Evaluation of the clinical effect of melatonin on oxidative stress markers in patients with lead poisoning

التق م ألسر ري لتأث ر الم لاتون ن على مؤشرات الإج اد التاكسدي لمرضى التسمم بالرصاص

Mohammad Dakhel Al-rekabi College of Pharmacy, University of Kufa, Iraq
Ashwaq Najem Eldeen College of Pharmacy, University of alsulaimania , Iraq
Mohammad Mahmood Mohammed College of Pharmacy, University of Almustansiryah, Iraq

الخلاصة:

عتبر الرصاص من المعادن السامة التي مكن امتصاص ا بالجسم عن طر ق الرئة والمعدة. بصورة عامة حدث التسمم بالرصاص ببطى و نتج عن التراكم التدر جي للرصاص في العظام والخلا ا بعد تكرار التعرض ل . في حال عدم علاج حالات التسمم بالرصاص بشكل مبكر حدث تحط م في الأعضاء الداخل ة وتتضمن (الكلى، الج از العصبي والدماغ). أظ رت الدراسات موخرا ان التسمم بالرصاص حدث إج اد تأكسدي عن طر ق تول د الجذور الحرة وتقلل مانعات الأكسدة في الخلا ا .اشارت الدراسات موخرا الى أ[a]م ة مانعات الأكسدة في علاج التسمم بالرصاص عن طر ق قنص الجذور الحرة. تعرف مانعات الاكسدة على ان ا المواد التي تقلل من التحط م الناتج من الاكسدة مثل الذي تحدث الجذور الحرة، و تعرف الجذور الحرة على ان ا جز ئات فعالة جدا أحاد ة الإلكترون والتي تتحد مع جز ئات أخرى باخذ الكترون منه ا وتحور تركيب ا الكيمائ ة. عرف الم لاتون ان ا[a] رمون فرزمن الغدة النخام ة التي توجد بقاعدة الدماغ و عتبر الم لاتون مانع أكسدة قوي.

صممت [a] ذه الدراسة السر ر ة لتق م دور الم لاتون في إن اء تاثر الجذور الحرة الناتج من التعرض المستمر للرصاص.عشر ن مر ضا شخصوا بالتسمم المزمن بالرصاص شملوا في [a] ذه الدراسة مع عشر ن شخصا من الأصحاء، تضمن العلاج م لاتون ٣ ملغم ل لا لمدة ش ر ن. تم اخذ عنات دم من المرضى قبل العلاج وبعد ش ر وش ر ن من استخدام العلاج كما وتم سحب عنة من الأصحاء. تم ق اس كل من المالوندال اد في كر ات الدم الحمراء، مانع الاكسدة الكلي في البلازما، نسبة الرصاص في الدم، و نسبة الزنك في مصل الدم.

أظ رت نتائج الدراسة أ[a]م ة الم لاتون كمضاد للتأكسد في ازالة أو تقل ل اثار الاكسدة الناتجة من التعرض للرصاص، حيث سا[a]م الم لاتون بتقلل مستوات علامات الإج اد التاكسدي (المالوندال اد، الرصاص) ورفع نسبة مانعات الاكسدة في الجسم (مانع الاكسدة الكلي والزنك).

Abstract

Lead is a neurotoxic metallic element that can be absorbed by the body, primarily through the lungs and stomach. Generally, lead poisoning occurs slowly, resulting from the gradual accumulation of lead in bone and tissue after repeated exposure. Left untreated, lead poisoning can damage many internal organs, including the kidney, nervous system and brain. Recent studies have shown that lead causes oxidative stress by inducing the generation of reactive oxygen species (ROS) and reducing the antioxidant defense system of cells. This suggests that antioxidants may play an important role in the treatment of lead poisoning as a kind of excellent scavenger of free radicals.

Antioxidant any substance that reduces oxidative damage (damage due to oxygen) such as that caused by free radicals. Free radicals (FRs) are highly reactive chemicals that attack molecules by capturing electrons and thus modifying chemical structures. Melatonin (a powerful antioxidant) is a hormone produced naturally in the pineal gland at the base of the brain. This study was designed to evaluate the clinical

significance of melatonin in ameliorating the oxidative stress induced due to chronic exposure to lead.

Twenty male patients with chronic lead poisoning with their 20 aged matched controls were included in this study. Treatment included 3 mg capsule of melatonin antioxidant at night for two months. Heparinized venous blood samples were collected from patients before treatment and at one and two months after treatment as well as from controls to measure erythrocytes malondialdehyde (RMDA), plasma total antioxidant status (TAS), blood lead (Pb) and serum zinc (Zn)levels.

The result of the study showed a significant antioxidant activity of melatonin in eliminating the oxidative consequences of lead exposure revealed by significant reduction in oxidative stress markers (RMDA and Pb) with a significant increase in body antioxidant defense mechanisms(TAS and Zn).

Key words: lead toxicity, oxidative stress, antioxidant, melatonin, zinc

Introduction

Lead is a very toxic element, causing a variety of effects at low dose levels. The accumulation of lead usually is gradual, building up unnoticed until levels become dangerous and cause signs and symptoms [(1)]. Brain damage, kidney damage, and gastrointestinal distress are seen from acute (short-term) exposure to high levels of lead in humans. Chronic (long-term) exposure to lead in humans results in effects on the blood, central nervous system (CNS), blood pressure, kidneys, and Vitamin D metabolism[(1)]. Reproductive effects, such as decreased sperm count in men and spontaneous abortions in women, have been associated with high lead exposure [(2, 3)].

In any biological system where ROS production increases, antioxidant reserves are depleted. In this situation, the negative effects on the human system's ability to deal with increased oxidant stress occur via independent pathways. Once FRs interacts with a tissue, many changes can occur [(4)] which can defined in term of injury or oxidative stress to denote disturbances in the pro-oxidant antioxidant balance in favor of the former, leading to potential damage [(5)].

An antioxidant is a chemical that prevents the oxidation of other chemicals [(6)]. In normal biological systems, free radicals have the tendency to attack healthy molecules around them and turn them into free radicals like themselves, creating a chain reaction which could lead to massive cellular damage[(7)]. It is the job of the antioxidant to keep free radicals from turning healthy molecules into free radicals. The antioxidant breaks off the free radical chain, thus preventing damage in the cells. In addition, antioxidants may also have properties that enable them to repair damages that might have been incurred [(8)].

Melatonin is the principal hormone produced by the pineal gland at the base of the brain. It's practically nontoxic and exhibits almost no toxic side effects [(9)]. Normally, the production of melatonin by the pineal gland is inhibited by light and permitted by darkness. Many biological effects of melatonin are produced through activation of melatonin receptors[(10)], while others are due to its role as a pervasive and extremely powerful antioxidant[(11)] with a particular role in the protection of nuclear and mitochondrial DNA by capturing electrons and thus modifying chemical structures. The antioxidant activity of melatonin may reduce damage caused by some types of Parkinson's disease, may play a role in preventing cardiac arrhythmia and may increase longevity; it has been shown to increase the average life span of mice by 20% in some studies[(12-14)]. The therapeutic efficacy of melatonin was studied in previous study. Its ability to restore altered haematopoietic, hepatic and other biochemical variables indicative of tissue oxidative stress in male rats was proved. Administration of

melatonin provided significant protection to lead induced disturbed antioxidant defense that may significantly compromise normal cellular function [15]. Administration of melatonin also provided a significant protection to thiobarbituric acid reactive substances (TBARS) levels, reduced glutathione (GSH) and oxidized glutathione (GSSG) contents in tissues, suggesting their ability to act as a free radical scavenger and in protecting cells against toxic insult [16-17].

Lead-induced oxidative stress has been identified as the primary contributory agent in the pathogenesis of lead poisoning[1] .ROS generated as a result of lead exposure have been identified in lung, endothelial tissue, testes, sperm, liver, and brain [2].Consequently, the potential role of using antioxidants of various types to provide protective effects became a major task in this respect.

Subjects and methods

1- Subjects

study group comprised of total of forty subjects, 20 patients with chronic lead poisoning and their 20 aged matched normal controls with age range (35-45) years . Patients involved in this study were under medical supervision. They were non-smokers, non-alcoholics and free from apparent other diseases. Patients were selected on the basis that they were on direct exposure to lead and have been employed for at least one year before this study was carried out. Treatment schedules included 3 mg capsule of antioxidants (melatonin) at night continued for two months. Heparinized venous blood samples were collected from patients before treatment and at one and two months after treatment as well as from controls.

2- Principle

Erythrocytes MDA levels were measured according to the method of Stocks and Dormandy [18] as modified by Gilbert et al [19] .Serum zinc levels were measured by atomic absorption spectrometry according to Taylor and Bryant method [20] .Blood lead level were measured by atomic absorption spectrometry according to Brown et al [21], and blood hemoglobin content was assayed according to the method of Drapkin and Austin [22]. Total antioxidant status levels (TAS) were measured using Randox TAS kit. Statistical analysis of data was done by using student t-test.

Results

The results of this study showed a significant elevation in basal (before treatment) erythrocyte MDA and lead levels with a significant reduction in TAS, hemoglobin and Zinc levels in lead exposed workers compared with their controls, table (1).

Two months treatment with melatonin significantly decrease erythrocyte MDA and lead levels with a significant increase in TAS, hemoglobin and Zinc levels, table (1),figure(1-5).

Table (1): case-control comparison of oxidative stress markers at base line level and after one and two month's treatment with 3 mg melatonin antioxidant.

Parameter	Control group	Treated group		
		Baseline	After 1 Month	After 2 Months
Erythrocyte MDA (OD/gm Hb)	0.200 ± 0.038	0.928 ± 0.055 ^{*a}	0.330 ± 0.055 ^{*b}	0.167 ± 0.034 ^{*c}
Serum Zinc(µg/dl)	94.35 ± 7.29	64.25 ± 4.56 ^{*a}	78.35 ± 5.96 ^{*b}	89.50 ± 1.87 ^{*c}
Plasma TAS(mMol/L)	1.27 ± 0.34	0.60 ± 0.05 ^{*a}	0.83 ± 0.09 ^{*b}	1.32 ± 0.12 ^{c}
Serum Lead (µg/dl)	12.04 ± 0.70	58.50 ± 1.60 ^{*a}	52.34 ± 1.49 ^{*b}	43.24 ± 2.17 ^{*c}
hemoglobin level (gm)	30.18 ± 2.35	26.00 ± 2.07 ^{*a}	27.22 ± 1.88 ^{*b}	29.82 ± 1.31 ^{c}

-Data are presented as mean ± SD
– N= number of patients
– *P<0.05 with respect to control group.
– Non-identical superscripts (a, b, c) among treated group considered significantly different, P<0.01.

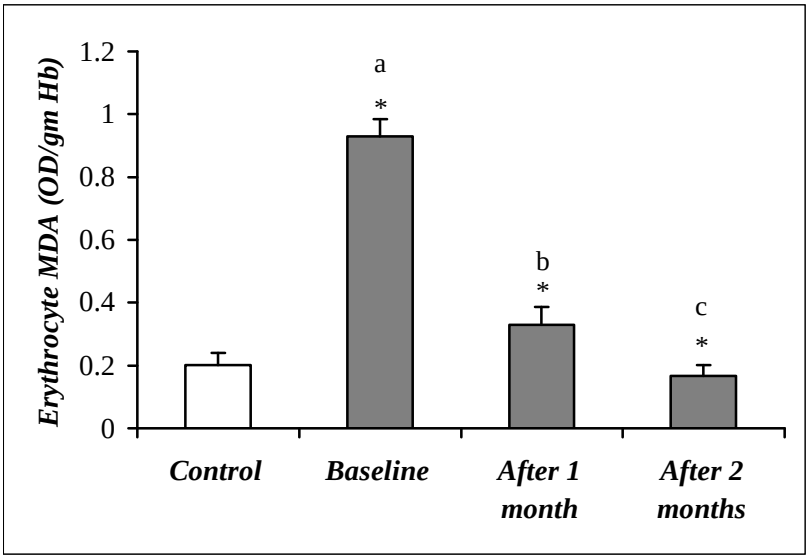

Figure (1): Erythrocyte MDA level in control and in group treated with 3 mg melatonin (before, after 1 and 2 months treatment). Data are presented as mean ± SD. * P<0.05 with respect to control, non-identical superscripts (a, b, c) considered

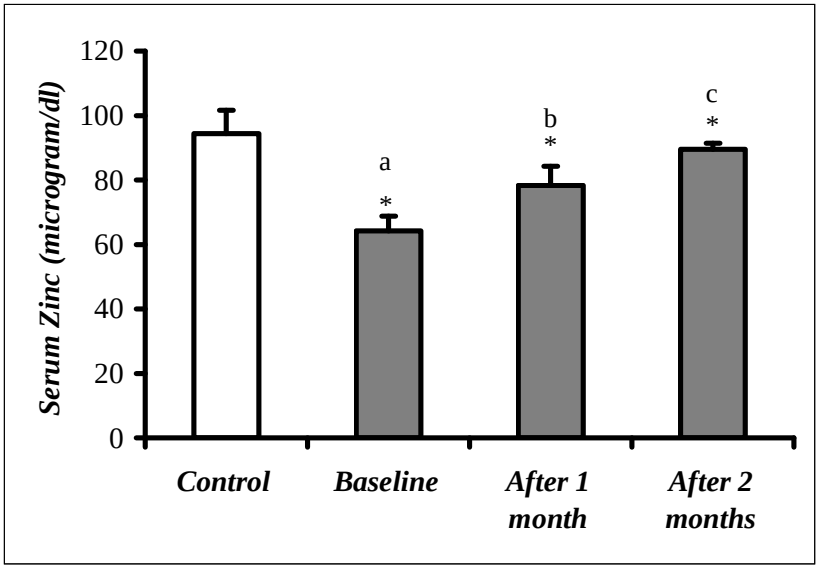

Figure (2): Serum Zinc level in control and in group treated with 3 mg melatonin (before, after 1 and 2 months treatment). Data are presented as mean ± SD. * P<0.05 with respect to control, non-identical superscripts (a, b, c) considered

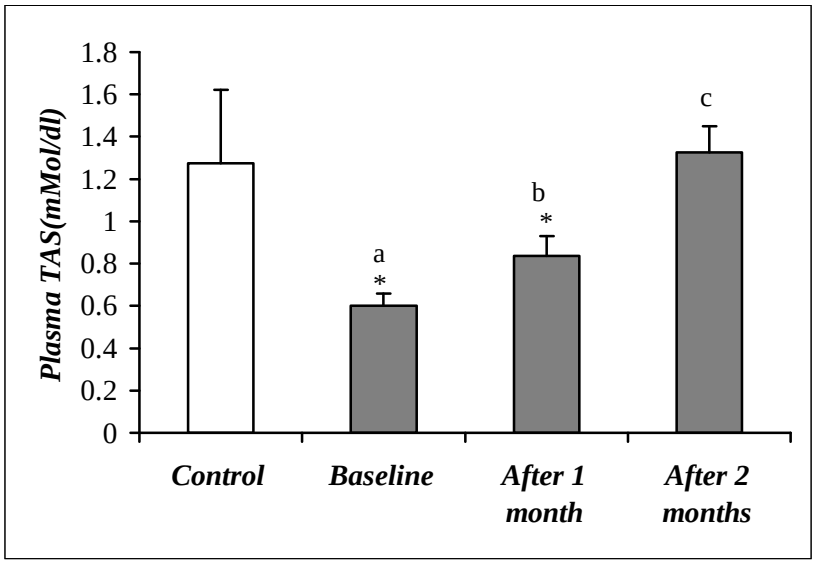

Figure (3): Plasma TAS level in control and in group treated with 3 mg melatonin (before, after 1 and 2 months treatment). Data are presented as mean ± SD. * P<0.05 with respect to control, non-identical superscripts (a, b, c) considered

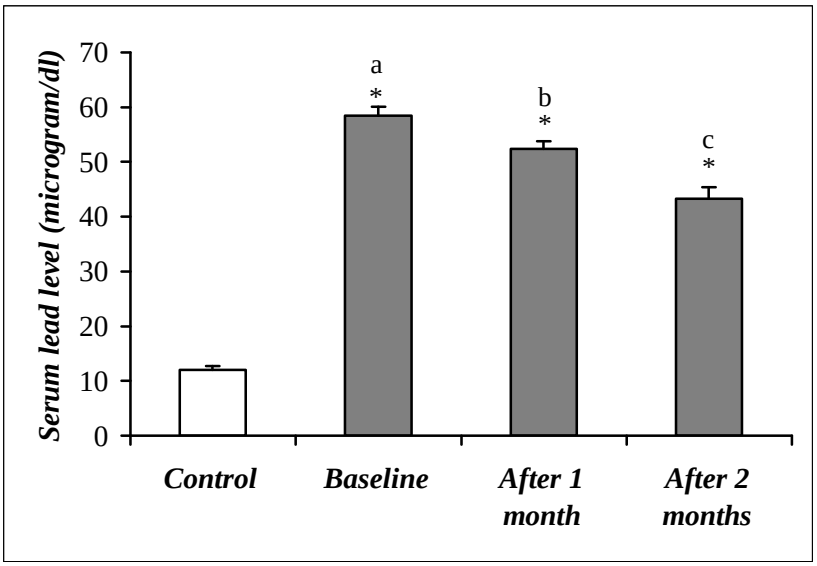

Figure (4): serum lead level in control and in group treated with3 mg melatonin (before, after 1 and 2 months treatment). Data are presented as mean ± SD. * P<0.05 with respect to control, non-identical superscripts (a, b, c) considered

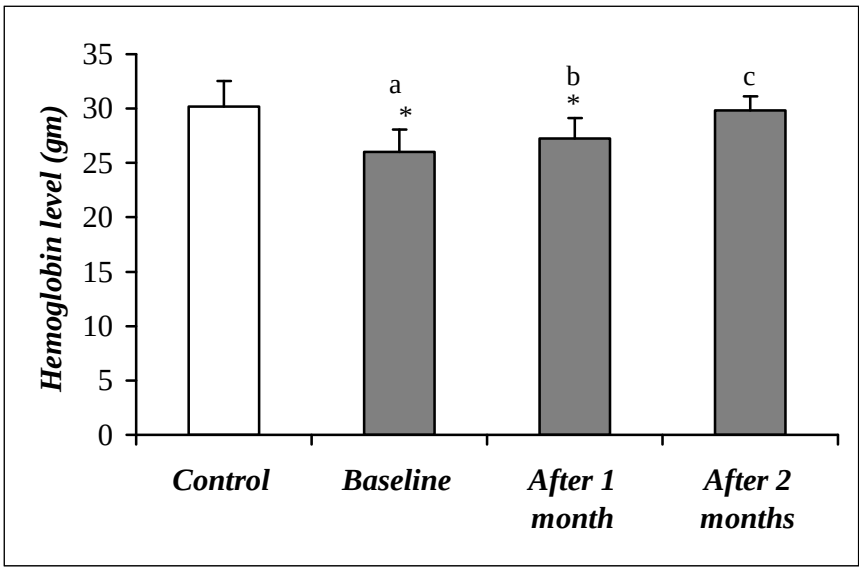

Figure (5): Hemoglobin level in control and in group treated with 3 mg melatonin (before, after 1 and 2 months treatment). Data are presented as mean ± SD. * P<0.05 with respect to control, non-identical superscripts (a, b, c) considered

Discussion

People with higher lead levels have a greater risk of long-lasting health problems, and must be followed carefully [23]. A complete recovery from chronic lead poisoning may take months to years. Symptoms resembling chronic intoxication may be develop with over a period weeks or months. By removing or avoiding lead sources or with early detection and treatment, we can prevent or limit the harmful effects of lead poisoning [23].

Total antioxidant status (TAS) is an important markers of disease since reactive oxygen species circulate freely in the body, and can have a serious repercussions throughout the body .The body utilizes antioxidant reserve to cope with free radicals, and monitoring antioxidants levels may be conductive to the early detection of disease .Therefore, a complete antioxidant profile may ensure accurate detection of variations in antioxidant levels caused by disease onset [24].

This study showed alterations in oxygen free radical scavenging process in the blood of patients with lead poisoning manifested by decreases body antioxidant defence mechanism(TAS, Hb and Zn) with an increase in oxidative stress markers (RMDA and Pb)table(1), suggesting the presence of a generalized decrease in antioxidant status in the patients which may be attributed to the utilization of body antioxidant in neutralizing the increased endogenous free radicals which is produced by exposure to lead.

Lead toxicity leads to FR damage via two separate, although related, pathways: (1) the generation of ROS, including hydroperoxides, singlet oxygen, and hydrogen peroxide, and (2) the direct depletion of antioxidant reserves [25].

Lead perturbs multiple enzyme systems. As in most heavy metals, any ligand with sulfhydryl groups is vulnerable. Perhaps the best-known effect is that on the production of heme. Lead interferes with the critical phases of the dehydration of aminolevulinic acid and the incorporation of iron into the protoporphyrin molecule; the result is a decrease in heme production. Because heme is essential for cellular oxidation, deficiencies have far-reaching effects [1] [26].

Two months treatment with melatonin (3 mg capsule at night) correct body antioxidant status in the patients which manifested by increase body antioxidant defense mechanism (TAS and ZN) with a significant reduction in oxidative stress markers (RMDA and lead). The action of melatonin on lead-induced changes was attributed to protection of the antioxidant capacity in cells in addition to the ability of melatonin to scavenge free radicals [27],table1, figure 1-3).

Melatonin is the most potent antioxidant known .It protects cells from free radicals damage, improves and enhances the functioning of the immune system, and delays the aging process. The antioxidant effect of melatonin has been shown to be much more potent than any of the well-known antioxidants (more than twice as effective as vitamin E at scavenging peroxyl radicals).Melatonin has scavenging activity, which in turn decrease both oxidative damage and utilization of glutathione in neutralization phagocytes induced free radicals [17].

Prophylactic effect of melatonin on lead-induced inhibition of heme biosynthesis and deterioration of antioxidant systems was stated in male rats[28].The pre-administration of melatonin reduced the inhibitory effect of lead on both enzymatic and non-enzymatic antioxidants [27]. This was accompanied by marked normalization of lipid peroxidation and modulation of copper and zinc levels in liver.

According to the results obtained by this study which suggest the potential clinical significant of antioxidants (melatonin) in improving oxidative stress markers in

patients with lead poisoning and because of that when antioxidants were combined with chelating agents showed a synergism that improved chelating ability resulted in significantly higher urinary lead excretion [28], so we recommend the use of melatonin in combination with chelating agent by patients with lead poisoning.

References

1. Patrick, Lyn .Lead toxicity Part II: the role of free radical damage and the use of antioxidants in the pathology and treatment of lead toxicity. Alternative Medicine Review .2006.
2. Hsu PC, Guo YL. Antioxidant nutrients and lead toxicity. Toxicology 2002; 180:33-44.
3. Levin SM, Goldberg M. Clinical evaluation and management of lead-exposed construction workers. AM J Ind Med 2000; 37:23-43.
4. Sies-H. Oxidative stress: introductory remarks in oxidative stress.Sies-H.ed.academic Press, New York.1985, 1.
5. Sies-H. Oxidative stress Introduction in oxidative stress and antioxidants. Sies-H. Academic Press, San Diego, 1991; XV.
6. Feri-B,et al. Ascorbate in an outstanding antioxidant in human blood plasma.proc.nati.acad.asci.USA.1989;86:6377-6381.
7. Halliwell B; Gutteridge JM., ''Free radicals in biology and medicine''. Second edition.Oxford: clarendon Press, 1989.
8. Andersen-H.R.et al. Antioxidative enzyme activities in human erythrocytes. Clinical chemistry.1997; 43(4):562-568.
9. Reiter RJ, Tan DX, Manchester LC, Q1W. Biochemical reactivity of melatonin with reactive oxygen and nitrogen species: a review of the evidence .Cell biochem biophys 2001; 34:237-56.
10. Maestroni GJ.the immunotherapeutic potential for melatonin.expert Opin Investig Drugs.2001;10(3):467-76.
11. Reiter RJ,et al .a review of the evidence supporting melatonins role as antioxidant.j Pineal Res.1995 Jan;18(1):1-11.
12. Ward Dean, John Morgenthaler, Steven William Fowkes (1993). Smart Drugs II: The Next Generation: New Drugs and Nutrients to Improve Your Memory and Increase Your Intelligence (Smart Drug Series, V. 2). Smart Publications. ISBN 0-9627418-7-6.
13. Anisimov V, Alimova I, Baturin D, Popovich I, Zabezhinski M, Rosenfeld S, Manton K, Semenchenko A, Yashin A (2003). "Dose-dependent effect of melatonin on life span and spontaneous tumor incidence in female SHR mice.". Exp Gerontol 38 (4): 449-61. PMID 12670632.
14. Oaknin-Bendahan S, Anis Y, Nir I, Zisapel N (1995). "Effects of long-term administration of melatonin and a putative antagonist on the ageing rat.". Neuroreport 6 (5): 785-8. PMID 7605949.
15. Gamal H.El-Sokkary,Gamal H.Abdel-Rahman and Esam S.Kamel.melatonin protects against lead induced hepatic and renal toxicity in male rats. Toxicology 213, issues 1-2, 15 september 2005, pages 25-33.
16. Al-Gaff AN,Humadi S,Wohaieb SA.Effect of melatonin on oxidative stress markers in patients with alopecia areata .Iraqi Journal Of Pharmacy;College of Pharmacy, Mosul.2005;Vol 5,No 1.
17. Flora SJ, pande M, Kannan GM,Mehta A.lead induced oxidative stress and its recovery following co-administration of melatonin or N-acetylcysteine during chelation with succimer in male rates.cell Mol Biol 2004;Suppl 50:OL 551.

18. Stocks-J and Dormandy-TL.the auto-oxidation of human red blood cell lipids induced hydrogen peroxide.Brit-J-Hematol.1971; 20:95-111.
19. Gilbert-HS;Stump-DD and Roth –EF. A method to correct for errors caused by generation of interfering compounds during erythrocyte lipid peroxidation.anal-Biochem.1984; 137:282-286.
20. Burtis-A.C; and Ashwood-R.E,Eds; Tietz'' Text Book Of Clinical Chemistry''3 ed edition W,B Sannders Company;London.1999;P482-484.
21. Dawser Khalil Ismaiel .effect of zinc and allpurinol in ameliorating oxidative stress in lead – exposed workers. Iraqi Journal Of Pharmacy;College of Pharmacy, Mosul.2005;Vol 5,No 1.
22. Drabken-DL; Austen-JH. Spectrophotometer studies II preparation from washed blood cells : nitric oxide hemoglobin and sulfhemoglobin.J-Biol-Chem.1935;112:51-65.
23. Bleecker ML, et al. Differential effects of lead exposure on components of verbal memory. *Occup Environ Med.* 2005 Mar; 62(3):181-7.
24. Ashwaq N.A. Evaluation of oxidative stress indicators in lymphocytes of patients with alopecia areata. Ph.D thesis, 2005.
25. Ercal N, Gurer-Orhan H, Aykin-Burns N. Toxic metals and oxidative stress. Part 1. Mechanisms involved in metal-induced oxidative damage. Curr Top Med Chem 2001; 1:529-539.
26. Geetu Saxena, S.J.S. Flora.lead –induced oxidative stress and hematological alterations and their response to combined adminsteration of calcium disodium EDTA with a thiol chelator in rats.Journal of biochemical and molecular toxicology.volume 18,issue 4,page 221-233.
27. Mohammed A El-missiry.prophylactic effect of melatonin on lead –induced inhibition of heme biosynthesis and deterioration of antioxidant systems in male rates. Journal of biochemical and molecular toxicology,volum 14,issue 1,pages 57-62,1999.

Flora SJ, Pande M, Kannan GM, Mehta A. Lead induced oxidative stress and its recovery following co-administration of melatonin or N-acetylcysteine during chelation with succimer in male rats. Cell Mol Biol(Noisy-le-grand) 2004;50:OL543-OL551.