Efficacy of Curcumin on Lead Induced- Nephrotoxicity in Female Albino Rats

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Abstract: Lead (Pb^{+2}) toxicity remains a significant public health problem because of its global pervasiveness and its adverse effects on the renal system. Oxidative stress plays a key role in lead-induced nephrotoxicity. The aim of the present study to clarify the possible corrective role of curcumin on the nephrotoxicity of lead acetate. Thirty mature female albino rats (190 - 225 g) were randomly divided into equal five groups, first group received 500 mg lead acetate / L in drinking water, second group orally received 100 mg curcumin / Kg B. W. dissolved in 1% carboxymethyle cellulose 3 times a week, third group received lead acetate and curcumin as previously mentioned with regard to dose and route for each of them. Fourth and fifth groups were kept as positive and negative controls orally received 1% carboxymethyl cellulose dissolved in distilled water 3 times a week and drinking water daily for 2 months respectively.

The results revealed that lead increased levels of serum urea and creatinine and renal MDA concentration but decreased renal antioxidant enzymes comparing with the control groups meanwhile, the co-treated group with lead and curcumin evoked a significant amelioration. There was a significant increase in the renal lead concentration which lowered after concomitant treatment with curcumin. The histopathological investigation confirmed the aforementioned findings. The study indicates that curcumin, an effective antioxidant, may have a protective effect against lead acetate exposure. The current study concluded that curcumin mitigate the nephrotoxic, oxidative, histopathological and residual impacts of lead acetate exposure however the detailed role of metallothionein in the nephrotoxicity mediated by co-exposure to lead and curcumin still remains to be elucidated.

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Key words: Curcumin, Lead, Nephrotoxicity.

1. Introduction

Lead (Pb) is a toxic metal that induces a wide range of behavioral, biochemical and physiological effects in humans. Lead toxicity is probably the most common form of heavy metal intoxication. It is well-documented as one of the most dangerous and insidious poisons. Its continuous environmental and occupational exposure may contribute to renal, nervous, hepatic, hematological and reproductive disorders in man and animals **(Flora *et al.,* 2006; El-Sayed and El-Neweshy, 2009 and Ashry *et al.,* 2010)**.

The absorbed Pb is conjugated in the liver and passed to the kidney, where a small quantity is excreted in urine and the rest accumulates in various body organs and affects many biological activities at the molecular, cellular and intercellular levels, which may result in morphological alterations that can remain even after Pb levels have fallen **(Jarrar, 2003; Sidhu and Nehru, 2004; Taib *et al.,* 2004 and Flora *et al.,* 2006)**.

The kidney is the primary site for the initial accumulation of lead and the critical target organ of chronic lead exposure following oral or inhalation exposure in humans and animals **(Nolan and Shaikh, 1992)**.

Lead is reported to cause oxidative stress by generating the release of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide and hydroxyl radicals and lipid peroxides **(Hsu *et al.,* 1997, Xu *et al.,* 2005 and El-Nekeety *et al.,* 2009).**

The possible molecular mechanism involved in lead toxicity is oxidative stress (OS), which is a consequence of an unbalance between oxidants and the antioxidant systems **(Flora *et al.,* 2009)**.

As oxidative stress has been mainly implicated in the lead toxicity, reducing the possibility of lead acetate interacting with cellular metabolism biomolecules and decreasing the reactive oxygen species generation by the use of antioxidant nutrients has received considerable attention in the recent past **(Gurer and Ercal, 2000, Patra *et al.,* 2001 and Hsu and Guo, 2002).**

Metallothioneins (MTs) are low molecular weight proteins (6-7 KDa), rich in cysteine which confers them with a high capacity to bind heavy metal ions in biological systems **(Schmitt *et al.,* 2007)**.The induction of MT synthesis represents a

sensitive biomarker of heavy metals exposure **(Tom *et al.*, 2004).**

Curcumin from turmeric, a well known biologically active compound, has been shown to ameliorate oxidative stress and it is considered to be a potent antioxidant **(Eybl *et al.*, 2006)**. Curcumin is a potent inducer of detoxifying enzymes and thereby counters the toxicity induced by chemical carcinogens **(Singletary *et al.*, 1998)**. Having a polyphenolic structure and diketone functional groups, curcumin is a stronger antioxidant inhibitor of lipid peroxidation than other flavonoids, which have a single phenolic hydroxyl group **(Phan *et al.*, 2001)**. The effective antioxidant property of curcumin inhibits the utilization of vitamins C and E in the liver, thus maintaining their levels **(Rukkumani *et al.*, 2003)**. It has been used as an antioxidant in toxicity studies of several metals including cadmium **(Daniel *et al.*, 2004)**, copper (**Nair *et al.*, 2005**), iron **(Manjunatha and Srinivasan, 2006)**, lead **(Dairam *et al.*, 2007)** and selenium **(Padmaja and Raju, 2004)**.

Recently, previous studies shown that simultaneous treatment with curcumin protects against alterations and oxidative stress in rat brain, liver and immunosuppressive effects of lead **(Daniel *et al.*, 2004 & Taghred, 2012)**. However the molecular mechanism of lead induced kidney injury and nephroprotective effects of curcumin are not yet completely understood.

We therefore carried out this work to evaluate the efficacy of curcumin in ameliorating lead toxicity and the antioxidant potential of curcumin co-treatment with lead exposure in rats. The effect of lead toxicity on the expressions of metallothionein (MT-I and MT-II) mRNAs in kidney tissues and the role of co-treatment with curcumin on metallothionein mRNA expression levels were also studied by RT-PCR analysis

2. Material and Methods

Tested compounds:

Lead acetate: is purchased from Sigma- Aldrich Co. (St. Louis, Mo, USA)

Chemical formula: Pb $(C_2H_3O_2)_2$

Curcumin: is purchased from Sigma- Aldrich Co. (St. Louis, Mo, USA).

Curcumin is a low molecular weight polyphenol derived from the rhizomes of turmeric (*Curcuma longa* which is a member of the ginger family (Zingiberaceae) **(Aggarwal *et al.*, 2007)**.

Appearance: bright yellow to orange color powder.

Solubility: it is insoluble in water but soluble in other solvents such as ethyl oleat **(Asali *et al.*, 2011)**, 50% ethanol **(Daniel *et al.*, 2004)** and carboxymethyl cellulose **(Kumar *et al.*, 2009)**.

Animals and dosing:

Thirty mature female albino rats with an average body weight ranging from 190 - 225 g were obtained from the Animal Research Unit of the Faculty of Veterinary Medicine, Zagazig University. Animals were kept in metal cages during the whole experimental period under hygienic conditions, fed on well balanced ration and provided with water *ad-libtum*, through the experiment. The animals were randomly divided into equal five groups, first group received 500 mg lead acetate / L in drinking water daily **(Liu *et al.*, 2010)**, second group orally received 100 mg curcumin / Kg B. W. dissolved in 1% carboxymethyle cellulose 3 times a week for 2 months **(Shukla *et al.*, 2003)**, third group received lead acetate and curcumin as previously mentioned with regard to dose and route for each of them, fourth and fifth groups were kept as positive and negative controls orally received 1% carboxymethyl cellulose dissolved in distilled water 3 times a week and drinking tap water daily without for 2 months respectively.

Biochemical study

Blood samples were collected from medial canthus of the eyes of all rats for serum separation. The serum samples were preserved at -20°C till used for estimation of serum kidney function parameters (creatinine and urea) using Kits provided from Biodiagnostic Co. (Giza, Egypt) using Spectrophotometer (Schimadzu). Kidney samples were preserved at-20°C for determination of renal antioxidant enzymes after preparation of the homogenate **(Sidu *et al.*, 2004)**, for estimation of SOD **(Nishikimi *et al.*, 1972)**, GST **(Habig *et al.*, 1974)**, GR **(Goldberg and Spooner, 1983)**, GPx **(Paglia and Valentine, 1967)** & CAT **(Aebi, 1984)** and MDA as previously mentioned by **Ohkawa *et al.*, 1979)**. .Small parts of the kidney tissues were immediately frozen in liquid nitrogen for RNA extraction for MT-I & MT-II genes using RT-PCR.

Residual study

Estimation of Pb residues in the frozen kidney samples were applied using Flame Atomic Absorption Spectrophotometer (FAAS), Model 210 VGP according to **(Julshman, 1983)**.

Histopathological study

Kidney specimens were fixed in 10 % neutral buffered formalin for histopathological examination **(Bancroft and Stevens, 1996)**.

Expression of Metallothionein gene (MT) in kidney

RNA extraction

Total RNA was extracted from frozen kidney tissues using the protocol supported by RNeasy Mini Kit. The isolated RNA was reverse transcribed using RevertAid- reverse transcriptase. Briefly, the RNA was denatured by heating for 5 minutes at 65 C°, cooled on ice, and incubated with reverse transcriptase reaction mixture. The standard mixture contained 2 µg of total RNA, 25 U of RNAase inhibitor, 0.5 mM each of dNTPs, 1.5 µM reverse primer, and 200 U of RevertAid-reverse transcriptase in a total volume of 25 µl. For reverse transcription, tubes were incubated at 42 °C for 60 minutes, followed by rapid cooling as was previously mentioned by **(Wang *et al.,* 2010)**.

Semi- quantitative RT-PCR

Reverse transcription PCR (RT-PCR) was performed using the one-step Dream Taq-Green PCR Master Mix. The RT-PCR was performed in a 50-µL reaction mixture. Two pairs of specific primers were designed according to the alignments of the published cDNA sequences of MT-1, MT-2 and GAPDH (internal control) genes in liver of rats. PCR amplification conditions were as follows: denaturation at 95 °C for 2 minutes and 40 cycles at 95 °C for 10 seconds, 60 °C for 20 seconds, and 72 °C for 30 seconds. The reaction was then subjected to a melting protocol from 67 °C to 95 °C with a 0.5 °C increment and 30 seconds holding at each increment to check the specificity of the amplified products. Amplification products were electrophorized in 1.5% agrose gel containing 0.5 X Tris-Buffer EDTA (TBE) at 70 volts for 60 minutes and visualized under Ultra violet light **(Sambrook *et al.,* 1989)**.

Gel Picture analysis:

The expression levels of the gene bands on gel were analyzed using Image J software (version 1.24o) for measurement of band intensity in Pixels and determined the fold increase in intensity between different bands. Image J is a public domain image analysis program that was developed at the National Institutes of Health. Image J folder can be copied from lab computer or download this program from the source (http://rsb.info.nih.gov/ij/) for use on any computer. Updates to this program are available at the ImageJ homepage at frequent intervals. Macintosh and Linux versions of ImageJ are also available. ImageJ software is commonly used in analysis of different biological pictures according to **(Michael *et al.,* 2010)**.

Statistical analysis

The results were analyzed using statistical analysis system (SAS) computer program for obtaining means and standard errors. The data were analyzed using one- way ANOVA to determine the statistical significance of differences **(SAS, 1997)**.

3. Results and discussion

Serum biochemical parameters

Female rats administered 500 mg lead acetate / L in drinking water daily for 2 months elicited a significant ($p < 0.05$) increase in sera levels of both urea and creatinine comparing with control groups meanwhile, the co-treated group with lead and curcumin evoked a significant amelioration in those parameters (Table 1).Our results indicated that the kidney function was impaired which are consistent with **Ahmed *et al.,* (2010)**. This may be attributed to the mechanism of action of lead-induced kidney damage were due to increase the production of reactive oxygen species **(Upasani and Balaraman, 2003)** which confirmed by our histopathological findings. Our results corroborate this suggestion, since we observed lipid peroxidation along with histopathological damage with alterations in SOD, GPx and CAT activities in kidney tissues.

Table (1): Effect of Pb on serum kidney enzymes of rats treated with Pb, Curcumin and both comparing with control group (Means ± SE).

Groups / Parameter	Control	Curcumin	Solvent	Pb	Pb+ Curcumin
Urea (mg/dL)	23.72 ± 0.26^{c}	23.09 ± 0.09^{c}	23.26 ± 0.21^{C}	45.76 ± 0.16^{a}	37.32 ± 0.27^{b}
Creatinine (mg/dL)	0.39 ± 0.01^{c}	0.36 ± 0.01^{c}	0.34 ± 0.01^{c}	2.20 ± 0.13^{a}	1.23 ± 0.16^{b}

Means within the same row have different superscripts are significantly different at ($p \leq 0.05$).

Effect on antioxidant enzymes activity and MDA concentration

On measuring the enzyme activity of GPx, GR, GST, CAT and SOD ; our study revealed an obvious significant ($p < 0.05$) decrease in antioxidant enzymes in Pb treated rats kidneys comparing with the control ones, while in co- treated group ; our data recorded a significant ($P< 0.05$) improvement compared to Pb treated one . The toxic action produced by lead might be attributed to its ability to generate reactive oxygen species (ROS) which induce oxidative damage in the renal tissues by enhancing lipid peroxidation (Ademuyiwa *et al.,* 2009) and depletion of antioxidant reserve (Ercal *et*

al., 2001) whereas Lead has affinity for sulphydryl groups or metal cofactors in antioxidant enzymes and molecules which resulted in a reduction in the antioxidant enzyme activities, such as SOD and CAT (Patrick, 2006) and glutathione (Christie and Costa, 1984) . CAT is a major antioxidant enzyme having heme as the prosthetic group .Lead is known to reduce iron absorption and inhibit heme biosynthesis (Sivaprasad *et al.,* 2004) .Concerning to estimation of MDA concentration ; the current experiment showed significant ($P < 0.05$) increase in Pb treated group comparing with the control one . Our results are in agreement with Upasani and Balaraman (2003) who found significant increase in the lipid peroxidation and decrease in the levels of endogenous antioxidants in the kidney of lead exposed rats. Previous studies showed that oxidative stress is involved in nephrotoxicity induced by lead exposure **(Ercal *et al.,* 1996 and Roels *et al.,* 1999).**

In co-treated rats, curcumin evoked a significant ($P < 0.05$) amelioration (Table 2) which may be postulated to its powerful antioxidant mechanism of curcumin which returned to antioxidant properties of naturally occurring phenolic Compounds as the phenolic hydroxyl and the methoxyl groups on the phenyl ring and the 1,3-diketone system seem to be important structural features that can contribute to these effects **(Jayaprakasha *et al.,* 2005)**.

Table (2): Effect of Pb on GPx, GR, GST, CAT & SOD activities & MDA concentration in kidney of rats treated with Pb, curcumin and both comparing with control group (Means ± SE).

Parameter \ Group	Control	Curcumin	Solvent	Pb	Pb+ curcumin
GPx (U/g tissue)	1.55 ±0.06 ^{a}	1.45 ±0.07 ^{a}	1.48±0.05^{a}	0.88±0.05^{c}	1.12±0.06^{b}
GR (U/g tissue)	44.16±1.02^{a}	45.71 ±1.06^{a}	44.58±1.36^{a}	24.17±0.90^{c}	37.11±1.05^{b}
GST(U/g tissue)	11.93±0.42^{ab}	12.10 ±0.72^{a}	11.71±0.69^{ab}	7.77±0.20^{c}	10.36±0.28^{b}
CAT(U/g tissue)	2.06 ±0.10^{a}	2.11±0.07^{a}	2.02±0.08^{a}	1.23±0.06^{b}	1.43±0.07^{b}
SOD(U/g tissue)	34.01 ±1.32^{a}	35.64 ±0.70^{a}	33.99±1.22^{a}	19.45±1.19^{c}	25.36±1.29^{b}
MDA (nmol/gtissue)	130.59±0.43^{c}	132.07±1.0^{c}	132.47±0.62^{c}	202.13±0.52 ^{a}	171.43±0.45^{b}

Means within the same row have different superscripts are significantly different at ($p \leq 0.05$).

Histopathological findings

The kidneys of lead treated female rats showed congestion of the renal blood vessels, degenerative changes in the epithelial cells of some renal tubules. Some epithelial cells of renal tubules were hypertrophied (karyo and cytomegaly) with eosinophilic intranuclear inclusion bodies (Fig 2). Multifocal areas of coagulative necrosis were detected and characterized by complete disappearance of the nuclei (Fig 3). The renal epithelia were exofoliated in the form of cellular casts (Fig 4) which became mineralized forming irregular laminated yellowish-brown bodies inside the lumen of some renal tubules and in the interstitial tissue. Periglomerular and interstitial aggregations of round cells were visualized (Fig 5). The wall of some renal arterioles was thickened and hyalinized, and their lumen was partially obliterated. Cloudy swelling and vacuolations of the convoluted tubular epithelia were also observed. Kidney of lead & curcumin treated group showing few interstitial aggregation of round cells (Fig 6) .

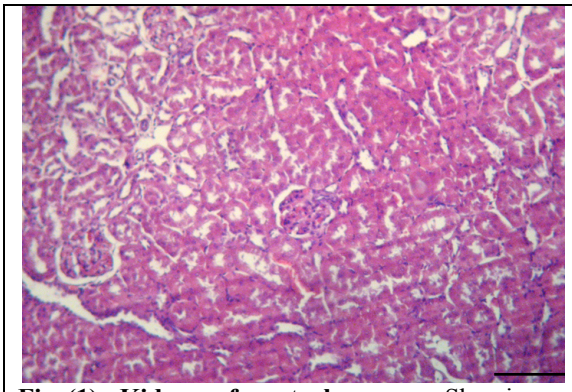


Fig (1): Kidney of control group: Showing normal nephrone of glomerular and tubular structures, HE (Bar = 100 µm).

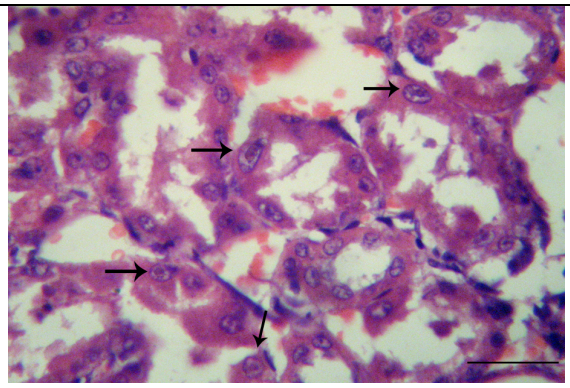


Fig (2): Kidney of lead group: Showing hypertrophied renal epithelia (karyo and cytomegaly) with eosinophilic intranuclear inclusion body, HE (Bar = 100 µm).

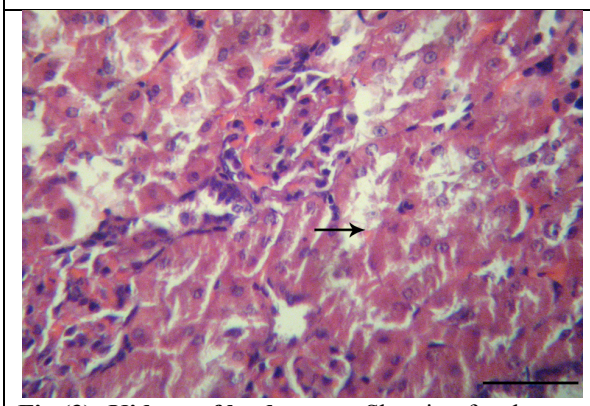


Fig (3): Kidney of lead group: Showing focal area of coagulative necrosis, HE (Bar = 100 µm).

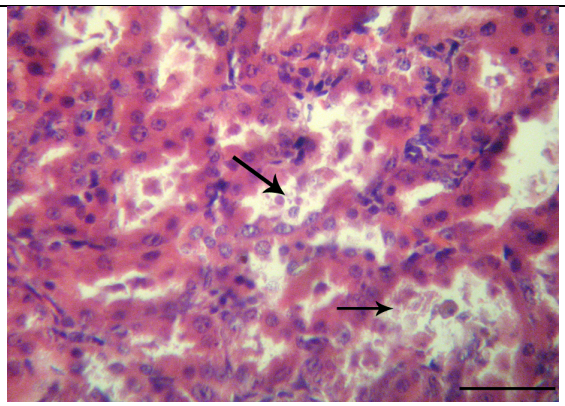


Fig (4): Kidney of lead group: Showing exofoliated epithelium in the form of cellular casts, HE (Bar = 100 µm).

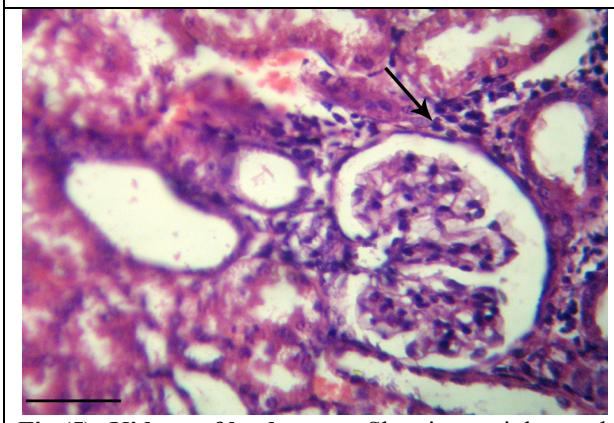


Fig (5): Kidney of lead group: Showing periglomerular and interstitial aggrgations of round cells, HE (Bar = 100 µm).

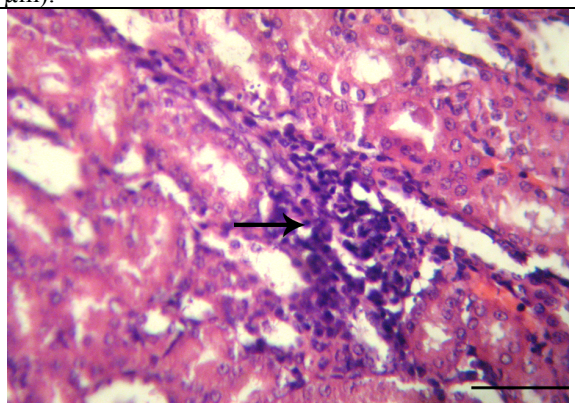


Fig (6): Kidney of lead & curcumin group: Showing few interstitial aggregation of round cells, HE (Bar = 100 µm).

Effects of lead and/or curcumin on the expression of metallothionein (MT) gene in the of rats kidney

Our results revealed that lead mild up-regulated MT-1& MT-2 mRNA expression in kidneys of Pb teated rats compared to control groups (Figs. 7, 8). Our result is consistent with the previous study, which suggested that lead is a weak inducer of MTs **(liu *et al.*, 2005)**. MTs are widely considered as biochemical environmental indicators and especially of metal contamination **(Alvarez-Barrientos *et al.*, 2001)** and as most well-known antioxidant that protects against metal toxicity (**Chan and Cherian**, **1992 and Klaassen and Liu, 1998)**. or the oxidative stress caused by ROS generated by lead toxicity and alteration in LPO and GSH levels and in the activities of SOD, GPx, CA in kidney tissues which returned to lead is a relatively hard metal, it has a lower sulfhydryl-binding affinity than softer metals, e.g., Cd, Cu, and Hg. As a consequence, proteins such as MT may provide a more efficient protection against the toxicity of soft metals than hard metals such as lead (**Maracine and Segner, 1998**).

We found some induction of MT-I and MT-II mRNA expressions in the kidney tissues after curcumin treatment alone, which may be due to ROS normally present in the animals that might be scavenged from the body by curcumin treatment. This elucidated the antioxidative property of curcumin (**Agarwal *et al.*, 2010)**.

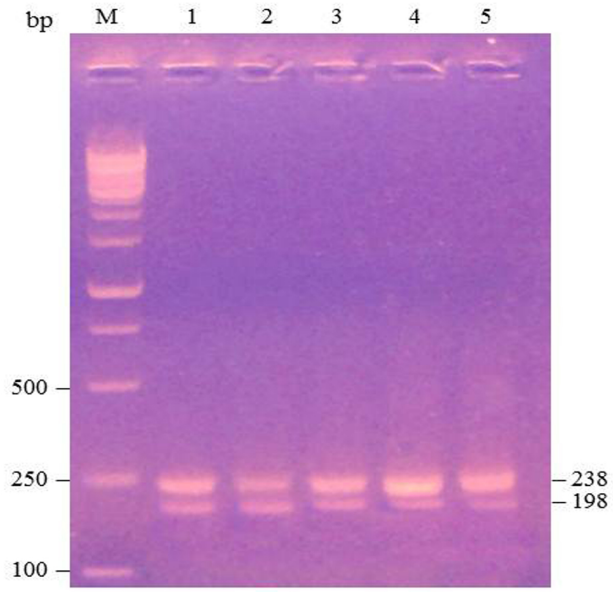


Fig (7): The electrophoretic profile of MT-1&MT-2genes in kidney of rats treated with pb, curcumin and both comparing with the control group using RT-PCR
M: 1000 bp DNA marker; Lane 1: MT-1 (238 bp) &MT-2 (198 bp) in solvent group, Lane 2: MT-1&2 in control group; Lane 3: MT-1&2 in curcumin group; Lane 4: MT-1&2 in Pb-treated group & Lane 5: MT-1&2 in Pb+curcumin group.

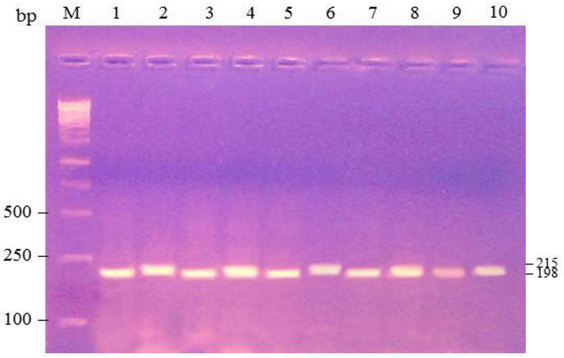


Fig (8): The electrophoretic profile of MT-2gene in kidney of rats treated with pb, curcumin and both comparing with the control group using RT-PCR.
M: 1000 bp DNA marker; Lane 1: MT-2 in solvent group,198 bp; Lanes (2, 4, 6, 8, 10): GAPDH, 215 bp; Lane 3: MT-2 in control group; Lane 5: MT-2 in curcumin group; Lane7: MT-2 in Pb-treated group & Lane 9: MT-2 in Pb+curcumin group.

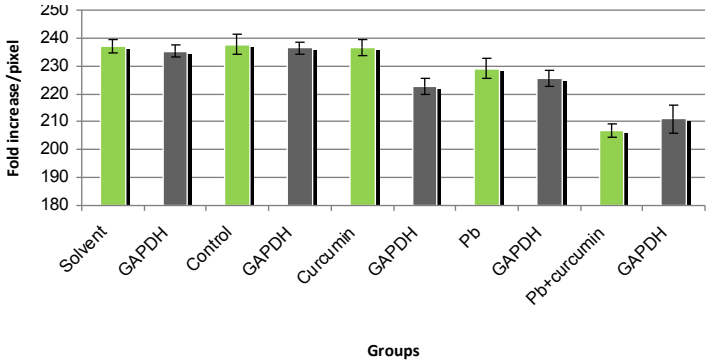
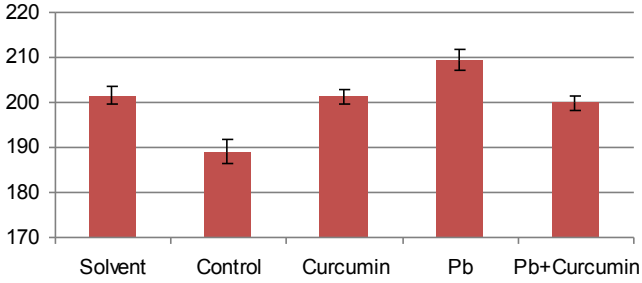


Fig (9): Metallothionine -2mRNA expression level with GAPDH in kidney of rats treated with Pb, curcumin and both comparing with control group.



Fig(10): Metallothionine -1mRNA expression level in kidney of rats treated with Pb, curcumin and both comparing with control group.

Residual analysis

There was a significant increase in the renal lead concentration in female treated rats while the combined treatment with curcumin mitigated this picture (Table 5& Fig 10) which may be attributed to the strong metal ligand binding between curcumin and lead which was previously explored **(Daniel *et al.*, 2004)** whereas the reduction potentials of lead and curcumin which are more negative metal alone; the high negative potential shift forming species between metal and the ligand is harder to reduce than metal alone.

Table (5): The residue levels of Pb (ppm) in kidney of rats treated with Pb, curcumin and both comparing with control groups (Means +SE).

Groups	Control	Curcumin	Solvent	pb	Pb +curcumin
Pb concentration (ppm)	0.97± 0.01^{c}	0.91± 0.01^{c}	0.91± 0.01^{c}	7.25± 0.13^{a}	3.21± 0.11^{b}

Means within the same row have different superscripts are significantly different at ($p \leq 0.05$).

From our findings, it is clear that curcumin prevents lead toxicity in terms of attenuated LPO, decreased GPx, GR, GST, CAT & SOD, creatinine and BUN levels. However, statistically significant lead concentration reductions in the kidney were found in curcumin co-treatment. Curcumin treatment showed no protective e□ect against histopathological changes in the kidney tissues. This may be due to the fact that the time required for the repair of damage at the cellular level may be short, during which reversal of histopathological changes is not possible. We found some induction of MT-I and MT-II mRNA expressions in the kidney tissues after curcumin treatment alone, which may be due to ROS normally present in the animals that might be scavenged from the body by curcumin treatment. This elucidated the antioxidative property of curcumin. The present work suggests that curcumin intake should be helpful in the prevention of lead toxicity and that curcumin can be used as a therapeutic agent for lead intoxication.

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References

Ademuyiwa, O.; Agarwal, R.; Chandra, R. and Behari, J.R. (2009): Lead-induced phospholipidosis and cholesterogenesis in rat tissues. Chem. Biol. Interact., 179 (2–3): 314–20.

Aebi, H. (1984): Catalase *in vitro*. Methods in Enzymol.105: 121-126.

Agarwal, R.; Sudhir, K.; Goel and Jai, Raj Behari (2010): Detoxification and antioxidant effects of curcumin in rats experimentally exposed to mercury. J. Appl. Toxicol.; 30: 457–468.

Aggarwal, B.B. and Sung, B. (2009): Pharmacological basis for the role of curcumin in chronic diseases: an age old spice with modern targets. Trends Pharmacol. Sci.; 30 (2):85–94.

Aggarwal, B.B.; Sundarome, C.; Malani, N. and Ichikawo, H. (2007): "Curcumin: the median solid gold". Adv.Exp.Med.Biol., 595:1-75.

Alvarez-Barrientos, A.; O'Connor, J.E.; Nieto Castillo, R.; Moreno Moreno, A.B. and Prieto, P. (2001): Use of flow cytometry and confocal microscopy techniques to investigate early CdCl2-induced nephrotoxicity *in vitro*. Toxicol *In Vitro,* 15:407–412.

Asali, M.; Roshan, V. D.; Hosseinzadeh, S.; Mahjoub, S. and Moghaddam, A. H. (2011): The role of Exercising and Curcumin on the treatment of lead- induced Cardiotoxicity in rats. Indian Journal of Health and Physical Activity, 2 (1): 1-5.

Ashry, K.M.; El-Sayed, Y.S.; Khamiss, R.M. and El-Ashmawy, I.M. (2010): Oxidative stress and immunotoxic effects of lead and their amelioration with myrrh (Commiphora molmol) emulsion. Food Chem Toxicol; 48(1):236–41.

Bancroft, J. D. and Stevens, A. (1996): Theory and Practice of Histological Technique. 4th Ed., Churchill, Livingston, New York, London, San Francisco, Tokyo.

Chan, H.M. and Cherian, M.G. (1992): Protective roles of metallothionein and glutathione in hepatotoxicity of cadmium. Toxicology 72:281–290

Chan, H.M. and Cherianm, M.G. (1992): Protective roles of metallothionein and glutathione in hepatotoxicity of cadmium. Toxicology, 72:281–290

Christie, N. T. and Costa, M. (1984): *In vitro* assessment of the toxicity of metal compounds IV. Disposition of metals in cells: interactions with membranes, glutathione, metallothionein and DNA ,
Biol. Trace Elem. Res., 6: 139- 158.

Dairam,A.; Limson, J.L.;Watkins, G.M.; Antunes, E and Daya, S. (2007): Curcuminoids, curcumin and demethoxycurcumin reduce lead-induced memory deficits in male Wistar rats. J. Agic. Food Chem., 55(3): 1039–1044

Daniel, S.; Limpson, J.L.; Dairam,A. ;Watkin, G.M. and Daya S. (2004) : Through metal binding, curcumin protects against lead and cadmium-induced lipid peroxidation in rat brain homo-genates and against lead-induced tissue damage in rat brain. J. Inorg. Chem.; 98:266–75.

El-Nekeety, A.A.; El-Kady, A.A.; Soliman, M.S.; Hassan, N.S. and Abdel-Wahhab, M.A. (2009): Protective effect of Aquilegia vulgaris (L.) against lead acetate-inducedoxidative stress in rats. Food Chem. Toxicol. , 47:2209-15.

El-Sayed, Y.S. and El-Neweshy, M.S. (2009): Impact of lead toxicity on male rat reproduction at ''hormonal and histopathological levels''. Toxicol. Lett. ; 189 (Suppl.1):S219–20.

Ercal, N.; Treeratphan, P.; Hammond, T.C.; Mattews, R.H.; Grannemann, N. and Spitz, D. (1996): *In vivo* indices of oxidative stress in lead exposed C57BL/6 mice are reduced by treatment with meso-2, 3-dimercaptosuccinic acid or N-acetyl cysteine. Free Rad. Biol. Med., 21:157–161

Ercal,N. ; Gurer-Orhan,H. and Aykin-Burns, N.(2001): Toxic metals and oxidative stress Part 1.Mechanisms involved in metal –induced oxidative damage . Curr. Top. Med. Chem., 1:529-539.

Eybl ,V.; Kotyzova, D. and Koutensky, J. (2006): Comparative study of natural antioxidants – curcumin, resveratrol and melatonin – in cadmium-induced oxidative damage in mice. Toxicology, 225: 150–156.

Flora, S.; Flora, G. and Saxena, G. (2006): Environmental occurrence, health effects and management of lead poisoning. In: Casas JS, Sordo J, editors. Lead: chemistry, analytical aspects, environmental impact and health effects. Amsterdam, Netherlands: Elsevier Science; 158–228.

Flora, S.J.S.; Mittal, M. and Mehta, A. (2009): Heavy metal induced oxidative stress and its possible reversal by chelation therapy. Indian J. Med. Res. , 128: 501–523.

Goldberg, D. M. and Spooner, R. J. (1983): In methods of enzymatic analysis (Bergmeyen, H. V. Ed.) 3rd edn. Vol 3, pp 258- 266, Verlog Chemie, Deerfield beach, F1.

Gurer, H. and Ercal, N. (2000): Can antioxidants be beneficial in the treatment of lead poisoning? Free Radical Biol and Med. , 29:927-45.

Habig, W.H. ; Pabst, M.J. and Jakoby, W.B. (1974): Glutathione –S- transferases: the first enzymatic step in mercapturic acid formation.J. Biol. Chem., 249: 7130-7139.

Henry, R.J. **(1974)**: Clinical chemistry principals and techniques.2nd ed.,Harper and Row Pupl.,New Yourk. P 525.

Hsu, C.P. and Guo, L.Y. (2002): Antioxidant nutrients and lead toxicity. Toxicol. , 180:33-44.

Hsu, P.C.;Liu, M.Y.; Hsu, C.C.; Chen, L.Y. and Leon, G.Y. (1997): Lead exposure causes generation of reactive oxygen species and functional impairment in rat sperm. Toxicol., 122:133-43.

Jarrar, B.M. (2003): Histological and histochemical alterations in the kidney induced by lead. Ann Saudi Med .; 23(1–2):10–5.

Jayaprakasha, G.K.; Jagan Mohan, Rao, L. and Sakariah, K.K. (2005): Chemistry and biological activities of *C. longa*. Trends Food Sci. Tech., 16(12):533-548.

Julshman, K. (1983): Analysis of major and minor elements in mollusks from Norway. Ph. D. Thesis Institute of Nutrition Directorate of Bergen Nygardsangen, Norway. Fisheries. P.O. Box 4285, N-5013.

Klaassen CD, Liu J (1998): Induction of metallothionein as an adaptive mechanism affecting the magnitude and progression of toxicological injury. Environ Health Perspect., 106:297–300.

Kumar, A.; Dogra, S. and Prakash, A. (2009): Protective effect of curcumin (*Curcuma longa*) against aluminium toxicity: possible behavioral and biochemical alterations in rats. Behavioural Brain Research, 205: 384-390.

Liu,C.M. ; **Ma,J .Q. and Sun,Y.Z.(2010)**: Puerarin protects the rat liver against oxidative stress-mediated DNA damage and apoptosis induced by lead. Exp. Toxicol. Pathol.(in press).

Liu,C.G.; Zhang,L.; Jiang,Y.; Chatterjee,D.; Croce,C.M.; Huebner,K. & Fong L.Y. (2005): Modulation of gene expression in precancerous rat esophagus by dietary zinc deficit and replenishment. Cancer Research, 65. 17: 7790-7799.

Lu, J.; Jin, T.; Nordberg, G. and Nordberg,G.(2005): Metallothionein gene expression in peripheral lymphocytes and renal dysfunction in a population environmentally exposed to cadmium. Toxicol Appl Pharmacol., 150:156-206

Manjunatha, H. and Srinivasan, K. (2006):. Protective effect of dietary curcumin and capsaicin on induced oxidation of low-density lipoprotein, iron-induced hepatotoxicity and carrageenan-induced inflammation in experimental rats. FEBS, 273(19): 4528–4537**.**

Maracine, M. and Segner, H. (1998): Cytotoxicity of metals in isolated fish cells: importance of the cellular glutathione status. Comp Biochem Physiol A., 120:83–88.

Michael, J. M. ; Fischer, S. U. and Karl, M. (2010): Measurement of meningeal blood vessel diameter *in vivo* with a plug-in for Image J. Microvascular Research, 80 (2) : 258–266.

Nair,J.; Strand, S.; Frank, N.; Knauft, J.; Wesch, H.; Galle, P.R. and Bartsch H. 200 Apoptosis and age-dependant induction of nuclear and mitrochondrial etheno-DNA adducts in Long–Evans Cinnamon (LEC) rat enhanced DNA damage by dietary curcumin upon copper accumulation. Carcinogenesis, 26(7): 1307–1315.

Nair, J.; Strand, S.; Frank, N.; Knauft, J.; Wesch, H.; Galle, P.R. and Bartsch, H. (2005): Apoptosis and age-dependant induction of nuclear and mitrochondrial etheno-DNA adducts in Long–Evans Cinnamon (LEC) rats: enhanced DNA damage by dietary curcumin upon copper accumulation. Carcinogenesis, 26(7): 1307–1315.

Nishikimi, M.; Roa, N. A. and Yogi, K. (1972): the occurrence of superoxide anion in the reaction of reduced Phenazine methosulphate and molecular oxygen.Biochem. Bioph. Res. Common., 46: 849-854.

Nolan, C.V. and Shaikh, Z.A. (1992): Lead nephrotoxicity and associated disorders: biochemical mechanisms. Toxicology, 73:127–146.

Ohkawa, H.; Ohishi, N. and Yagi, K. (1979): Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction ; Anal. Biochem, 95: 351-358.

Padmaja, S. and Raju, T.N. (2004): Antioxidant e□ect of curcumin in selenium induced cataract of Wistar rats. Ind. J. Exp. Biol., 42(6): 601–603.

Paglia, D. E. and Valentine, W. N. (1967): Studies on quantitative and qualitative characterization of erythrocyte gluatathione perioxidase. J. Lab. Clin. Med., 70: 158-169.

Patra, R.C.; Swarup, D. and Dwivedi, S.K. (2001): Antioxidant effects of a-tocopherol, ascorbic acid and L-methionine on lead induced oxidative stress to the liver, kidney and brain in rats. Toxicol ., 162:81-88.

Patrick, L., (2006): Lead toxicity, a review of the literature. Part 1: Exposure, evaluation and treatment. Alt. Med. Rev., 11: 2-22.

Phan, T.T.; See, P.; Lee, S.T. and Chan, S.Y. (2001): Protective effects of curcumin against oxidative damage on skin cells *in vitro*: its implication for wound healing. J. Trauma, 51: 927–931.

Roels, H.A.; Hoet, P. and Lison, D. (1999) Usefulness of biomarkers of exposure to inorganic mercury, lead, or cadmium in controlling occupational and environmental risk of nephrotoxicity. Ren Fail., 21:251–262

Rukkumani, R.; Balasubashini, S. and Menon, V.P. (2003): Protective effects of curcumin and photoirradiated curcumin on circulatory lipids and lipid peroxidation products in alcohol and polyunsaturated fatty acid induced toxicity. Phytother. Res., 17: 925–92

Sambrook, J.; Fritsch, A. and Maniatis, T. (1989): Molecular cloning: a laboratory manual, Second edition. Cold Spring Harbor Laboratory Press.

SAS Institute, Inc (1997): Statistical analysis system for windows 6-12. Ed, Cary, NC.USA.

Schmitt, C.J.; Whyte J.J.; Robert, A.P.; Aniss, M.L.; May, T.W. and Tillitt, D.E. (2007): Biomarkers of metals exposure in fish from lead-zinc mining areas of South eastern Misshouri, USA. Ecotoxicol Environ Saf., 67: 31-47.

Shukla, P.K.; Khanna, V.K.; Ali, M.M.; Khan,M.Y. and Srimal, R.C. (2008): Anti-ischemic effect of curcumin in rat brain. Neurochem Res.; 33:1036–43.

Shukla, P. K.; Khanna,, V. K.; Khan, M. Y. and Srimal, R. C. (2003): Protective effect of curcumin against lead neurotoxicity in rat. Experimental Toxicology, 22 (12): 653-658.

Sidhu, P. and Nehru, B. (2004): Lead intoxication: histological and oxidative damage in rat cerebrum and cerebellum. J. Trace Elem. Exp. Med. ;17(1):45–53.

Sidu, P.; Garg, M. L. and Dhawan, J. (2004): Protective effects of zinc on oxidative stress enzymes in liver of protein deficient rats. Nutr. Hosp. XIX, 6:341- 347.

Singletary, K.; MacDonald, C.; Lovinelli,M.; Fisher, C. and Wallig, M. (1998): Effect of the diketones diferuloylmethane (curcumin) and dibenzoylmeth-ane on rat mammary DNA adducts and tumor induced by 7,12-dimethylbenz(a)anthracene. Carcinogenesis, 19: 1039–1043.

Sinha, A.K. (1972): Colorimetric assay of catalase. Anal. Biochem., 47: 389– 394.

Sivaprasad, T.R., Malarkodi, S.P. and Varalakshmi, P. (2004): Therapeutic efficacy of lipoic acid in combination with dimercaptosuccinic acid against lead-induced renal tubular defects and on isolated brush-border enzyme activities. Chem. Biol. Interact., 147(3): 259-271.

Strimpakos, A.S. and Sharma, R.A. (2008): Curcumin**:** preventive and therapeutic properties in laboratory studies and clinical trials. Antioxid Redox Signal ; 10(3):511–45.

Taghred, S.M. (2012): Non domestic birds as biomarker for some environmental pollutants in Sharkia Province. Ph. D. Thesis, Dept. of Med. Toxicology Zagazig University,Egypt.

Taib, N.T.; Jarrar, B.M. and Mubarak, M. (2004): Ultrastructural alterations in hepatic tissues of white rats (Rattus norvegicus) induced by lead experimental toxicity. Saudi J Biol Sci.;11:11–20.

Tom, M., Chen, N., Segev, M., Herut, B., and Rinkevich, B. (2004): Quantifying fish metallothionein transcript by real - time PCR for its utilization as an environmental biomarker . Mar Pollut. Bol., 48: 705- 710.

Upasani, C.D. and Balaraman, R. (2003): Protective effect of Spirulina on lead induced deleterious changes in the lipid peroxidation and endogenous antioxidants in rats. Phytother Res., 17: 330–334.

Wang, L.; Li, J. Li, J. and Liu, Z. (2010): Effects of lead and/ or Cadmium on the oxidative damage of rat kidney cortex mitochondria. Biol. Trace Elem Res., 37: 69- 78.

Xu, Y.; Li, G.; Han, C.; Sun, L.; Zhao, R. and Cui, S. (2005): Protective effect of Hippophae rhamnoides L. juice on lead-induced neurotoxicity in mice. Biol Pharm Bull, 28:490-94.