), while non significant difference between groups (IIa and IIb), (IIIa and IIIb) and (IVa and IVb) were observed (Table 6).

In the current work, there was significant negative correlation of blood lead level and the biochemical parameters (total serum protein, total serum thiol, glutathione peroxidase, superoxide dismutase concentrations). Also, there was significant positive correlation of blood lead level and serum TBARS (Table 7).

In the present study the histopathological findings in the brain sections of rats in group I (positive control) showed cerebral oedema (60%) , neuronal degeneration forming Rosenthal fibers (90%) , necrosis (80%) , congestion in blood vessels (70%) , peri-vascular lymphocytic infiltration (100%) , intraventricular and sub-dural haemorrhages (70%). The use of antioxidants (before and after lead acetate exposure) resulted in improvement of the histological changes in the brain but the improvement was more better in prophylactic groups led to non significant difference comparing to control group while the treatment groups showed significant difference in some pathological parameters when compared to control group (Table 8 and Figures 1 and 2) .

DISCUSSION

Lead is a pervasive environmental pollutant with no beneficial biological role. The higher concentration of lead in different tissues and RBCs following occupational or experimental exposure was associated with increased oxidative reaction which might be responsible for lead- induced toxic effects (Patra and Swarup, 2000).

It is well known that lead deteriorates cellular functions through increasing the production of reactive oxygen species (ROS) and/or inhibiting antioxidant activities, directly or indirectly leading to oxidative injuries (Hsu et al., 1998-b , Hamed et al., 1999). Oxidative stress is proposed as a molecular mechanism in lead toxicity, which suggests that antioxidants might play a role in the treatment of lead poisoning (Gürer et al., 2001).

Regarding the biochemical changes, in the current study the highly significant increase in blood lead level and serum TBARS level as well as highly significant decrease in total serum protein concentration, total serum thiol concentration, glutathione peroxidase concentration and superoxide dismutase concentration in rats received lead acetate intraperitoneally indicates the oxidative stress induced by exposure to the lead. Supportting these results are the studies of El-Missiry (2000), Pande and Flora (2002) and Sivaprasad et al. (2003).

The current results indicate the protective role of N-acetylcysteine and L-methionine alone, combined together or combined with ascorbic acid and α- tocopherol either before or after lead acetate administration in reducing the lipid peroxidation and lead-induced oxidative stress. This is evidenced by significant decrease serum TBARS concentration, increase in total serum protein concentration, total serum thiol concentration, glutathione peroxidase concentration and superoxide dismutase concentration, and decrease in blood lead level in comparison to positive control group.

It has long been known that sulfhydryl-containing compounds have the ability to chelate metals. The sulfur-containing amino acids methionine and cysteine, N-acetylcysteine, S-adenosylmethionine, alpha-lipoic acid, and the tri-peptide glutathione (GSH) all contribute to the chelation and excretion of metals from the human body (Miller, 1998). Also, Tandon et al. (2002) explained that the reversal of lead-induced changes in blood and brain by N-acetylcysteine was independent of its lead mobilizing capability, but ought to be due to its strong antioxidant property.

Flora et al. (1991) stated that methionine was effective in reducing the accumulation of lead in blood, liver and kidney and they concluded its use as preventive was more effective than treatment after lead intoxication exposure which may be caused by a decrease in the absorption of lead from the gastrointestinal tract.

Regarding ascorbic acid effects, Dawson et al. (1999) concluded that ascorbic acid may provide an economical and convenient method of reducing blood lead levels, possibly by reducing the intestinal

absorption of lead. In addition, Fotherby et al. (2000) stated that it forms the first line of antioxidant defense and further it has been shown to protect the membrane and other cellular compartments by regenerating the antioxidants form of α- tocopherol. Tandon et al. (2001) stated that the daily intake of ascorbic acid may prevent the accumulation of lead and reduce its toxic effects particularly in those regularly exposed to lead.

In addition, Hathcock et al. (2005) reported that robust database shows that dietary supplements of ascorbic acid and α- tocopherol are safe for the general population. Because these nutrients supply antioxidant and other functions for homeostasis and protection against free radical damage, supplementation has been intensively needed.

In disagreement of our results, Mori and Hirayama (2000) concluded that excess L-methionine intake may induce oxidative stress in the liver if used in a high dose (16 g/kg diet) for 1, 3, 6 and 9 months. In addition, Pande et al. (2001) stated that the administration of N-acetylcysteine alone was mildly effective in preventing lead absorption in the blood and tissues.

The comparison between the prophylactic groups (subgroups a) and the treatment groups (subgroups b), showed a significant differences in serum TBARS concentration and total serum protein concentration between all groups which means that the effect of all antioxidants is more effective if given before than after lead acetate exposure as regards the oxidative stress on lipid and protein. N-acetylcysteine and L-methionine have the same better effect on the lead oxidative stress as regards total serum thiol concentration whether they given before or after lead acetate exposure . N-acetylcysteine, L-methionine and a combination of ascorbic acid, α-tocopherol, L-methionine have the same better effect on the lead oxidative stress as regards glutathione peroxidase concentration whether they given before or after lead acetate exposure . Antioxidants have the same better effect on the lead oxidative stress in all groups as regards superoxide dismutase concentration whether they given before or after lead acetate exposure .

The previous results indicate that the use of antioxidants as a prophylaxis considered better than its use as a treatment and this may be explained by the short period that the treated test groups are exposed to the antioxidants (7 days). These results are in accord with the studies of Dhawan et al. (1989) and Pande et al. (2001).

In the present work, the groups received different combined antioxidants be-

fore lead acetate exposure showed improvement in the biochemical parameters and the best response in biochemical parameters was observed with the group VIIa that received combination of ascorbic acid, α-tocopherol, N-acetylcysteine and L-methionine before lead acetate exposure than other groups received combined antioxidants before lead acetate exposure (groups IVa, Va and VIa).

In agreement of the current study, Christen (2000) stated that antioxidants work best in combination. Although there is value in supplementing with extra amounts of one or two antioxidants, better results are always obtained when a "cocktail" is administered. The reason is that different antioxidants neutralize different free radicals.

In the current work, serum TBARS is the most significant predictor affect lead followed by total serum thiol concentration, glutathione peroxidase and superoxide dismutase. The significant negative correlation of blood lead level and the biochemical parameters (total serum protein, total serum thiol, glutathione peroxidase, superoxide dismutase concentrations) and the significant positive correlation of blood lead level and serum TBARS coincide with the study of Siddiqui et al. (2002).

In the current study the histopathological findings of the brain observed in rats received lead acetate (positive control group) was improved with the use of antioxidants (before and after lead acetate exposure) but the improvement was more better in prophylactic groups similar to results obtained by Kelly (1998), Upasani and Balaraman (2001) and Shalan et al. (2005).

Finally a study done by Banerjee and Bagchi (2001) reviewed that health is our most important asset and one of the most effective means of protecting health is by taking a complimentary combination of antioxidants. The finest antioxidant formula provides an excellent combination of antioxidants that are highly effective in guarding our bodies from a wide spectrum of illnesses caused by toxic chemicals, poisons, poor diet, polluted air and water, stressors, and free radicals. It stimulates the body's immune response to help overcome existing disorders. Antioxidants are uniquely different from one another and each may have a specific function in the body. However, they are also synergistic, and will work most effectively when they are used together. In the proper combination, they can perform a wide range of metabolic activities, free radical scavenging and preventative actions.

CONCLUSION & RECOMMENDATIONS

It is concluded from this work that lead exposure results in varying degree of oxi-

dative stress with tissue specific changes. In addition serum TBARS, total serum thiol concentration, glutathione peroxidase levels and superoxide dismutase levels can act as significant predictors that reflect blood lead level. It can be concluded also that supplementation with adjusted doses of antioxidants N-acetylcysteine, L-methionine, ascorbic acid and α-tocopherol in combinations as pretreatment has the most protective role in lead exposure.

It is recommended that high risk individuals who are more vulnerable to the toxic effects of lead must increase consumption of diets rich in vitamin C and vitamin E as well as sulfur-containing foods, such as eggs, garlic, and onion. In addition they must be supplemented with extra amounts of one or two antioxidants, better in a "cocktail" administered because different antioxidants neutralize different free radicals.

Table (1): Statistical comparison of serum Thio Barbituric Acid Reactive Substance (TBARS) concentrations (nmol/ml) in all studied groups.

TBARS nmol/ml	Control	Group I	Group IIa	Group IIb	Group IIIa	Group IIIb	Group IVa	Group IVb	Group Va	Group Vb	Group VIa	Group VIb	Group VIIa	Group VIIb
Mean	0.88	6.05	3.25	4.3	3.43	4.13	2.12	4.02	2.2	3.9	2.25	3.9	1.76	3.95
SD	0.47	1.01	0.56	0.67	0.75	0.76	0.42	0.68	0.79	0.53	1.05	0.56	0.46	0.64
t_1		14.66	10.23	13.19	9.14	11.57	6.29	11.99	4.52	13.49	3.77	13.18	4.24	12.21
p		0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
t_2			7.64	4.55	6.57	4.8	11.34	5.25	9.5	5.96	8.24	5.85	12.18	5.54
p			0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
t_3			3.78		2.07		7.49		5.67		4.42		8.73	
p			0.001*		0.05*		0.001*		0.001*		0.001*		0.001*	

t_1: Student's *t* test with control negative group

t_2: Student's *t* test with control positive group (group I).

t_3: Student's *t* test with subgroups a and b of each group.

*Significance at ($P<0.05$).

Table (2): Statistical comparison of total serum protein concentrations (g/dl) in all studied groups.

Protein g/dl	Control	Group I	Group IIa	Group IIb	Group IIIa	Group IIIb	Group IVa	Group IVb	Group Va	Group Vb	Group VIa	Group VIb	Group VIIa	Group VIIb
Mean	15.38	3.27	8.98	6.55	8.54	6.34	9.75	6.74	9.37	6.42	9.45	6.76	11.5	6.9
SD	2.07	1.26	1.16	1.74	1.1	1.32	0.89	1.64	1.21	1.4	0.74	1.74	2.51	1.75
t_1		15.81	8.55	10.35	9.33	11.66	7.92	10.36	7.95	11.32	8.54	10.09	3.76	9.89
p		0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
t_2			10.52	4.84	10.11	5.32	13.26	5.31	11.05	5.26	13.37	5.13	9.22	5.36
p			0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
t_3				3.67		4.102		5.103		5.03		4.5		4.72
p				0.002*		0.001*		0.001*		0.001*		0.001*		0.001*

t_1: Student's *t* test with control negative group

t_2: Student's *t* test with control positive group (group I).

t_3: Student's *t* test with subgroups a and b of each group.

*Significance at ($P<0.05$).

Table (3): Statistical comparison of total serum thiol concentrations (mmol/L) in all studied groups.

Thiol mmol/L	Control	Group I	Group IIa	Group IIb	Group IIIa	Group IIIb	Group IVa	Group IVb	Group Va	Group Vb	Group VIa	Group VIb	Group VIIa	Group VIIb
Mean	1.63	0.13	0.79	0.53	0.69	0.53	0.88	0.57	0.98	0.65	0.97	0.63	1.18	0.71
SD	0.33	0.1	0.39	0.24	0.33	0.3	0.43	0.23	0.48	0.21	0.42	0.3	0.46	0.2
t_1		13.9	5.25	8.57	6.42	7.9	4.39	8.41	3.6	8.02	3.94	7.2	2.56	7.57
P		0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.02*	0.001*
t_2			5.11	4.86	5.23	3.99	5.4	5.59	5.49	7.16	6.21	5.01	7.05	8.05
P			0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
t_3			1.74		1.19		2.03		1.97		2.14		2.93	
P			0. 1		0.2		0.05*		0.05*		0.05*		0.01**	

t_1: Student's *t* test with control negative group

t_2: Student's *t* test with control positive group (group I).

t_3: Student's *t* test with subgroups a and b of each group.

*Significance at ($P<0.05$).

Table (4): Statistical comparison of glutathione peroxidase concentrations (U/L) in all studied groups.

GPx U/L	Control	Group I	Group IIa	Group IIb	Group IIIa	Group IIIb	Group IVa	Group IVb	Group Va	Group Vb	Group VIa	Group VIb	Group VIIa	Group VIIb
Mean	5765.2	1312.9	3499.6	2801.2	3665.8	2959	4291	2816.9	4535	3580	4835.7	3514.6	5270.7	4054.6
SD	654	282	771	943	872	1146	1210	478	1244	1413	1182	1389	928	998
t_1		19.77	7.1	8.17	6.1	6.73	3.39	11.51	2.77	4.44	2.18	4.64	1.38	4.53
p		0.001*	0.001*	0.001*	0.001*	0.001*	0.003*	0.001*	0.01*	0.001*	0.04*	0.001*	0.1	0.001*
t_2			8.42	4.78	8.12	4.41	7.58	8.58	7.99	4.98	9.17	4.91	12.91	8.36
p			0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
t_3			1.81		1.55		3.58		1.604		2.29		2.82	
p			0.08		0. 1		0.002*		0.1		0.03*		0. 01*	

t_1: Student's *t* test with control negative group
t_2: Student's *t* test with control positive group (group I).
t_3: Student's *t* test with subgroups a and b of each group.
*Significance at ($P<0.05$).

Table (5): Statistical comparison of superoxide dismutase concentrations (U/ml) in all studied groups.

SOD U/ml	Control	Group I	Group IIa	Group IIb	Group IIIa	Group IIIb	Group IVa	Group IVb	Group Va	Group Vb	Group VIa	Group VIb	Group VIIa	Group VIIb
Mean	413	187	275.8	233	271.8	248.5	310.5	289.6	313.6	288.1	300	281.4	338	289
SD	117.5	71.5	45.8	50.9	54.2	47	36.5	53.3	30.9	48.8	49.4	51.5	79.2	44.3
t_1		5.19	3.43	4.14	3.44	4.1	2.62	3.01	2.58	2.58	3.09	2.79	1.67	3.11
p		0.001*	0.003*	0.001*	0.003*	0.001*	0.017*	0.007*	0.019*	0.019*	0.006*	0.012*	0.1	0.006*
t_2			3.31	1.45	2.99	2.27	4.87	3.64	5.139	5.14	3.69	4.12	4.48	3.85
p			0.004*	0.01*	0.008*	0.03*	0.001*	0.002*	0.001*	0.001*	0.002*	0.001*	0.001*	0.001*
t_3			1.6		1.03		1.02		0.001		0.55		1.69	
p			0. 1		0.3		0.3		0.9		0.5		0.1	

t_1: Student's *t* test with control negative group
t_2: Student's *t* test with control positive group (group I).
t_3: Student's *t* test with subgroups a and b of each group.
*Significance at ($P<0.05$).

Table (6): Statistical comparison of blood lead levels (µg/dl) in all studied groups.

Lead µg/dl	Control	Group I	Group IIa	Group IIb	Group IIIa	Group IIIb	Group IVa	Group IVb	Group Va	Group Vb	Group VIa	Group VIb	Group VIIa	Group VIIb
Mean	8.6	56.1	36.4	40.2	34.1	39.2	28.6	34.7	26.9	37.5	27.8	39.3	24.7	32.4
SD	2.17	16.66	7.46	12.4	9	14.46	5.34	18.99	7.36	11.1	7.9	14.4	5.1	8.5
t_1		8.94	11.32	7.93	8.67	6.62	10.98	4.32	7.55	8.11	7.4	6.67	9.22	8.58
p		0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
t_2			3.41	2.42	3.67	2.42	4.97	2.68	5.07	2.94	4.85	2.41	5.7	4.008
p			0. 001*	0.01*	0. 001*	0.01*	0.001*	0.01*	0. 001*	0.01*	0. 001*	0.01*	0.001*	0. 01*
t_3			0.83		0.95		0.98		2.52		2.21		2.46	
p			0.4		0.3		0.3		0.02*		0.04*		0.02*	

t_1: Student's *t* test with control negative group
t_2: Student's *t* test with control positive group (group I).
t_3: Student's *t* test with subgroups a and b of each group.
*Significance at ($P<0.05$).

Table (7): Correlation between blood lead levels and the different biochemical parameters in all groups.

Variables	TBARS conc.	Total thiol conc.	Total protein conc.	GPx conc.	SOD conc.
Blood lead levels	0.582*	- 0.51*	- 0.488*	- 0.428*	- 0.381*
	P=000	P=000	P=000	P=000	P=000

Table (8): Histopathological findings of the brain of studied groups.

Group \ Findings		Cerebral cortex				Vascular changes	
		Degeneration	Necrosis	Oedema	Lymphocytic infiltration	Haemorrhages	Congested blood vessels
Control (n=10)	Occurrence	-	-	-	-	-	+
	%						20
I (n=10)	Occurrence	+	+	+	+	+	+
	%	90***	80***	60**	100***	70**	70**
IIa (n=10)	Occurrence	+	+	+	+	-	+
	%	60**	30	30	60**		40
IIb (n=10)	Occurrence	+	+	+	+	+	+
	%	80***	70**	50*	80***	60**	60**
IIIa (n=10)	Occurrence	+	+	+	+	-	+
	%	50*	20	30	60**		50*
IIIb (n=10)	Occurrence	+	+	+	+	+	+
	%	80***	70**	50*	80***	60**	60**
IVa (n=10)	Occurrence	+	-	-	+	-	+
	%	30			40		30
IVb (n=10)	Occurrence	+	+	+	+	+	+
	%	70**	60**	40	70**	50*	50*
Va (n=10)	Occurrence	+	-	-	+	-	+
	%	40			40		30
Vb (n=10)	Occurrence	+	+	+	+	+	+
	%	70**	70**	30	80***	50*	60**
VIa (n=10)	Occurrence	+	-	-	+	-	+
	%	40			50*		40
VIb (n=10)	Occurrence	+	+	+	+	+	+
	%	80***	60**	40	70**	50*	50*
VIIa (n=10)	Occurrence	+	-	-	+	-	+
	%	10			20		20
VIIb (n=10)	Occurrence	+	+	+	+	+	+
	%	60**	50*	30	60**	40	40

$p< 0.05$* significant.
$p<0.01$** highly significant.
$p<0.001$*** extremely significant.

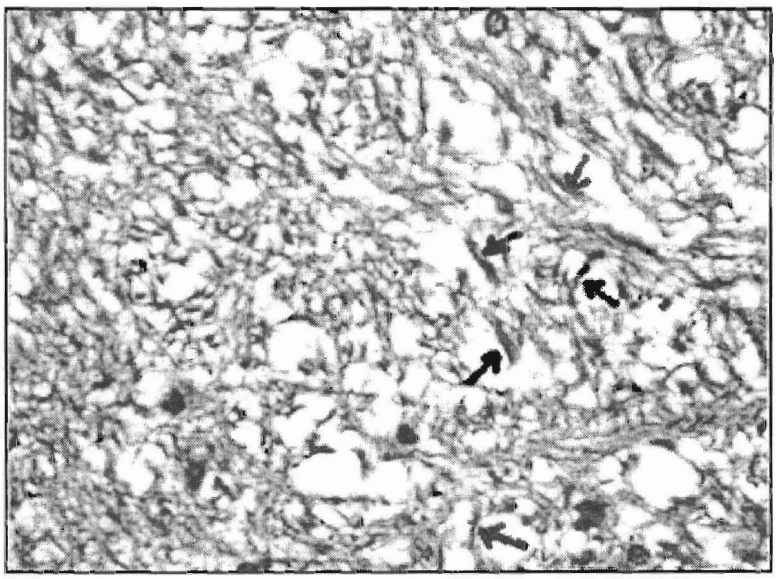

Fig. (1) : A photograph of the brain in group I (positive control) showing severe neuronal degeneration forming Rosenthal fibers *(H&E x400).*

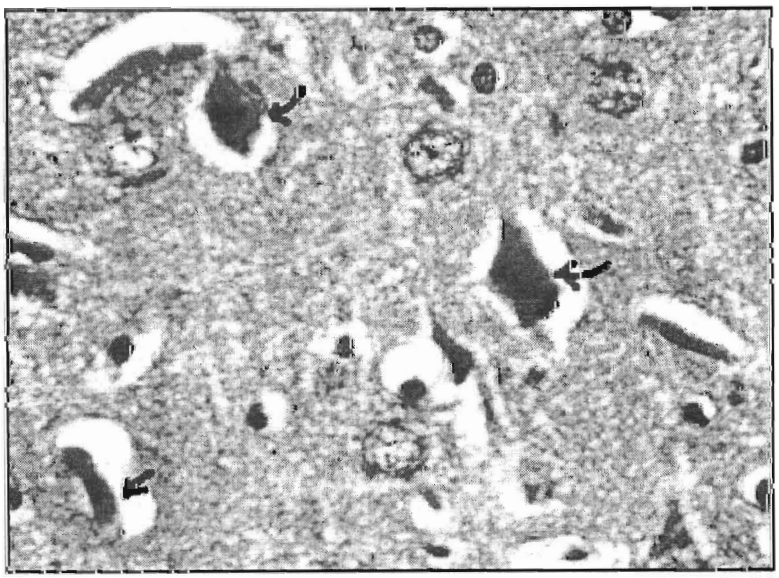

Fig. (2) : A photograph of the brain in group VIIa showing normal brain with some enlarged nuclei *(H&E x400).*

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تأثير أسيتيل سيستايين وميثايونين بمفردهم أو مجتمعين مع حامض الاسكوربيك وألفا-توكوفيرول على الخطورة التأكسدية الناتجة عن الرصاص فى ذكور الجرذان البيضاء

المشتركون فى البحث

د. عصام ممدوح على أحمد د. عادل عبدالقادر زلطه*
أ.د. عادل محمود المنصورى أ. د. إبراهيم الدسوقى محمد**
ط. عفاف محمود عطيه حسن

من أقسام الطب الشرعى والسموم الإكلينيكية والكيمياء الحيوية*، والباثولوچى**
كلية الطب - جامعة المنصورة

إستهدف هذا البحث دراسة التأثير الوقائى لمضادات الأكسدة أسيتيل سيستايين وميثايونين بمفردهم أو مجتمعين معاً أو مع حامض الأسكوربيك وألفا-توكوفيرول فى مواجهة الخطورة التأكسدية الناجمة عن التعرض المزمن للرصاص فى ذكور الجرذان البيضاء.

وقد أجرى هذا البحث على عدد ١٤٠ من ذكور الجرذان البيضاء والتى تم تقسيمها إلى ١٤ مجموعة كل منها ١٠ جرذان. المجموعة الضابطة تم حقنها بمحلول ملحى داخل التجويف البريتونى يومياً لمدة ثمانية أسابيع. المجموعة الأولى تم حقنها بخلات الرصاص داخل التجويف البريتونى يومياً بجرعة ١مج/كجم من وزن الجسم لمدة ثمانية أسابيع. المجموعات الثانية أو الثالثة أو الرابعة أو الخامسة أو السادسة أو السابعة ، أعطيت مضادات الأكسدة يومياً عن طريق الفم قبل حقن جرعة خلات الرصاص، المجموعات الثانية ب والثالثة ب والرابعة ب والخامسة ب والسادسة ب والسابعة ب أعطيت مضادات الأكسدة يومياً عن طريق الفم فى الأسبوع التاسع بعد حقن خلات الرصاص يومياً لمدة ثمانية أسابيع داخل التجويف البريتونى.

أعطى أسيتيل سيستايين بجرعة ٨٠٠مج/كجم من وزن الجسم عن طريق الفم يومياً وأعطى كل من ميثايونين وحامض الأسكوربيك وألفا-توكوفيرول بجرعة ١٠٠مج/كجم من وزن الجسم عن طريق الفم يومياً. أعطيت مضادات الأكسدة كالآتى أسيتيل سيستايين فى المجموعات الثانية أ والثانية ب، ميثايونين فى المجموعات الثالثة أ والثالثة ب، أسيتيل سيستايين وحامض الأسكوربيك وألفا توكوفيرول مجتمعين معاً فى المجموعات الرابعة أو الرابعة ب، ميثايونين وحامض الاسكوربيك وألفاتوكوفيرول مجتمعين معاً فى الجموعات الخامسة أ والخامسة ب، أسيتيل سيستايين وميثايونين مجتمعين معاً فى المجموعات السادسة أ والسادسة ب، أسيتيل سيستايين وميثايونين وحامض الأسكوربيك وألفا توكوفيرول مجتمعين معاً فى المجموعات السابعة أ و السابعة ب.

تم ذبح الجرذان فى نهاية التجربة وأخذت عينات من الدم لقياس الشوارد الجامحة النشطة بالمصل ومستوى البروتين الكلى ومستوى الثيول الكلى بالمصل وإنزيم بيروكسيد الجلوتاثيون وإنزيم سوبر أوكسيد الدسميوتاز بالدم وقياس مستوى الرصاص فى الدم. وكذلك أخذت عينات من

المخ للفحص الهستوباثولوچى.

وأظهرت نتائج هذا البحث أن مضادات الأكسدة سواء تم إعطائها بمفردها أو مجتمعة قبل التعرض لخلات الرصاص فى المجموعات (أ) أو بعد التعرض لخلات الرصاص فى المجموعات (ب) لها تأثير واقى على التسمم بالرصاص من خلال تحسن ذا دلالة إحصائية فى التغيرات الكيميائية الناتجة عن الخطورة التأكسدية فى صورة إنخفاض الشوارد الجامحة النشطة بالمصل وارتفاع كل من مستوى البروتين الكلى ومستوى الثيول الكلى بالمصل وإنزيم بيروكسيد الجلوتاثيون وإنزيم سوبر أوكسيد الدسميوتاز بالدم بالإضافة إلى إنخفاض مستوى الرصاص بالدم وكذا تحسن فى التغيرات الهستوباثولوچية على المخ وكان التأثير الوقائى أفضل فى المجموعات أ. كانت نتيجة التأثير الواقى لمضادات الأكسدة الأربعة عندما أستخدمت مجتمعة قبل التعرض للجرعة اليومية لخلات الرصاص فى المجموعة السابعة أ أفضل من نتائج المجموعات الأخرى،

وعلى ضوء هذه النتائج يوصى بتناول مضادات الأكسدة أسيتيل سيستايين وميثايونين وحامض الأسكوربيك وألفا-توكوفيرول مجتمعة معاً بالجرعة المناسبة قبل التعرض للرصاص كوسيلة حماية فعالة من التسمم بالرصاص كما يوصى بالإكثار بالأطعمة الغنية بهذه العناصر فى للأشخاص الأكثر تعرضاً للرصاص.