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Original Article

The protective effects of *Curcuma longa* extract on oxidative stress markers in the liver induced by Adriamycin in rats

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Abstract

Introduction: The aim of the study was to investigate the effects of *Curcuma longa* (*C. longa*) extract on Adriamycin-induced hepatotoxicity in rat.

Methods: Animals were divided in six groups: Control (CO), Adriamycin (ADR), Adriamycin with Vitamin C (ADR+VitC), Vitamin C (Vit C), *C. longa* with Adriamycin (CL+ADR) and without Adriamycin (CL-ADR). Hepatotoxicity was induced by Adriamycin 5mg/kg and rats were treated with *C. longa* 1000 mg/kg and Vitamin C 100 mg/kg , per day, orally for 4 weeks.

Results: In the liver tissue of ADR group, Malonyldialdehyde (MDA) level was increased significantly compared to CO group, ($p < 0.05$). MDA level in the treatment groups, Vit C, CL+ADR and CL-ADR were increased significantly compared to ADR group ($p < 0.05$, for all three groups), and compared to ADR+VitC group ($p < 0.01$). Thiol level in ADR, ADR+VitC and CL+ADR groups were decreased compared to CO group ($p < 0.001$), and also thiol level in CL-ADR and Vit C were increased significantly compared to ADR group ($p < 0.01$ and $p < 0.001$ respectively). The activity of catalase in liver tissue in ADR group was lower compared to CO group ($p < 0.01$), though was increased in CL-ADR, ADR+VitC and Vit C groups in comparison with ADR group ($p<0.05$, $p < 0.01$ and $p < 0.001$, respectively).

Conclusion: The results showed that chronic administration of *C. longa* hydroalcoholic extract in Adriamycin-induced hepatotoxic rats could decrease the oxidative stress injuries in the liver tissue.

Keywords:
Adriamycin;
Curcuma longa;
Hepatotoxicity;
Oxidative stress;
Hydroalcoholic extract;
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Introduction

Adriamycin is an anthracycline antibiotic with cytotoxic effect on tumor cells. Anthracyclines act by intercalating into nuclear DNA, impairing transcription and cell division, inhibiting topoisomerase II, reactive oxygen species (ROS) generators and further injuring DNA as well as mitochondria and cell membranes

(Minotti et al., 2004).

Studies showed that Adriamycin is rapidly cleared from the plasma of the rodents following intravenous administration, and accumulates in the liver, spleen, kidney, lung, and heart. Drug elimination occurs predominantly through the liver over a prolonged time (Carter et al., 1975).

Curcuma longa (*C. longa*), belongs to the Zingiberaceae family, possesses numerous active compounds including, curcumin and zingiberin which are widely used in the Middle Eastern countries and other regions of Asia to prepare delicacies. *C. longa* is also used as a food preservative (Krishnaswamy, 2006), a food colorant and as a dye (Chainani-Wu, 2003). It also has a long history of usage in herbal remedies particularly in China, India and Indonesia (Tayyem et al., 2006).

C. longa has antioxidant (Mohebbati et al., 2016), anti-inflammatory, anti-parasitic, antispasmodic, as well as cardiovascular, gastrointestinal and hepatoprotective properties (Adaramoye et al., 2010). It also inhibits carcinogenesis (Araujo and Leon, 2001). Curcumin, the main effective component of *C. longa*, is a powerful antioxidant agent. *C. longa* is used as an anti-carcinogenic agent, delays premature aging and possesses liver protection. In addition *C. longa* inhibits the growth of various types of cancer cells and decreases liver injury in experimental models of animals (Ramsewak et al., 2000; Akram et al., 2010).

The present study was designed to study the effects of hydroalcoholic extract of *Curcuma longa* on Adriamycin-induced hepatotoxicity.

Materials and methods

Plant material and preparation of the extract

Curcuma longa rhizomes was purchased from a local herbal shop in Mashhad, Khorasan province, Iran and identified by botanists in the herbarium of Ferdowsi University of Mashhad.

Curcuma longa rhizomes,100 grams, was grounded, weighed, and homogenized in 70% ethanol at a ratio of 1:10 of plant to ethanol and left to be soaked for 3 days at 37°C with occasional shaking and stirring. The mixture was then filtered and the resulting liquid was concentrated under reduced pressure at 45°C in an EYELA rotary evaporator (7%, w/w). The concentrated extract was then kept in the incubator at 45°C for 3 days to evaporate the ethanol residue yielding the crude extract (Salama et al., 2013). Extract was then dissolved in 96% ethanol (0.5%, w/w) before being orally administrated to animals (Mohebbati et al., 2016).

Chemicals and drugs

All chemicals and drugs were of analytical grade (Merck). Adriamycin was obtained from EBO pharma Iran.

Animals and treatments

Forty eight male Wistar rats (220 - 250 gr, 10 weeks old) were kept on a 12 h light-dark cycle, under constant temperature (22 ± 2°C), and were allowed to have free access to standard laboratory diet and drinking water. All experiments were approved by Animal Experimentation Ethics Committee of Mashhad University of Medical Sciences (MUMS).

Animals were randomly assigned to six groups (n = 8 in each group) including:

1. Control group (CO), which received normal saline via a tail vein on the first day of the study.
2. Adriamycin group (ADR), which received Adriamycin (5mg/kg, B.W) (Zima et al., 1998) via a tail vein on the first day of the study.
3. Vitamin C group (Vit C), which received Vitamin C (100 mg/kg, B.W) (Antunes et al., 2000) in drinking water for 28 consecutive days.
4. Vitamin C plus Adriamycin (ADR+VitC) group, which received Adriamycin (5mg/kg, B.W) via a tail vein on the 1st day of the study and received Vitamin C (100 mg/kg) in drinking water for 28 consecutive days.
5. *C. longa* extract without Adriamycin (CL-ADR) group, which received *Curcuma longa* extract (1000 mg/kg, B.W) (Khorsandi and Orazizadeh, 2008) in drinking water for 28 consecutive days.
6. *C. longa* extract plus Adriamycin group (CL+ADR) which received Adriamycin (5mg/kg, B.W) via a tail vein on the 1st day of the study and received *C. longa* extract (1000 mg/kg, B.W) in drinking water for 28 consecutive days.

Preparation of the rat liver tissue

At the end of the study period, the animals were

anesthetized with ether and euthanized by decapitation. The liver tissue was rapidly dissected out and after washing, stored at -80 ° C.

Malondialdeyde (MDA) and thiol assessment

Liver sample was homogenized with ice-cold KCl (150 mM) for the determination of MDA and thiol levels

Determination of MDA concentration

MDA level is an index of lipid peroxidation. MDA reacts with thiobarbituric acid (TBA) as a TBA reactive substance (TBARS) and produces a red complex. Briefly, 1 mL of homogenates was added to 2 mL of a complex solution containing TBA/trichloroacetic acid (TCA)/(hydrochloric acid (HCL) and it was then boiled in a water bath for 40 minutes. The solution was allowed to reach the room temperature and then, centrifuged at 1000 g for 10 minutes. The absorbance was read at 535 nm (Janero, 1990). The MDA concentration was calculated according to the following equation.

MDA concentration (M) = Absorbance / (1.56 × 105 cm-1 M-1)

The MDA levels results are expressed per gram of tissue.

Determination of thiol concentration

DTNB (2, 2'-dinitro-5, 5'-dithiodibenzoic acid) reagent, which reacts with the SH group, was used to determine the concentration of total thiol groups. The produced yellow complex has a peak absorbance at 412 nm.

Briefly, 50 µL of tissue homogenates was added to 1 mL Tris-EDTA (ethylenediaminetetraacetic acid) buffer (pH = 8.6) and he absorbance ofTris-EDTA buffer alone (blank) was read at 412 nm (A1). Then, 20 µL of 10 mM solution of DTNB was mixed with the aforementioned solution and was stored at room temperature for 15 minutes and the absorbance was read again (A2). The absorbance of DTNB reagent was also read as blank (B) (Sharma et al., 2006). The thiol levels were determined by a spectrophotometric method using Ellman's reagent and the results were expressed as per gram of tissue.

Total thiol concentration (mM) = (A2 – A1 – B) × 1.07 / (0.05 × 14,150).

Determination of catalase (CAT) activity

Catalase activity was measured according to the Aebi method. The principle of the assay is based on the determination of the rate constant, k, (dimension: s-1, k) of hydrogen peroxide decomposition. By measuring the decrease in absorbance at 240 nm per minute, the rate constant of the enzyme was determined. Activities were expressed ask (rate constant) per 100 g tissue (Aebi, 1984).

Statistical analysis

All data were expressed as means ± SEM. Normality test (Kolmogorov–Smirnov) was done. Different groups were compared by one way ANOVA followed by tukey's Post hoc comparison test. Differences were considered statistically significant when $p<0.05$.

Results

According to present results the percentage of weight changes in different groups were lower than control groups. The percent of weight changes in VitC and CL-ADR ($p < 0.05$ for both), and in ADR ($p < 0.01$) were decreased significantly compared to control group. The percent of weight changes in ADR+VitC and CL+ADR ($p < 0.001$ for both), were reduced significantly compared to control group (Table1).

The malondialdehyde levels of the liver tissue

In the liver tissue, MDA level in the ADR group was increased significantly compared to CO group, ($p<0.05$). MDA level in the treatment groups, Vit C, CL+ADR and CL-ADR were decreased significantly compared to ADR group, ($p<0.05$, for three groups) and ($p<0.01$) compared to ADR+VitC group (Figure 1).

The thiol levels of the liver tissue

Thiol level in the ADR group was decreased compared to CO group ($p < 0.001$). Thiol level in the CL-ADR and Vit C were increased significantly compared to ADR group ($p<0.01$, $p<0.001$) respectively (Figure 2).

Table 1: Weight changes in Adriamycin-induced oxidative stress in the liver of rat.

Group	Weight changes (%)
CO	33.75±6.13
ADR	13.75±0.78**
Vit C	23.75±1.26*
ADR+VitC	0.62±2.03***
CL-ADR	23.75±2.08*
CL+ADR	8.50±0.59***

Values are means ± SEM (n =8). * p < 0.05, ** p < 0.01, *** p < 0.001 compared to control group. Statistical analyses were made using the one-way ANOVA followed by the Tukey's test post hoc.

The catalase activity in the liver tissue

The activities of CAT in liver tissue of the ADR group were lower than CO group (p < 0.01), and increased in CL-ADR, ADR+VitC and Vit C groups in comparison with ADR group (p < 0.05, p < 0.01 and p< 0.001, respectively) (Figure 3).

Discussion

Administration of Adriamycin caused oxidative excess in the liver tissue of rats. In the Adriamycin group, MDA level was increased and total thiol level and catalase activity were decreased in the liver compared to control group. Some experimental studies have reported that hepatic superoxide formation, MDA, and protein carbonyl levels were increased, and GSH levels decreased, in mice after binge ethanol administration (Kim et al., 2008; Develi et al., 2014).

It has been reported that MDA level significantly increased and glutathione level significantly decreased in Adriamycin treated rat liver tissue compared to control group (Singla et al., 2014). Several studies indicated that the side effects of Adriamycin could cause systemic injury, in which reactive oxygen species (ROS) play an important role. ROS are a large family of oxygen free radical and non-free radical active oxygen-containing molecules, including superoxide radical, hydrogen peroxide and hydroxyl radical, which contribute to oxidative stress (Qin et al., 2008). Adriamycin enhances NADPH-dependent lipid peroxidation in liver microsomal membranes via the formation of superoxide anion radicals (O2) and hydrogen peroxide (H2O2). So, Adriamycin stimulated malondialdehyde production (Sterrenberg et al., 1984).

The results of present study showed that Vit C and *C. longa* possess pronounced antioxidant effects in ADR-induced hepatotoxic. Vit C influenced mitochondrial function by decreasing the ROS formation via stimulating the activity of manganese superoxide dismutase as well as glutathione peroxidase and by altering the activity of the oxidative phosphorylation in electron transport chain, mainly through declining the Phosphate/Oxygen Ratio (P/O ratio) (Valdecantos et al., 2010).

Vitamin C is a potential antioxidant that has been hypothesized to antagonize the effects of ROS–generating antineoplastic drugs including methotrexate, vincristine, cisplatin and Adriamycin (Heaney et al., 2008).

Hydro-alcoholic extract of curcumin exhibit antioxidant activity, comparable to vitamins A, E and C. The study of ischemia indicated that pretreatment with curcumin reduced ischemia-induced changes in the heart (Akram et al., 2010). Hepatoprotective effect of *C. longa* is mainly a result of its antioxidant properties, as well as its ability to decrease the generation of pro-inflammatory cytokines such as TNF-α and IL1ß. In the rats with CCl4-induced acute liver injury, curcumin administration significantly

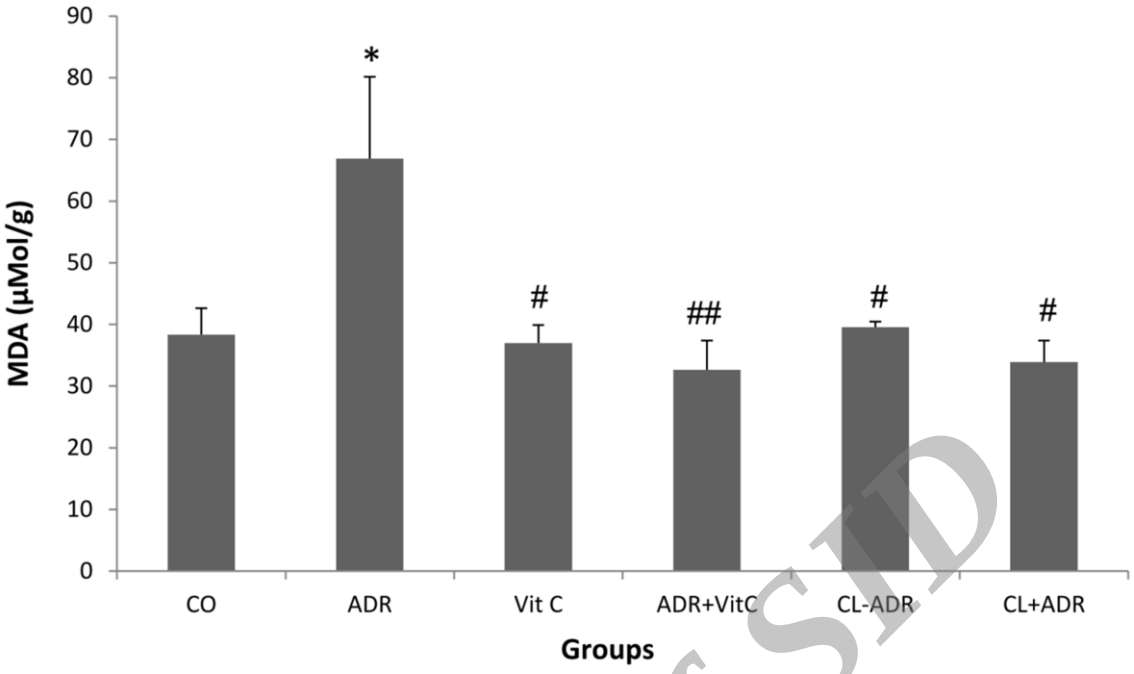

Fig.1. The malondialdehyde (MDA) level of liver tissue in CO: control, ADR: Adriamycin, ADR+VitC: Adriamycin with Vitamin C, Vit C: Vitamin C, CL-ADR: Curcuma longa without Adriamycin, CL+ADR: Curcuma longa with Adriamycin. Values are means ± SEM (n = 8). * $p < 0.05$, compared to control group. # $p < 0.05$ ## $p < 0.01$ compared to Adriamycin group. Statistical analyses were made using the one-way ANOVA followed by the Tukey's test post hoc.

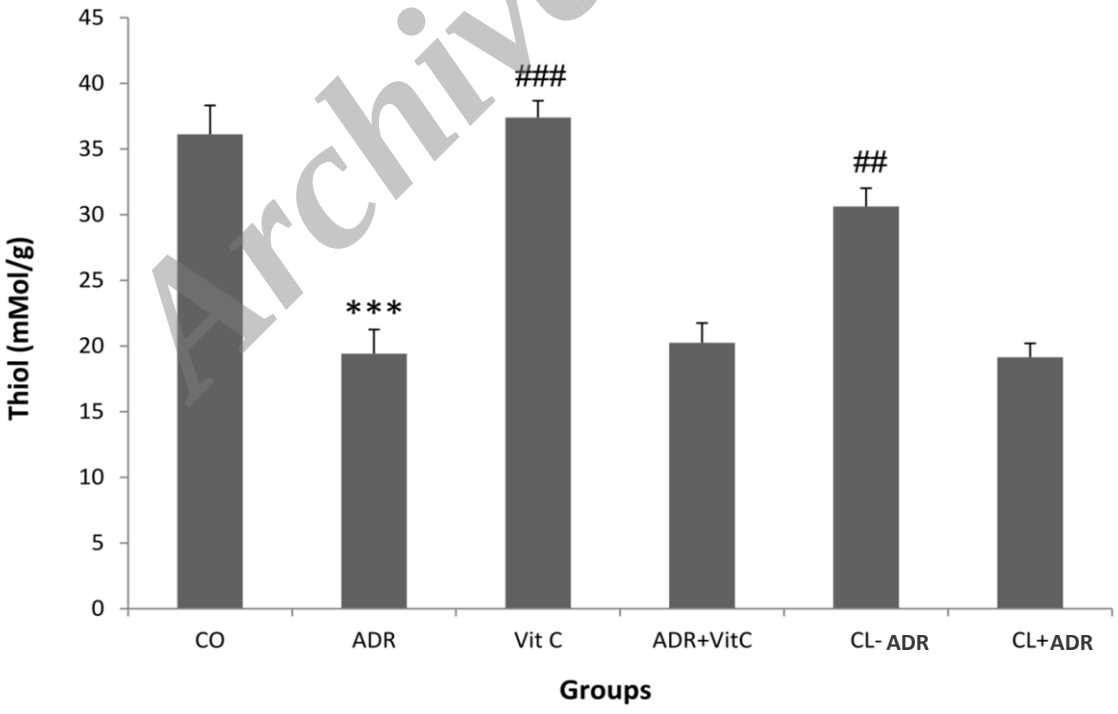

Fig.2. The thiol level of liver tissue in CO: control, ADR: Adriamycin, ADR+VitC: Adriamycin with Vitamin C, Vit C: Vitamin C, CL-ADR: Curcuma longa without Adriamycin, CL+ADR: Curcuma longa with Adriamycin. Values are means ± SEM (n = 8). *** $p < 0.001$ compared to control group. ## $p < 0.01$ ###$p < 0.001$ compared to Adriamycin group. Statistical analyses were made using the one-way ANOVA followed by the Tukey's test post hoc.

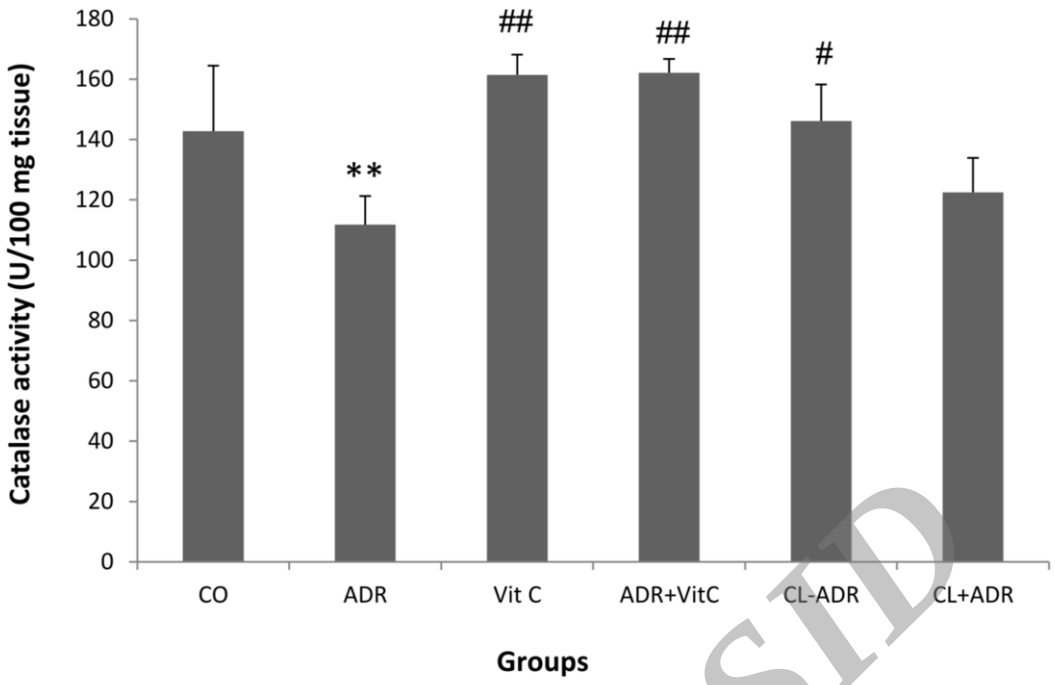

Fig.3. The catalase (CAT) activity (U/100 mg tissue) CO: control, ADR: Adriamycin, ADR+VitC: Adriamycin with Vitamin C, Vit C: Vitamin C, CL-ADR: *Curcuma longa* without Adriamycin, CL+ADR: Curcuma longa with Adriamycin. Values are means ± SEM (n = 8). ** $p < 0.01$ compared to control group. # $p < 0.05$, ## $p < 0.01$ compared to Adriamycin group. Statistical analyses were made using the one-way ANOVA followed by the Tukey's test post hoc.

decreased liver injury in treated animals compared to controls (Akram et al., 2010). While Curcumin, an effective compound of *C. longa*, has antioxidant effect, this compound may show pro-oxidative properties in high doseages s. However, according to our finding it seems that *C. longa* extract can be useful for protecting against Adriamycin induced-hepatotoxicity.

Conclusion

The results showed that administration of hydroalcoholic extract of *C. longa* could decrease the oxidative stress related injuries that were increased by Adriamycin in the liver tissue of rats.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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