Efficacy of Curcumin on Lead Induced Nephrotoxicity in Female Albino Rats

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Abstract: Lead (Pb) 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 is to clarify the possible corrective role of curcumin on the nephrotoxicity induced by lead acetate. Thirty mature female albino rats (190 - 225 g) were randomly divided into five equal groups, First and second groups were kept as positive and negative controls orally received 1% carboxymethyl cellulose dissolved in distilled water 3 times a week and drinking water respectively. Third group received orally 100 mg curcumin / Kg B. W. dissolved in 1% carboxymethyl cellulose 3 times a week, fourth group received 500 mg lead acetate / L in drinking water, fifth group received lead acetate and curcumin as previously mentioned with regard to dose and route for each of them. All rats were decapitated after two months from previous treatments. The results revealed that lead acetate toxicity induced significant increase in the levels of serum urea and creatinine as well as malondialdehyde (MDA) and lead acetate residue in kidney tissues meanwhile, renal antioxidant enzymes decreased significantly when compared with the control groups. On the level of metallothionein (MT1-MT-2) mRNA expression, lead induced mild up regulation when compared to control groups. The co-treated group with lead and curcumin evoked a significant amelioration. The histopathological investigation confirmed the aforementioned findings. 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 study had shown that simultaneous treatment with curcumin protects against alterations and oxidative stress in rat brain and immunosuppressive effects of lead **(Daniel *et al.,* 2004)**. However the molecular mechanism of lead induced kidney injury and nephroprotective effects of curcumin are not yet completely understood.

Therefore we 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 their 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 five equal groups, First and second groups were kept as positive and negative controls orally received 1% carboxymethyl cellulose dissolved in distilled water 3 times a week and drinking water respectively. Third group received orally 100 mg curcumin / Kg B. W. dissolved in 1% carboxymethyl cellulose 3 times a week **(Shukla *et al.,* 2003).** Fourth group received 500 mg lead acetate / L in drinking water **(Liu *et al.,* 2010)**.Fifth group received lead acetate and curcumin as previously mentioned with regard to dose and route for each of them. All rats were decapitated after 2 months from previous treatment.

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 (Shimadzu). 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 superoxide dismutase (SOD) **(Nishikimi *et al.,* 1972)**, glutathione S-transferase (GST) **(Habig *et al.,* 1974)**, glutathione reductase (GR) **(Goldberg and Spooner, 1983)**,glutathione peroxidase (GPx) **(Paglia and Valentine, 1967)** & catalase (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 estimation the expression of 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 genes (MT) in kidney RNA extraction and cDNA formation

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

PCR reaction was performed using the Dream Taq-Green PCR Master Mix. The 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 with sequence as follow forward primer 5 CAC CGT TGC TCC AGA TTCAC-3, reverse primer 5- GCA GCA GCA CTG TTCGTCAC-3 (gene bank accession number RATMETI),MT-2 forward primer 5-ATC TCC AAC TGCCGCCTCC-3, reverse primer 5- TGC ACT TGT CCGAAGCCTCT-3 (gene bank accession number XM_001062488) and GAPDH (internal control) forward primer 5- CCT TCA TTG ACC TCA ACT ACATG-3, reverse primer 5-CTT CTC C A TGG TGG TGAAGAC-3 (gene bank accession number NM_017008). 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% agarose 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 1.24) for measurement of band intensity in Pixels and determined the fold increase in intensity between different bands. 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 and alterations in GPx, GR, GST, CAT SOD activities in kidney tissues.

Table (1). Showing serum parameters of rats treated with Pb, Curcumin and both comparing with control group (Means ± SE).

Groups / Parameter	Solvent	Control	Curcumin	Pb	Pb+ Curcumin
Urea (mg/dL)	23.26±0.21^{c}	23.72±0.26^{c}	23.09±0.91^{c}	45.76±1.02^{a}	37.32±1.01^{b}
Creatinine (mg/dL)	0.34±0.01^{c}	0.39±0.02^{c}	0.36±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 ($p \leq 0.05$) decrease in antioxidant enzymes in Pb treated rats kidneys comparing with the control ones, while in co- treated group ; our data recorded a significant ($P \leq 0.05$) 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 sulfhydryl 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 ($P \leq 0.05$) 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 ($P \leq 0.05$) 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, curcumin and both on GPx, GR, GST, CAT & SOD activities & MDA concentration in kidney homogenate of rats comparing with control group (Means ± SE).

Parameter \ Group	Solvent	Control	Curcumin	Pb	Pb+ curcumin
GPx (U/g tissue)	14.80±0.52^{a}	15.51 ±0.63 ^{a}	14.52 ±0.77^{a}	8.81±0.53^{c}	11.22±0.67^{b}
GR (µM /g tissue)	44.58±1.36^{a}	44.16±1.02^{a}	45.71 ±1.06^{a}	24.17±0.90^{c}	37.11±1.05^{b}
GST(µ mole /g tissue)	11.71±0.69^{ab}	11.93±0.42^{ab}	12.10 ±0.72^{a}	7.77±0.20^{c}	10.36±0.28^{b}
CAT (µmole H2O2 decomposed /g tissue)	20.02±0.81^{a}	20.06 ±1.12^{a}	21.11±0.73^{a}	12.23±0.62^{b}	14.43±0.76^{b}
SOD (U/g tissue)	33.99±1.22^{a}	34.01 ±1.32^{a}	35.64 ±1.70^{a}	19.45±1.19^{c}	25.36±1.29^{b}
MDA (nmole /g tissue)	132.47±3.69^{c}	130.59±3.43^{c}	132.07±3.05^{c}	202.13±4.52 ^{a}	171.43±2.45^{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) .

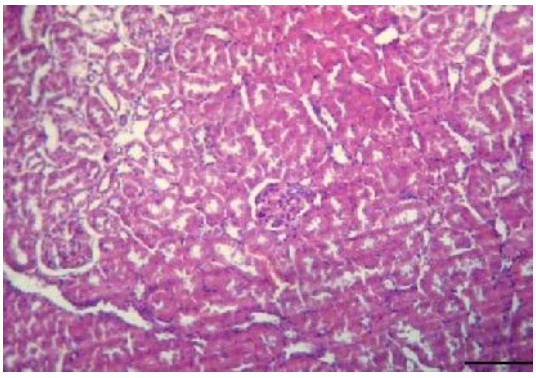


Fig (1): Kidney of control group: Showing normal nephrone of glomerular and tubular structures, HE (Bar = 100 µm).

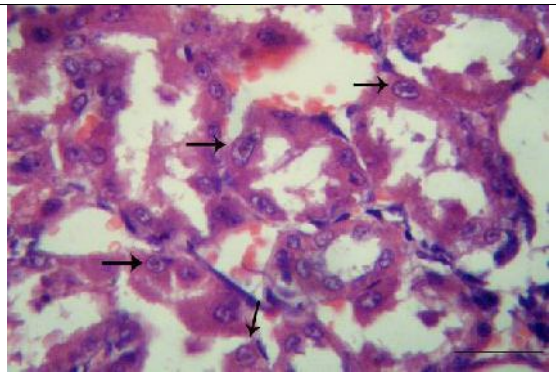


Fig (2): Kidney of lead group: Showing hypertrophied renal epithelia (karyo and cytomegaly) with eosinophilic intranuclear inclusion body, HE (Bar = 100 µm).

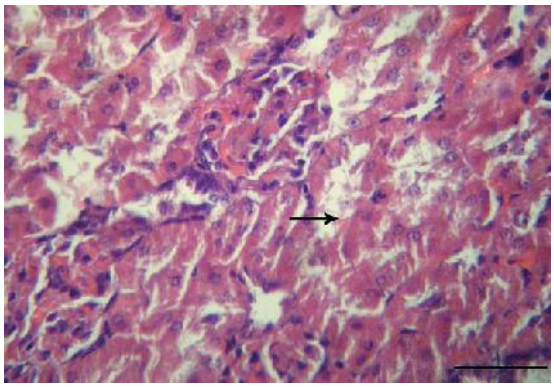


Fig (3): Kidney of lead group: Showing focal area of coagulative necrosis, HE (Bar = 100 µm).

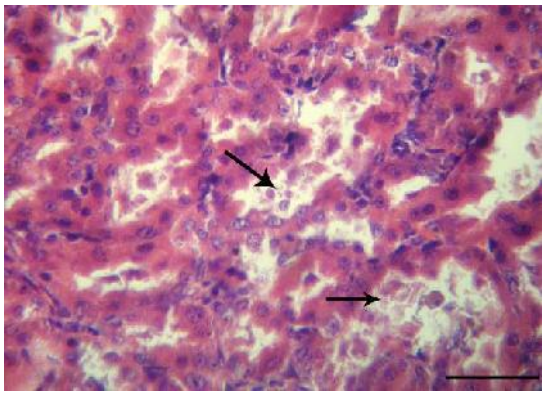


Fig (4): Kidney of lead group: Showing exofoliated epithelium in the form of cellular casts, HE (Bar = 100 µm).

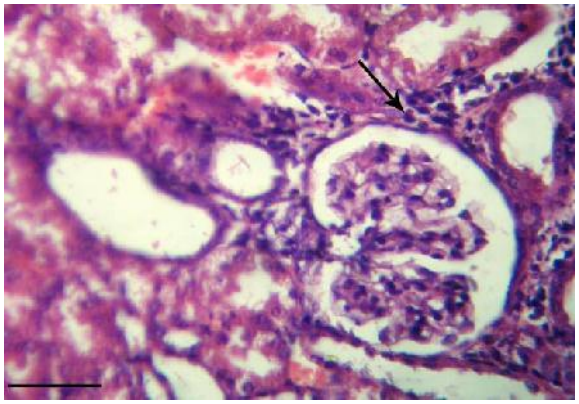


Fig (5): Kidney of lead group: Showing periglomerular and interstitial aggrgations of round cells, HE (Bar = 100 µm).

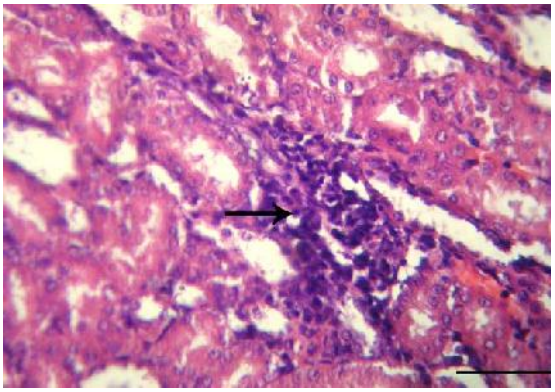


Fig (6): Kidney of lead & curcumin group: Showing few interstitial aggregation of round cells, HE (Bar = 100 µm).

Effects of lead and/or curcumin on the expression of metallothionein (MT) genes in rat's kidney

Our results revealed that lead mild up-regulated MT-1&MT-2 mRNA expression in kidneys of Pb treated rats compared to control groups and this level was down regulated on curcumin treatment (Figs. 7-10). Our results are 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 especially for metal contamination **(Alvarez-Barrientos *et al.*, 2001)** and protects against metal toxicity (**Chan and Cherian**, **1992 and Klaassen and Liu, 1998)** which returns to the oxidative stress caused by ROS generated by lead toxicity. The alterations in LPO and in the activities of antioxidant enzymes in kidney tissues were returned to lead as it is a relatively hard metal and 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 little 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)**.

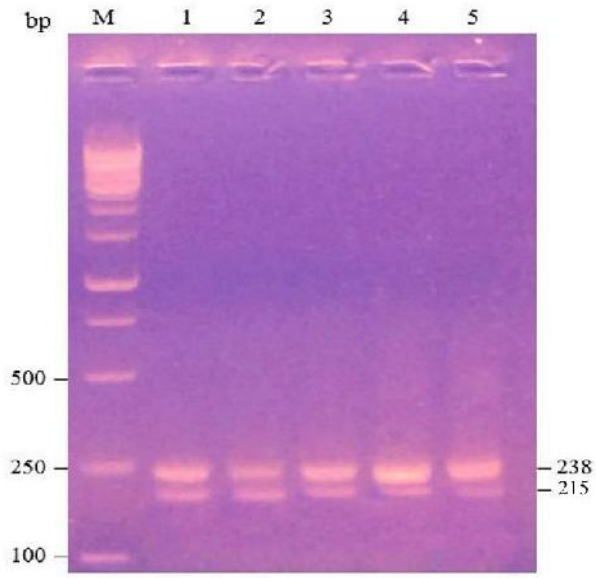


Fig (7): The electrophoretic profile of MT-1(238bp) and GAPDH (215bp) expression in kidney of rats treated with pb, curcumin and both comparing with the control group using RT-PCR
M: DNA marker; Lane 1: MT-1 and GAPDH in solvent group, Lane 2: MT-1 and GAPDH in control group; Lane 3: MT-1 and GAPDH in curcumin group; Lane 4: MT-1 and GAPDH in Pb-treated group & Lane 5: MT-1 and GAPDH in Pb+curcumin group. Densitometric analysis was carried for 3 different rat. (a) $p < 0.05$ vs. control while (b) $p < 0.05$ vs. lead treated group.

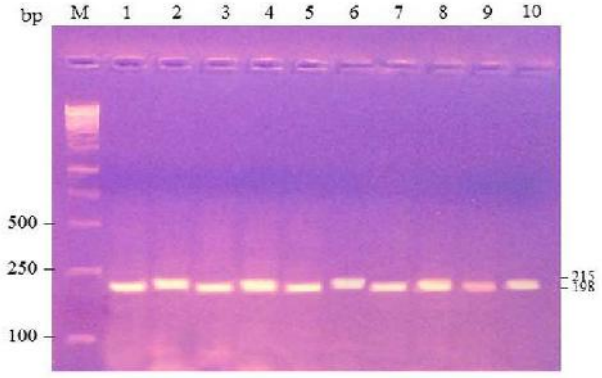


Fig (8): The electrophoretic profile of MT-2 expression in kidney of rats treated with pb, curcumin and both comparing with the control group using RT-PCR.M: DNA marker; Lane 1: MT-2 in solvent group,198 bp; Lanes (2, 4, 6, 8, 10): GAPDH, 215 bp; Lane 3: MT-2 in control group; Lane 5: MT-2 in curcumin group; Lane7: MT-2 in Pb-treated group & Lane 9: MT-2 in Pb+curcumin group.Densitometric analysis was carried for 3 different rat. (a) $p < 0.05$ vs. control while (b) $p < 0.05$ vs. lead treated group.

Fig(9): Metallothionine -1mRNA expression level in kidney of rats treated with Pb, curcumin and both comparing with control group.

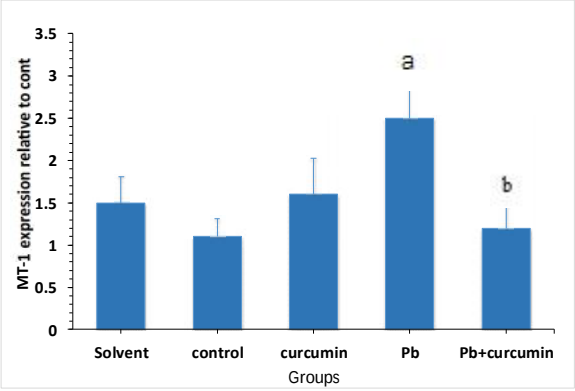
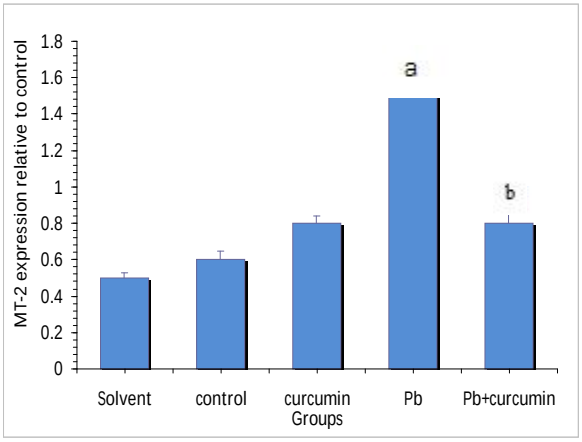


Fig (10): Metallothionine-2 mRNA expression level with GAPDH in kidney of rats treated with Pb, curcumin and both comparing with control group.



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) 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 lead, curcumin and both comparing with control groups (Means ±SE).

Groups	Solvent	Control	Curcumin	pb	Pb + curcumin
Pb^{+2} concentration (ppm)	0.91 ± 0.01^{c}	0.97 ± 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, increased GPx, GR, GST, CAT & SOD and decreased creatinine and urea levels. However, statistically significant lead residue reductions in the kidney were found in curcumin co-treatment. Curcumin treatment showed slight protective effect 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 little 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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