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Research Article

THE PROTECTIVE EFFECT OF THYMOQUINONE AGAINST LEAD ACETATE INDUCED DNA DAMAGE AND ALTERATIONS IN TUMOR INITIATION GENES

SALEHA YM ALAKILLI[1], WAGDY KB KHALIL[2], KARIMA F MAHROUS[2]

[1]Department of Biology, King Abdulaziz University, Jeddah, Saudi Arabia. [2]Department of Cell Biology, National Research Centre, 33 Bohouth St., 12622 Dokki, Giza, Egypt.

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ABSTRACT

Objective: Several pollutants represent a significant ecological and public health concern due to their toxicity and their ability to accumulate in living organisms in which lead is one of them. The present investigation was designated to assess the modulating effect of thymoquinone (TQ) against lead acetate (LA) toxicity.

Methods: Several endpoints were considered to design this study such as: The gene expression of tumor initiation genes (cytochrome P450 3A [CYP3A], cyclooxygenase 2 [COX2], BAX and Bcl_2), DNA damage and alterations in the levels of glutathione (GSH), lipid oxidation (malondialdehyde [MDA]), and protein oxidation (protein carbonyl [PC]) in male rats. About 60 male rats were used in this study which allocated in six groups (10 animal each) and treated with LA (200 mg/kg diet), TQ (5 and 10 mg/kg b.wt.), and LA + TQ.

Results: The results revealed that LA induced significant DNA damage and alteration in the expression of CYP3A, COX2, BAX, and Bcl_2 as well as induced changes in GSH content and MDA and PC levels in male rats. Meanwhile, TQ was decreased significantly the toxic effect of LA in male rats which decreased the alterations in the gene expression and DNA damage as well as GSH content and MDA and PC levels.

Conclusion: The results suggested that TQ treatment confers protection against toxicity inflicted by LA and support the contention that TQ protection is achieved by its ability as a scavenger for free radicals generated by LA.

Keywords: Thymoquinone, Lead acetate, Gene expression, DNA damage, Rats.

INTRODUCTION

In the developing country, exposure to few of toxic metals causes many of the classic occupational diseases. One of these toxic metals is lead, which has many uses in the industry including pipes, paints, hair dyes, enamels, glazes, motor industry, and others [1]. The predominant hazard in industry arises from the inhalation of dust and fume, but the organic compounds may also be absorbed through the skin. Ingestion is a much less serious problem in the industry, although in the general environment it is the predominant route of entry [1].

Lead is transported following absorption to the red cell, and considerably <10% of the total blood lead concentration represents lead in the plasma. All the soft tissues have their complement of lead [2]. The total blood lead concentration is not raised in organic lead poisoning, although the fraction which is lipid-bound is usually elevated. It is technically rather difficult to estimate the lipid-bound fraction, so biological monitoring of organic lead workers tends to be based on urinary lead analysis; the aim should be to keep the urinary concentration below 150 μg/L (0.7 mol/L) [2].

Lead represents a significant ecological and public health concern due to its toxicity and its ability to accumulate in living organisms. Numerous studies have demonstrated that lead can pass through the blood-testis barrier, accumulate in the testis and/or epididymis and affect the germinal cells at different levels of differentiation (spermatogonia, primary spermatocytes, spermatids or spermatozoa) [3]. Several studies assessed the genotoxic effect of lead acetate (LA) by means of chromosomal aberrations and micronucleus test.

Regarding the induction of chromosomal aberrations, LA induced significant increase of aberrant cells and numerical aberrations in bone marrow cells of Wistar rats [4,5]. In additional, Aboul-Ela [1] detected a significant increase of structural chromosomal aberrations in the bone marrow cells and primary spermatocytes of albino mice treated with LA. In addition, LA proved to be a potent micronuclei inducer *in vivo* and *in vitro* test systems: LA induced micronuclei in kidney cells of Sprague–Dawley albino rats [6]; in human melanoma cell [7]; in Chinese hamster V79 cells [8]; in Wistar rats' leukocytes, reticulocytes and erythrocytes [9,10]; in rats' erythrocytes [11,12]; in peripheral blood erythrocytes, gill and fin epithelial cells of *Carassius auratus* [13], and in bone marrow cells of Algerian mice [14]. Moreover, positive results indicate the induction of sister chromatid exchange with LA was obtained by Poma *et al*. [7] in human melanoma cells and in bone marrow cells of Algerian mice [14].

The most of Asian and African countries are very rich in historical knowledge in particular about the medicinal use of herbs and spices. Of the most noted, is the historical reference to the remarkable use of black cumin seed (BCS) (*Nigella sativa*) which dates back to the burial of the Egyptian Pharaoh Tutankhame. Black cumin was also mentioned in ancient texts by Hippocrates and Dioscorides to treat headaches and toothaches [15]. *N. sativa* seed has a long history of unsurpassed medicinal value with versatility to treat a wide range of ailments, including headaches, asthma, colds, paralysis, pain, gastrointestinal problems, eczema, obesity, and diabetes [16]. Only in this century have we begun to explore the potential of *N. sativa* seed, which continues to be useful in cooking, baking, pastries, cheeses, wine, and an ingredient in bread referred to as Naan. Medicinally, it has been reported to treat amongst many things infections [17], hypertension [18], brain injury [19], opioid dependence [20], and cancer [21].

Thymoquinone (2-Isopropyl-5-methyl-1, 4-benzoquinone, TQ) is believed to be the main active constituent of BCS oil responsible for suppressing the proliferation of tumor cells including colon, breast, bone, ovarian, prostate, and pancreatic carcinoma [22,23].

The objective of this study is to assess the protective effect of TQ to modulate the frequency of LA-induced gene expression alteration, DNA damage, and biochemical changes in male rats.

METHODS

Chemicals

LA and reagents for gene expression analysis including kits, chemicals, and primers were purchased from and Sigma-Aldrich and Invitrogen (Germany). All reagents and chemicals were of the highest purity available.

Experimental animals

Swiss albino male rats purchased from the Animal House Colony, Jeddah, Saudi Arabia, were maintained on standard laboratory diet (protein - 16.04%; fat - 3.63%; fiber - 4.1%; and metabolic energy - 0.012 MJ), and water *ad libitum* at the were obtained from the Animal House Colony of the Department of Biology, King Abdulaziz University, Jeddah, Saudi Arabia. After an acclimation period of 1 week and at 50 days of age, animals were divided into nine groups (10 rats/group) and housed individually in filter-top polycarbonate cages housed in a temperature-controlled (23°C±1°C) and artificially illuminated (12 hrs dark/light cycle) room free from any source of chemical contamination.

Experimental design

Male albino rats (n=60) at 50 days of age were maintained on standard laboratory diet water *ad libitum*. The animals were allocated in different groups as follows: The first group: Untreated animal (control); the second group: Animals administered 200 mg LA/kg diet, once daily, 5 days for 4 weeks [1]; the third and fourth groups: Animals treated with LA were treated orally with 5 and 10 mg/kg of TQ 5 times per week for 2 months, respectively; fifth and sixth groups: Healthy animals were orally with 5 and 10 mg/kg of TQ 5 times per week for 2 months, respectively.

At the end of experimental treatment, the animals were sacrificed, and then the liver tissues, bone marrow cells, and brain tissues were extracted from all treatment groups. The liver tissues were kept at −80°C for molecular analysis, bone marrow cells were used immediately for comet assay and brain tissues were kept at −20°C for biochemical analysis.

Semi-quantitative - Reverse transcription-polymerase chain reaction (RT-PCR)

RNA extraction and first-strand cDNA synthesize

Liver tissue samples, which stored at −80°C prior to extraction, were used to extract the total RNA. Total RNA was isolated from 50 to 100 mg of tissues by the standard TRIzol extraction method (Invitrogen, Paisley, UK) and recovered in 100 µl molecular biology grade water. In order to remove any possible genomic DNA contamination, the total RNA samples were pre-treated using DNA-free™ DNase treatment and removal reagents kit (Ambion, Austin, TX, USA) following the manufacturer's protocol. The RNA concentration wasdetermined by spectrophotometric absorption at 260 nm.

To synthesize the first-strand cDNA, 5 µg of the complete Poly(A)$^+$ RNA isolated from rat samples was reverse transcribed into cDNA in a total volume of 20 µl using 1 µl oligo (poly(deoxythymidine)$_{18}$) primer [24]. The composition of the reaction mixture consisted of 50 mM $MgCl_2$, ×10 RT buffer (50 mM KCl; 10 mM Tris-HCl; pH 8.3), 200 U/µl reverse transcriptase (RNase H free), 10 mM of each dNTP, and 50 µM of oligo (dT) primer. The RT reaction was carried out at 25°C for 10 minutes, followed by 1 hr at 42°C, and finished with denaturation step at 99°C for 5 minutes. Afterward the reaction tubes containing RT preparations were flash-cooled in an ice chamber until being used for DNA amplification through PCR [25].

RT-PCR assay

The first strand cDNA from different rat samples was used as templates for the SQ RT-PCR with a pair of specific in a 25 µl reaction volume. The sequences of specific primer and product sizes are listed in Table 1. β-actin was used as a housekeeping gene for normalizing mRNA levels of the target genes. The reaction mixture for RT-PCR was consisted of 10 mM dNTP's, 50 mM MgCl2, 10x PCR buffer (50 mM KCl; 20 mM Tris-HCl; pH 8.3), 1U/µl taq polymerase, and autoclaved water. The PCR cycling parameters of the studied genes (Cytochrome P450 3A [CYP3A], cyclooxygenase 2 [COX2], BAX, and Bcl_2) were performed as the PCR condition summarized in Table 1. The PCR products were then loaded onto 2.0% agarose gel, with PCR products derived from β-actin of the different rat samples. Each reaction of the RT-PCR was repeated with 10 rats, generating new cDNA products at least ten times per each group.

Estimation of DNA damage using comet assay

The modified single-cell gel electrophoresis, comet assay [26] was used to estimate the DNA damage in isolated hepatic cells of male rats. To obtain the cells, a small piece of the liver was washed with an excess of ice-cold Hank's balanced salt solution (HBSS) and minced quickly into approximately 1 mm^3 pieces while immersed in HBSS, with a pair of stainless steel scissors. After several washings with cold phosphate-buffered saline (PBS) (to remove red blood cells [RBC]), the minced liver was dispersed into single cells using a pipette. In brief, the protocol for electrophoresis involved embedding of the isolated cells in agarose gel on microscopic slides and lysing them with detergent at high salt concentrations overnight (in the cold). The cells were treated with alkali for 20 minutes to denature the DNA and electrophoresis under alkaline conditions (30 minutes) at 300 mA, 25 V. The slides were stained with ethidium bromide and examined using a fluorescence microscope (Olympus BX60 F-3) with a green filter at ×40 magnification. For each experimental condition, about 100 cells (about 25 cells per fish) were examined to determine the percentage of cells with DNA damage that appear like comets. The non-overlapping cells were randomly selected and were visually assigned a score on an arbitrary scale of 0-3 (i.e. Class 0 = No detectable DNA damage and no tail; Class 1 = Tail with a length less than the diameter of the nucleus; Class 2 = Tail with length between 1x and 2x the nuclear diameter; and Class 3 = Tail longer than 2x the diameter of the nucleus) based on perceived comet tail length migration and relative proportion of DNA in the nucleus [26]. A total

Table 1: Primers and PCR thermocycling parameters

Gene	Primer sequence (5¢-3¢)	Conditions of the PCR assay	PCR amplicons (bp)
CYP3A	GAA GCA TTG AGG AGG ATC AC GGG TTG TTG AGG GAA TCC AC	25 cycles: 94°C, 40 seconds; 54°C, 40 seconds; 72°C, 45 seconds Final extension: 72°C, 5 minutes	376
COX2	CTG TAT CCC GCC CTG CTG GTG ACT TGC GTT GAT GGT GGC TGT CTT	25 cycles: 94°C, 30 seconds; 65°C, 30 seconds; 68°C, 1 minutes Final extension: 68°C, 2 minutes	279
BAX	ACA AAG ATG GTC ACG GTC TGC C GGT TCA TCC AGG ATC GAG ACG G	25 cycles: 94°C, 30 seconds; 65°C, 30 seconds; 68°C, 1 minute Final extension: 68°C, 2 minutes	429
Bcl_2	CTC AGT CAT CCA CAG GGC GA AGA GGG GCT ACG AGT GGG AT	25 cycles: 94°C, 30 seconds; 65°C, 30 seconds; 68°C, 1 minute Final extension: 68°C, 2 minutes	450
β-actin	GTG GGC CGC TCT AGG CAC CAA CTC TTT GAT GTC ACG CAC GAT TTC	25 cycles: 94°C, 30 seconds; 65°C, 30 seconds; 68°C, 1 minute Final extension: 68°C, 2 minutes	540

PCR: Polymerase chain reaction, COX2: Cyclooxygenase 2, CYP3A: Cytochrome P450 3A

damage score for each slide was derived by multiplying the number of cells assigned to each class of damage by the numeric value of the class and summing up the values. Slides were analyzed by one observer to minimize the scoring variability.

Estimation of glutathione (GSH) concentration

The GSH concentration in brain tissues was estimated using standard method of Luqman *et al*. [27] with slight modification. Briefly: A volume of 900 µl of phosphate solution was added to 100 µl of packed RBC. The tubes were centrifuged at 5000 rpm for 5 minutes, and the supernatant was discarded. 100 µl of phosphate solution was added to 100 µl of RBC (pellet). Afterward, 100 µl was taken from 200 µl of cell suspension and then 900 µl of distilled water was added to them followed by the addition of 1.5 ml precipitation solution. The tubes were centrifuged at 5000 rpm for 3 minutes. 200 µl phosphate solutions and 25 µl of freshly prepared 5, 5'-Dithiobis 2-nitro benzoic acid (DTNB) were added to the 50 µl of the supernatant. This method is based on the ability of the sulfhydryl group to reduce DTNB and form a yellow-colored anionic product which absorbed and measured at 412 nm. Concentration of GSH is expressed as µmol/ml of packed erythrocytes and was determined from a standard plot.

Lipids and protein oxidation analyses

Lipid oxidation analysis by determination of malondialdehyde (MDA) concentration

MDA formed during lipid peroxidation was measured in brain tissues according to the method of Luqman *et al*. [27]. Brain tissues (200 µg) were suspended in 3 ml of PBS-glucose solution (pH 7.4). To 1.0 ml of the suspension, 1.0 ml of 10% TCA was added. Centrifugation was done for 5 minutes at 5000 rpm. To 1.0 ml of supernatant, 1.0 ml of 0.67% TBA in 0.05 mol/L NaOH was added. Tubes were kept in boiling water bath for 20 minutes at temperature >90°C and cooled. Absorbance was measured at 532 nm (optical density [OD1]) and 600 nm (OD2) against a blank. The net OD was calculated after subtracting absorbance at OD2 from that of OD1. The concentration of MDA was determined from a standard plot and expressed as nmol/ml of cerebellum tissues.

Protein oxidation analysis by determination of protein carbonyl (PC)

Protein oxidation was analyzed through the cellular PC content in brain tissues in which it was determined according to the method described by Baltacioglu *et al*. [28] with a minor modification using the 2, 4-dinitrophenylhydrazine reagent. The carbonyl content was calculated from the peak absorbance at 340 nm, using an absorption coefficient of 22,000/M/cm.

Statistical analysis

Data for gene expression and biochemical analyses were analyzed using the general linear models procedure of statistical analysis system [29] followed by Scheffé-test to assess significant differences between groups. The values are expressed as a mean±standard error of mean. All statements of significant were based on the probability of $p<0.05$.

RESULTS

Determination of changes in the gene expression

Expression of tumor initiation genes including CYP3A, COX2, BAX, and Bcl_2 in liver tissues of male rats is summarized in Figs. 1-4, respectively. The expression levels of CYP3A, COX2, and BAX genes were significantly higher ($p\leq0.01$) in the liver tissues of rats exposed to LA compared with control rats (Figs. 1-3). However, the expression levels of Bcl_2 gene were significantly lower in rats exposed to LA compared with control rats (Figs. 1-3).

On the other hand, the expression levels of CYP3A, COX2, and BAX genes were significantly lower in male rats treated with *TQ compared with those exposed to* LA*, where, the expression levels of these genes in low or high dose of TQ treated rats were relatively similar to control rats (Figs. 1-3). In contrary,* the expression levels of Bcl_2 gene were significantly higher in male rats treated with *TQ compared with those exposed to* LA *(Fig. 4).*

Moreover, treatment of LA*-exposed rats with TQ was able to prevent the alterations in the expression induced by* LA *in the genes under study. Whereas, the expression* levels of CYP3A, COX2, and BAX genes decreased significantly in LA-*exposed rats* treated with low or high dose of *TQ compared with those exposed to* LA alone *(Figs. 1-3).* Furthermore*, the expression* levels of Bcl_2 gene increased significantly in LA-*exposed rats* treated with low or high dose of *TQ compared with those exposed to* LA alone *(Fig. 4).*

DNA damage assessed by comet assay

Determination of the DNA damage in this study as evident from Table 2 corresponds to DNA from control, LA, TQ, and LA + TQ animals, respectively. We have showed that treatment of male rats with LA resulted in significantly ($p\leq0.01$) higher DNA damage as compared to control and the both doses of TQ.

In contrary, DNA damage reduced significantly ($p\leq0.05$) in male rats treated with low or high doses of TQ compared with LA treated rats. This reduction was ever more pronounced in the high dose of TQ group than in rats treated with low dose of TQ.

In addition, treatment of LA exposed rats with low and high doses of TQ decreased significantly the DNA damage induced by LA treatment, moreover, the high dose of TQ was more effective than low dose in preventing the DNA damage (Table 2).

Effect of LA exposure and TQ treatment on GSH levels

The results attributed to LA exposure and TQ treatment on GSH levels in male rats are summarized in Fig. 5. The results revealed a significant decreasing in GSH levels in the brain tissues of male rats treated with LA as compared to those in the control rats (Fig. 5).

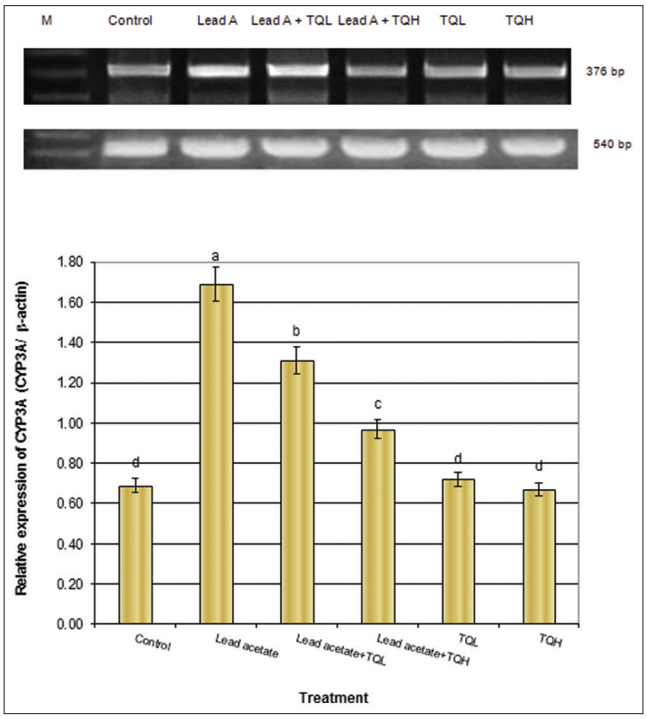

Fig. 1: The relative expression of CYP3A gene (a and b) in liver of male rats exposed to *lead acetate* and treated with low and high dose of thymoquinone (TQL: Low dose, TQH: High dose). Mean values in the same column with different superscript differ significantly ($p<0.05$)

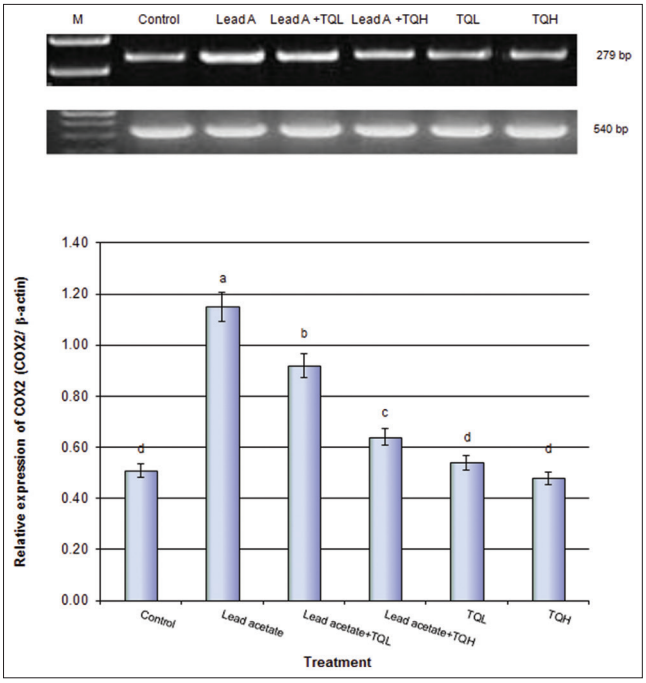

Fig. 2: The relative expression of cyclooxygenase 2 gene (a and b) in liver of male rats exposed to lead acetate and treated with low and high dose of thymoquinone (TQL: Low dose, TQH: High dose). Mean values in the same column with different superscript differ significantly (p<0.05)

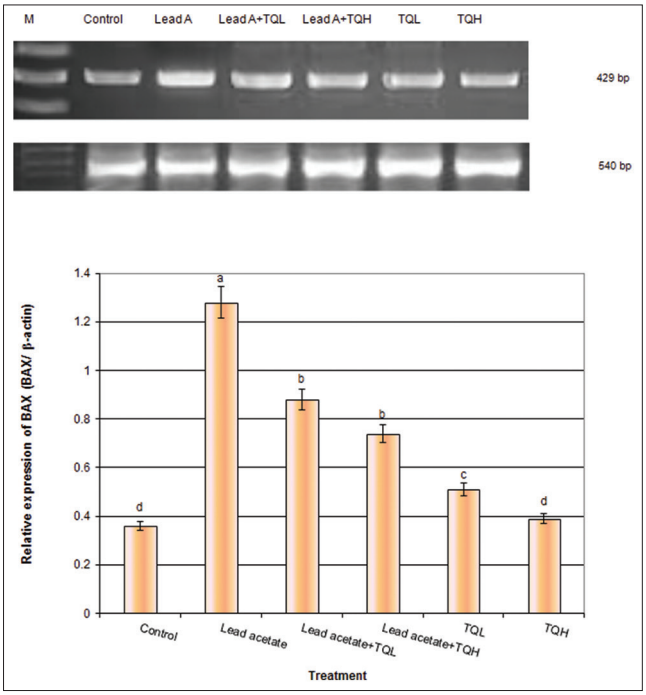

Fig. 3: The relative expression of BAX gene (a and b) in liver of male rats exposed to lead acetate and treated with low and high dose of thymoquinone (TQL: Low dose, TQH: High dose). Mean values in the same column with different superscript differ significantly (p<0.05)

However, reduced GSH levels in the brain tissues of male rats treated with all doses of TQ reached relatively similar values to those in the control group (Fig. 1).

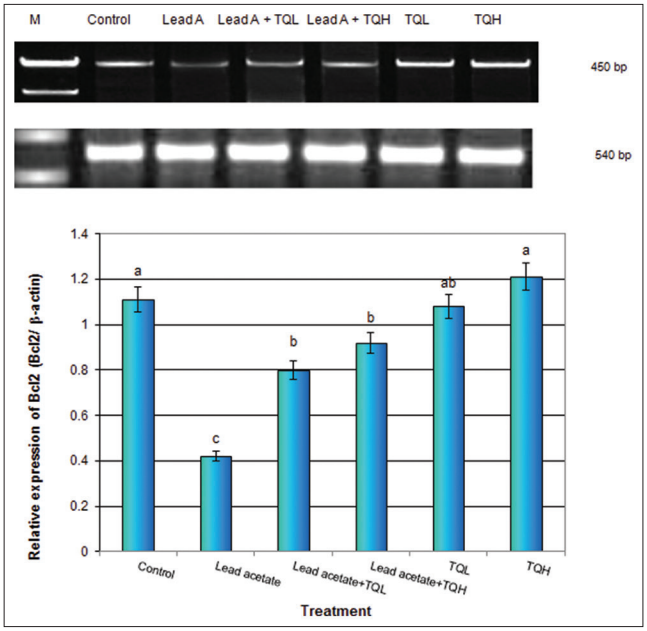

Fig. 4: The relative expression of Bcl_2 gene (a and b) in liver of male rats exposed to lead acetate and treated with low and high dose of thymoquinone (TQL: Low dose, TQH: High dose). Mean values in the same column with different superscript differ significantly (p<0.05)

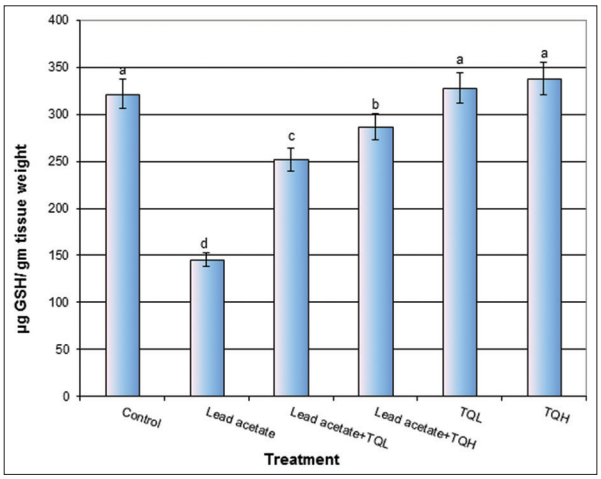

Fig. 5: Glutathione content in the brain tissues of male rats exposed to lead acetate and treated with thymoquinone. Data are presented as the mean ± standarWd error of the mean. Mean values in the same column with different superscript differ significantly (p<0.05)

Moreover, treatment of male rats with TQ inhibited the negative effect of LA on GSH levels in the brain tissues. Treatment of LA exposed rats with high dose of TQ increased the levels of reduced GSH in the brain tissues of male rats compared with those in rats treated with LA alone (Fig. 5).

Effect of LA exposure and TQ treatment on lipids and protein oxidation level

Levels of MDA content and PC in the brain tissues of male rats exposed to LA and TQ are summarized in Table 3. The results revealed that MDA and PC contents were significantly higher in the brain tissues of male rats treated with LA compared with those of control group (Table 3). However, the levels of MDA and PC decreased significantly

Table 2: DNA damage visual score in the brain tissues of male rats exposed to LA and treated with TQ using comet assay

Treatment	Number of animals[§]	Number of cells	Class[¥] of comet				DNA damaged cells (%)
		Total comets	0	1	2	3	
Control	5	21	479	17	4	0	4.2
LA	5	86	414	28	32	26	17.2
LA+TQL	5	53	447	21	17	15	10.6
LA+TQH	5	42	458	17	16	9	8.4
TQL	5	22	478	16	6	0	4.4
TQH	5	19	481	15	4	0	3.8

[§]Number of cells analyzed per animal were=100. [¥]Class 0=No tail; 1=Tail length<diameter of nucleus; 2=Tail length between 1x and 2x the diameter of nucleus; and 3=Tail length >2x the diameter of nucleus, *Number of cells analyzed were 100 per an animal, TQL: Thymoquinone low dose, TQH: Thymoquinone high dose, TQ: Thymoquinone, LA: Lead acetate

Table 3: Levels of malondialdehyde content (MDA, nmol/mL) and (PC, nmol/mg protein) content in the brain tissues of male rats exposed to LA and treated with TQ

Treatment	Mean±SD	
	MDA	PC
Control	2.82±0.6^{c}	1.32±0.03^{c}
LA	6.29±0.4^{a}	4.16±0.06^{a}
LA+TQL	3.91±0.2^{b}	2.50±0.05^{b}
LA+TQH	3.11±0.3^{bc}	1.83±0.04^{bc}
TQL	2.92±0.4^{bc}	1.67±0.03^{c}
TQH	2.31±0.5^{c}	1.23±0.04^{c}

Mean values in the same column with different superscript differ significantly ($p<0.05$), MDA: Malondialdehydem PC: Protein Carbonyl, TQ: Thymoquinone, TQL: Thymoquinone low dose, TQH: Thymoquinone high dose, LA: Lead acetate, SD: Standard deviation

in the brain tissues of male rats treated with all doses of TQ compared with those in LA exposed rats. In addition, the levels of MDA and PC in the brain tissues of male rats treated with all doses of TQ reached relatively those in the control group. Moreover, treatment of male rats with TQ inhibited the hazardous effect of LA on the brain tissues of male rats. Treatment of male rats treated with high dose of TQ plus LA decreased the levels of MDA and PC in the brain tissues of male rats (Table 3).

DISCUSSION

LA is recognized as the most soluble and most bioavailable lead salt [30]. The molecular mechanisms of lead toxicity are not fully understood, but strong evidences indicate that it can act by competing with endogenous cations on binding protein binding sites [31]. Inorganic lead is reported to be aneugenic, clastogenic, and in some cases mutagenic [8]. Studies on the clastogenic effects of lead, however, are contradictory [10].

This study showed that LA was able to induce DNA damage and alteration in the expression of tumor initiation genes (CYP3A, COX2, BAX, and Bcl_2) as well as induced biochemical alterations including GSH content and MDA and PC levels in male rats. In same line, several *in vitro* and *in vivo* studies have shown that different LA metabolic pathways result in reactive oxygen species (ROS) generation and alteration of antioxidant defense systems in animals which are responsible to induce genotoxicity [32,33]. Haleagrahara *et al*. [34] reported that exposure to LA caused a marked increase in lipid peroxidation and a reduction in free radical scavenging enzymes in bone marrow. In the present investigation, the genotoxic effect of LA on the gene expression and DNA damage could be due to the induction of lipid peroxidation and ROS which has been considered as one of the direct mechanisms underlying lead-mediated DNA damage [35]. The generation of highly ROS, such as superoxide radicals (O^{-}_{2}), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH) and lipid peroxides, and in the aftermath of heavy metal ions, is known to damage various cellular components including proteins, membrane lipids, and nucleic acids [36].

A variety of herbal and plant extracts or preparations are available today for treating any number of diseases affecting the human body [37]. Some preparations have been known for literally thousands of years while others are just being discovered to have curative effects. Effective plant extracts are highly desired as a "natural" way to treat a disease. It is believed that natural preparations will not have as much of an adverse effect on the body as synthetic preparations [37].

Black seed (*N. sativa* L.), has been employed for thousands of years as a spice and food preservative, as well as a protective and curative remedy for numerous disorders [38]. Several beneficial pharmacological effects have been attributed to various crude and purified components of black seed, including anti-histaminergic, antihypertensive, hypoglycemic, antimicrobial, mast cell stabilizing, antioxidant, and anti-inflammatory activities [39]. Black seed preparations have also demonstrated significant *in vitro* and *in vivo* antineoplastic activity [40].

N. sativa contain black seeds that possess immense medicinal value. The seeds contain fixed oils, essential oils and chemicals, such as TQ, which help treat a variety of conditions including gastrointestinal disorders, hypertension, diabetes, bronchitis, fever and dizziness [41]. TQ constitutes about 30-48% of essential oils extracted from *N. sativa* seeds and is extensively investigated at the preclinical level for its antioxidant, anti-inflammatory and anticancer activities [42].

In the current study, we found that TQ was able to decrease the toxic effect of LA in male rats. We showed that use TQ in different doses decreased the alterations in the gene expression of tumor initiation genes and DNA damage in male rats.

In additional, it has been reported that TQ also preserves the activity of various antioxidant enzymes such as catalase, GSH peroxidase and GSH-S-transferase thus acting as free radical and superoxide scavenger [43]. Our results were in same line with these findings where TQ increased the levels of GSH and decreased the lipids and protein oxidation levels in the form of MDA and PC levels.

TQ modulates numerous molecular targets (p53, p73, PTEN, STAT3, peroxisome proliferator-activated receptors [PPAR-c]), activates caspases and generates ROS that are implicated in tumor cell proliferation, apoptosis, cell cycle arrest, metastasis and angiogenesis [44] in different drug resistant cancers like osteosarcoma, uterine sarcoma and leukemia [45]. In the same line, the current results showed that TQ in different doses increased the expression of Bcl_2 gene in which it is known for its apoptotic activity on the tumor cells. In addition, treatment of male rats with TQ decreased the expression levels of CYP3A, COX2, and BAX genes which play an important role in the cancer pathway.

Lehman-McKeeman *et al*. [46] found that exposure of mice to the environmental pollution increased CYP3A mRNA level in liver. In addition, Badawi *et al*. [47] reported that COX2, PPARγ, BAX, Bcl_2,

Akt-1, and PKCα genes were found to be responsible for prostate cancer in aged rats.

CONCLUSION

This study showed that LA has a potent clastogenic effect and TQ has the ability to evacuate that clastogenic effect as recorded by the gene expression levels, DNA damage and alterations in GSH content and MDA and PC levels which retained to control levels.

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