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Turmeric Extracts as a Protective Natural Compound Against Hepatotoxicity Induced by Lead Nitrate in Male Albino Rat Model

Abd El-Fattah Abd El-Fattah[1], Medhat Abozid[2], Kamal Mahmoud[2*]
1- Ministry of Health and Population, Cairo, Egypt
2- Biochemistry Dept, Fac of Agric, Menoufia Univ, Postal Code 32516, Mostafa Kamel St., Eastern Bank, Shebin El-Kom, Menoufia, Egypt

* Corresponding author: esraamasria74@gmail.com

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Abstract: Excessive exposure to lead may damage the liver by increasing oxidative stress, leading to many serious diseases. Dietary antioxidants may protect the liver from damage. Therefore, the effect of ethanolic and aqueous turmeric extracts against lead-induced liver damage was evaluated by estimating liver function and antioxidant/oxidative stress biomarkers. Forty rats were divided into group (A) served as the control and group (B) was treated with 93 mg/kg b.wt. lead nitrate; while group (C) and group (D) were treated as group B in addition to 400 mg/kg b.wt. of turmeric aqueous and ethanolic extracts, respectively. Group B showed a significant increase in the activities of alanine transaminase by 10 folds, aspartate transaminase by 9 folds, alkaline phosphatase by 3 folds and the level of malondialdehyde by 10 folds, while showed a significant decrease in albumin by 60%, total protein by 54.5%, and superoxide dismutase by 80.5%, catalase by 75% and glutathione by 71.6% in comparison with those of the control group. On the other hand, turmeric treatments (groups C and D) led to a readjustment of levels for such biomarkers. Collectively, these results demonstrate the potential of turmeric extracts (particularly the ethanolic extract) to improve the fatal effect of lead in a rat model.

1 Introduction

Heavy metals such as Lead (Pb) are toxic materials and harmful pollutants in water (Karadede-Akin and Unlü 2007). Their presence not only affects human beings but also harms animals and vegetation because of their mobility. Lead is known to cause many harmful physiological and biochemical changes in animals as well as in humans (Florea and Büsselberg 2006, Ruff et al 1996).

In biological systems, lead affects many cellular components and organelles, such as the endoplasmic reticulum, cell membrane, nucleus, mitochondria, and many other enzymes. One of the toxic effects of this heavy metal is caused by the production of reactive oxygen species (ROS), which causes oxidative stress and ultimately leads to cell damage, especially liver cells (Wang and Shi 2001).

At the same time, the use of synthetic drugs to combat the toxic effect of these metals often becomes inappropriate due to their undesirable side effect especially when used either for long terms or when more than one of them is used simultaneously because of drug interactions (Jaishankar et al 2014). It was therefore necessary to look for natural therapeutic sources

that may reduce the harmful effects of these elements, and this makes attention to food plants rich in antioxidants increase (Abozid et al 2018).

One of these plants is the turmeric (*curcuma longa tinn*) rhizomes which have pharmacological activities that include antioxidant, anti-inflammatory, anticancer, immunomodulatory, neuroprotective, and antiaging effects (Pari and Prasath 2008). Curcumin is the most important compound in the turmeric plant in terms of therapeutic because of its positive effect as a natural antioxidant, as it has no negative side effects such as chemical drugs (Joe et al 2004, Mohajeri et al 2017). This study aims to evaluate the effect of ethanolic and aqueous extracts of turmeric rhizomes against lead on liver function tests and the antioxidant/oxidative stress biomarkers in rats.

2 Materials and Methods

2.1 Plant collection and identification

A bout ½ Kg Rhizomes of turmeric (*Curcuma longa* Linn) were obtained from Agriculture Research Center in El-qnater El-khairia, Kaliobia, Egypt in September 2016. Rhizome were Identified in the Horticulture department, Faculty of Agriculture, Menoufia University.

2.2 Preparation of aqueous and ethanolic extracts of turmeric

The turmeric rhizomes were kept at room temperature in a dry place prior to use. About 50g of air-dried rhizomes were powdered and extracted with five times the volume of distilled water or ethanol 80% for 48 h with continuous shaking (Mukhtar and Ghori 2012). The final extracts were filtrated with filter paper Whatman №1, concentrated with a rotary evaporator at 50±1°C to give solid residues and stored at 4°C for further use.

2.2.1 Determination of total phenolic compounds, total flavonoids and DPPH radical scavenging

The concentration of total phenolic compounds in each extract was determined colorimetrically as gallic acid equivalent based on the gallic acid standard curve (GAE; mg gallic acid/100 g) by the method of Folin-Ciocalteu's as described by Gülçin et al (2002), while the total flavonoids contents were determined and expressed as mg of quercetin (mg QCE/100 g) based on the quercetin standard curve using the method reported by Dewanto et al (2002) and DPPH (2, 2-diphenyl -1-pricrylhydrazyl) free radical was measured for aqueous or ethanolic extracts according to Lee et al (1996).

2.2.2 Determination of curcumin content in turmeric

Curcumin was separated and determined by using HPLC (A Shimadzu LC 20 AT HPLC), the mobile phase was (acetonitrile / acetic acid/double distilled water 9/2/89 v/v/v), the column was 5-µm C18 column (Waters, Milford, MA) and detector was SPD-20 UV Visible detector (At wavelength of 262 nm) according to Zuo et al (2002).

2.3 Biological experiment

2.3.1 Animals

Forty male Wister albino rats (120±10 g) were obtained from the Research Institute of "Laboratory Animals Research Center", Faculty of Veterinary Medicine, Benha University**,** Qalyubia, Egypt. All experimental procedures were completed according to the ethical guidelines of the International Association for the Study of Pain (Zimmermann 1983).

Animals were placed in laboratory conditions for 15 days as an adaptation period. Water and food were always available throughout the experiment. Randomized groups of rats were housed in cages containing wood shaving as bedding and were allocated into four groups (45 days), each having 10 male rats as follows: (A) control group without any treatment, (B) positive control group treated with 93 mg/kg b.wt. orally of lead nitrate, (C) treated with 93 mg/kg b.wt. orally of lead nitrate in addition to 400 mg/Kg b.wt. of water extract of turmeric, and (D) received 93 mg/kg b.wt. orally of lead nitrate in addition to 400 mg/Kg b.wt. of ethanolic extract of turmeric. At the end of the experiment, blood was collected to further determination.

2.3.2 Blood samples

After 45 days of treatment period, the animals were deprived of food overnight and anesthetized and then sacrificed by cervical decapitation. Blood samples were collected from the orbital sinus veins technique at the end of the experimental period, into (1.5 ml) Eppendorf tubes (containing heparin as an anticoagulant).

Blood samples were centrifuged for 15 min at 3600 rpm in a refrigerated centrifuge to separate plasma. Plasma samples were kept in a deep freeze at (-20°C), till the different assays were carried out.

2.3.3 Biochemical analysis

Kits for enzymes activity superoxide dismutase (SOD), catalase (CAT), alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and levels of total protein, albumin, glutathione (GSH) and malonaldehyde (MDA) purchased from the Bio-diagnostic Company, Cairo, Egypt.

Transaminases (ALT and AST) were measured according to Young (1997), while alkaline phosphatase (ALP) was determined in serum according to Moss et al (1987), total protein was determined in plasma as described by Schultze and Heremans (1966) and albumin was measured in plasma as described by Henry et al (1974).

Malondialdehyde (MDA) was measured according to Ohkawa et al (1979) also, superoxide dismutase (SOD) activity was determined by using the method of Nishikimi et al (1972), catalase (CAT) activity was determined as described by Aebi (1984) and determination of glutathione (GSH) level was done according to the method of Goldberg and Spooner (1983).

2.4 Statistical analysis

Duncan's multiple range test was used to compare the treatment means. The mean values within each column followed by the same letters are not significantly different at 5%.

3 Results and Discussion

3.1Total phenolic compounds and total flavonoids as well as DPPH-radical scavenging activity in aqueous and ethanolic extracts of turmeric

The obtained results as shown in **Fig 1** clarified that ethanolic extract contains an amount higher for both total phenolics content (76.26 mg GAE/100 g) and total flavonoids (46.7 mg/100 g) than aqueous extract where free phenolics content was 65.38 mg/100 g and total flavonoids were 29.5 mg/100 g.

Many factors cause the quantity and type of phenolic compounds to differ in the same plant type; the most important of which are the soil type, growing conditions, harvesting methods and timing, and climatic conditions in which the cultivation took place (Jeffery et al 2003). Also, the extraction method represents an important qualitative factor in the amount and type of phenolic compounds in the final extract (Gallardo et al 2006).

It is clear from the results that the alcoholic extract was better than the aqueous extract in the effect as an antioxidant, which in turn is linked to its high content of phenolic compounds. Liu et al (2003) reported that the antioxidant activity of the methanolic extract of turmeric was 6.3 mg GAE/g dry weight of the sample. In another study, the total phenolics in the ethanolic extract of turmeric was 7.124 mg GAE/g dry weight of the sample (Shan et al 2005).

The DPPH method is one of the best in vitro methods that evaluate the antioxidant activity (radical scavenging) of different extracts because of its accuracy and simplicity (Gallardo et al 2006).

Data in **Table 1** showed that both turmeric rhizome extracts recorded more than 20% inhibition in the DPPH assay, as well as IC_{50} for aqueous extract was 569.77 ppm while the IC_{50} for ethanolic extract was 430.67 ppm. The increase in the DPPH inhibition activity of the ethanolic extract compared to the aqueous extract may be due to the presence of higher total phenolic and flavonoid contents in the former extract as shown in **Fig 1**. The varied results obtained for the same extract in the DPPH method are due to the extreme sensitivity of this reaction to environmental conditions (pH, solvent purity, light exposure) (Schaich et al 2015).

3.2 The curcumin content in turmeric

The content of curcumin in ethanolic extract of turmeric was 19.2%. This result is close to what both (Yue et al 2016, Li et al 2018**)** found in previous studies of turmeric rhizomes. Curcumin is the main ingredient in turmeric plant extracts and is largely responsible for many of the vital and healthy roles (Nouzarian et al 2011, Wang et al 2015, Nawab et al 2019).

3.3 Effect turmeric ethanolic and aqueous extracts against lead on liver functions

The obtained results as shown in **Table 2** revealed that lead treatment (poisoned control groups) caused a significant increase in the activities of liver function indicators in plasma (ALT, AST and ALP), while a significant decrease in synthetic liver functions (albumin and total protein levels) was observed, comparing with normal control group.

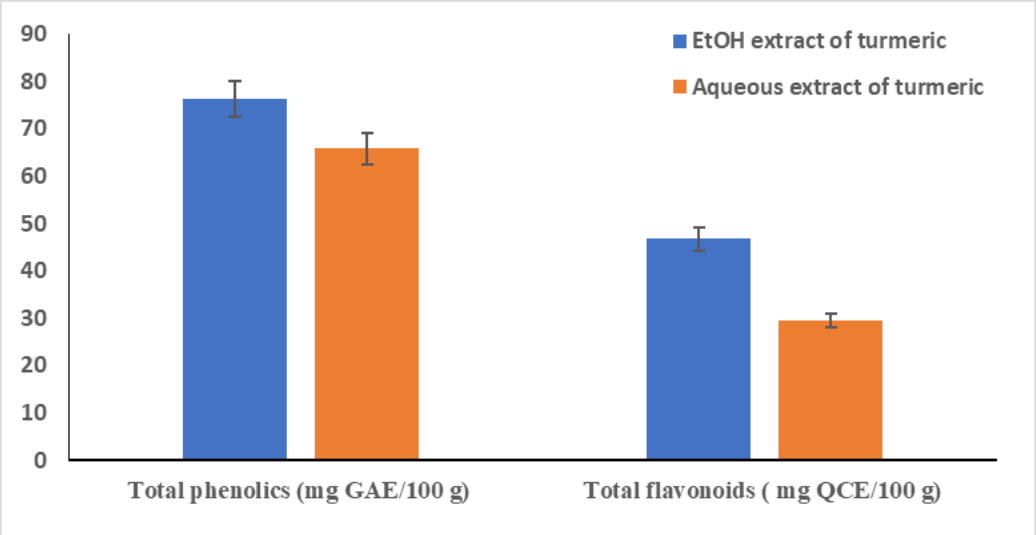

Fig 1. Total phenolic compounds and total flavonoids in turmeric aqueous and ethanolic extracts

Table 1. DPPH activity in turmeric aqueous and ethanolic extracts

		Turmeric aqueous extract			**Turmeric ethanolic extract**		
DPPH assay	Concentration	250ppm	500ppm	750ppm	250ppm	500ppm	750ppm
	% Inhibition	23.7	41.6	66.7	36	56.8	72.3
	IC_{50} (ppm)	569.77			430.67		

Table 2. Plasma liver functions in rats supplemented with turmeric aqueous and ethanolic extracts against lead nitrate

Groups	**AST** (U/L)	**ALT** (U/L)	**ALP** (U/L)	**Total Protein** (g/dl)	**Albumin** (g/dl)
Group A	14.0± 0.894 a	13.1 ± 1.16 a	68.0 ± 3.84 a	7.7 ± 0.200 b	4.6 ± 0.258 b
Group B	129.1±1.47 d	132.0±1.41 d	201.8 ± 2.31 d	3.5 ± 0.216 a	1.8 ± 0.250 a
Group C	111.3± 1.63 c	98.0±1.41 c	187.5 ± 1.87 c	4.3 ± 0.231 d	2.1 ± 0.216 d
Group D	79.6 ±1.63 b	74.0±1.41 b	154.5 ± 1.87 b	5.1 ± 0.216 c	3.0 ± 0.216 c

Each value represents a mean followed by a standard deviation for 10 rats. **A** = Control group, **B** = Treated with lead nitrate 93 mg/kg b.wt., **C**= Treated as group B + 400 mg/Kg b.wt. of aqueous turmeric extract, **D=** Treated as group B + 400 mg/Kg b.wt. of ethanolic turmeric extract.

Different letters (a, b, c and d) in the same column indicate a significant difference ($p \leq 0.05$), while the same letter in different cells in the same column indicates a non-significant difference ($p \leq 0.05$).

In contrast, the administration of ethanolic and aqueous extracts of turmeric showed a pronounced decrease in AST, ALT, and ALP activities associated with an increase in total protein and albumin levels compared with poisoned control and that ethanolic extract was more effective on the readjustment of all liver biomarkers than that of aqueous extract.

The liver is considered the most organ of the body exposed to toxins (including heavy metals such as lead) (Meyer and Kulkarni 2001). Lead toxicity studies have shown that the liver stores the equivalent of 33% of the lead in experimental animals compared to the rest of the soft tissues (Bechara 2004). One of the mechanisms that may explain how lead affects the liver depends on the idea of an imbalance between oxidants and antioxidants, which is called oxidative stress, which in turn leads to damage and breakdown of liver cells as a result of increased levels of free radicals (Liu et al 2013).

This study has recorded the occurrence of confirmed manifestations of damage to liver cells, and this was observed in the significant increase in activities of liver enzymes in the plasma (ALT, AST and ALP), which is consistent with what was published by many authors Taib et al (2004), Liu et al (2012), and Su et al (2017) about the association of the deterioration of liver cells and the increase in levels of liver enzymes in the plasma. Also, the structure of the protein is altered by the destruction of the triangular shape because of lead, which caused a decrease in the total protein level (Shalan et al 2005) and albumin levels (Lin et al 2016, Wu et al 2016).

Concerning the effect of curcumin on the liver of rats affected by lead toxicity, the results showed that two curcumin extracts decreased the activity of liver enzymes activities (AST, ALT and ALP); these results, are supported by what many scientists have found about the positive effect of turmeric on improving the activities of liver enzymes in plasma when treated with different toxins (Kang et al 2009, Essam and Ashraf 2013, Palipoch et al 2014).

The improvement of the plasma levels of albumin and total protein when treated with ethanol and an aqueous extract of turmeric in the face of the harmful effect of lead is supported by the results of both (Mathews et al 2012, García-Niño et al 2013).

In our expectation, the strong effect of the turmeric ethanolic extract on improving liver function is due to its high content of total phenolics and flavonoids (**Fig 1**).

Although the groups treated with turmeric extracts showed a significant and clear improvement in liver function parameters (**Table 2**), the values they showed were still far from the normal levels recorded in the control group. We believe that these results are promising and that it is possible to get closer to achieving normal levels of liver function parameters by increasing the duration of the experiment and increasing the doses used of turmeric extracts.

3.4 Antioxidant and protective effects of turmeric against lead

The effect of turmeric extract supplementation against the toxic effects of lead is presented in **Table 3,** where a variation between both controls and the other treated groups can be noticed. It can conclude that the activity of both SOD and CAT as well as levels of GSH in the poisoned control group was the lowest except for MDA which behave inversely compared with all other groups. The readjustment effect as a result of supplementation with turmeric extracts on the previous biomarkers could be clearly observed compared with lead treated group after 45 days of treatments.

These results are in accordance with those reported by many investigators including Tirkey et al (2005), as well as, those who stated that turmeric extracts increase SOD activity Mary et al (2018), CAT activity Eybl et al (2006) and GSH levels Daniel et al (2004). While the levels of MDA were improved by strong antioxidants of turmeric (Daniel et al 2004), and that the ethanolic extract was the best in restoring antioxidants to their normal level.

The oxidative damage by lead led to increased generation of free radicals and caused direct impoverishment of antioxidants (Flora et al 2004), the body's primary front lines, the superoxide dismutase (SOD) and catalase (CAT) enzymes, control, and counter free radicals (Ferrari 2001). SOD converts superoxide radical (highly toxic) into hydrogen peroxide (H_2O_2) (Li et al 2013), then CAT complete the work by converting (H_2O_2) into water molecule and oxygen (Hamadouche et al 2008). Glutathione (GSH) then works differently to resist toxic substances as it binds them to convert them into less dangerous compounds (Jurczuk et al 2006). The final product of lipid peroxidation is MDA, whose level increases by increasing the oxidative stress (Abozid et al 2018).

Table 3. Plasma antioxidant/oxidative stress parameters in rats supplemented with turmeric aqueous and ethanolic extracts against lead nitrate

Groups	MDA (nmol/ml)	SOD (U/L)	CAT (U/L)	GSH (U/ml)
Group A	12.3 ± 1.63 a	420.0 ± 1.41 c	545.0 ± 1.41 c	95.0± 3.57 c
Group B	126.3 ± 1.50 d	81.9 ± 2.04 a	136.8 ± 1.94 a	27.0 ± 1.54 a
Group C	113.0 ± 1.41 c	127.3 ± 1.63 b	407.3 ± 2.16 b	61.8 ± 1.47 b
Group D	82.0 ± 1.41 b	285.5 ± 1.87 d	506.6 ± 2.58 d	77.6 ± 1.63 d

Each value represents a mean followed by standard deviation for 10 rats. **A** = Control group, **B** = Treated with lead nitrate 93 mg/kg b.wt., **C**= Treated as group B + 400 mg/Kg b.wt. of aqueous turmeric extract, **D=** Treated as group B + 400 mg/Kg b.wt. of ethanolic turmeric extract.
Different letters (a, b, c and d) in the same column indicate significant deference ($p \leq 0.05$), while the same letter in different cells in the same column indicates a non-significant difference ($p \leq 0.05$).

The obtained results by Bokara et al (2008) showed that lead caused elevation in MDA and reduced the SOD activity (Wang et al 2012) and CAT (Han et al 2007), as well as, that the levels of GSH were decreased by lead treatment (Maiti and Chatterjee 2001). These results with numerous studies indicated that lead motivated oxidative stress in rats, possibly via the formation ROS (Hamadouche et al 2008), causing an increase in MDA levels, which is considered to be a marker of lipid peroxidation (Bokara et al 2008).

Previous studies indicate the presence of curcumin in polar solvent extracts, whether such as aqueous or alcoholic extracts as a major compound (Karioti et al 2011), which was found to have a very good effect as an antioxidant by scavenging free radicals or binding to heavy metals (Dinis et al 1994, Reddy and Lokesh 1994, Sreejayan and Rao 1994), which may explain many of the results of the current study.

4 Conclusion

From the results of such a study, it is obvious that lead nitrate caused elevation in the liver biomarkers (ALT, AST, and ALP activities) and (total protein and albumin levels), accompanied by an increase in MDA levels, together with marked decrees in antioxidant biomarker (SOD, CAT and GSH). All these effects can be ameliorated by turmeric (aqueous and ethanolic extract) treatment.

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