

# Hepatoprotective Effects of Combination Hydroalcoholic Extracts of *Nigella Sativa* and *Curcuma Longa* on Adriamycin-Induced Oxidative Stress in Rat

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## ABSTRACT

The ample of studies show that Adriamycin caused hepatotoxicity in rat. The present study investigated the effects of *Nigella Sativa* (*N. Sativa*) and combination with *Curcuma longa* (*C. longa*) extract on Adriamycin-induced hepatotoxicity in rat.

Rats were divided into eight experimental groups: Control (CO), Adriamycin (ADR), Vitamin C (Vit C), Adriamycin with Vitamin C (ADR+VitC), *N. Sativa* with and without Adriamycin (NS +ADR, NS -ADR), Combination extract of *C. longa* and *N. Sativa* with and without Adriamycin (NS+CL+ADR, NS+CL-ADR). Malondialdehyde (MDA) and thiol levels and also the activities of catalase (CAT) in liver tissue were evaluated.

MDA level in the liver tissue in ADR was increased compared to CO group but in NS+ADR group, ADR+VitC and NS -ADR groups decreased compared to ADR group. Thiol levels in ADR and ADR+VitC groups were decreased compared to CO group. Thiol levels in treatment groups were increased compared to ADR group. The activities of CAT in liver tissue of ADR group were lower than CO group, and increased in treatment groups comparison with ADR group.

The results showed that chronic administration of *N. sativa* hydroalcoholic extract in Adriamycin-induced hepatotoxicity rats could decrease the oxidative stress injuries in liver tissue.

## Introduction

Anticancer drugs are widely used against variety of human tumors. However, while they generate acceptable outcome in chemotherapy of some cancers, they also exhibit severe toxicity and undesirable side effects<sup>[1]</sup>. Pharmacological studies show that intravenous administration of Adriamycin is rapidly cleared from the plasma of rodents, with concentrated of the drug in the liver, spleen, lung, kidney, and heart. Drug excretion is prolonged and occurs predominantly via the liver.

<sup>[2]</sup> Adriamycin is a cytotoxic agent of anthracycline antibiotics. Anthracyclines activity by intercalating into nuclear DNA, impairing transcription and cell division, inhibiting topoisomerase II activity, producing reactive oxygen species (ROS), and further injuring DNA as well as mitochondria and cell membranes<sup>[3]</sup>.

In one study with ADR injection intraperitoneally (2 mg/kg) in sterile saline, once per week for 6 weeks on rats hepatotoxicity accrued. In this study ADR causes an increase in malondialdehyde (lipid peroxidation marker) and depletion of the antioxidant enzyme such as superoxide dismutase <sup>[4]</sup>. In other study with single dose ADR injection (10 mg/kg) in rat, hepatotoxicity accrued. Also, in this study ADR significantly increased the concentration of MDA and decreased the activities of SOD and CAT in hepatic tissue <sup>[5]</sup>.

The use of herbal plants for the treatment of many diseases is associated to folk medicine from different parts of the world.

*N. sativa* that be known as black seed is a plant from the Ranunculaceae family <sup>[6]</sup>. The seeds contain 36–38% fixed oils, 0.4–2.5% essential oil, proteins, alkaloids, and saponins. <sup>[7]</sup> Thymoquinone (TQ), the main effective component of *N. Sativa*, which is found abundantly (30-48%) in the black seeds, together with its derivatives such as dithymoquinone, thymohydroquinone and thymol <sup>[8]</sup>. The many therapeutic effects such as antidiabetic, <sup>[9-11]</sup> hypolipidemic, <sup>[10,11]</sup> antioxidant, <sup>[9, 11, 12]</sup> anti-inflammatory, antibacterial, anticancer, analgesic, antiulcer, diuretic and antihypertensive, bronchodilator, and hepatoprotective activities exists in *N. Sativa* extract. <sup>[11, 13-15]</sup> Hepatoprotective properties of

*N. Sativa* in liver injury of experimental rats are documented by reducing the oxidative stress<sup>[16, 17]</sup>. *C. longa* belongs to the Zingiberaceae family, exhibits antioxidant, anti-parasitic, antispasmodic, anti-inflammatory, cardiovascular, gastrointestinal and hepatoprotective effects; and also inhibits carcinogenesis and cancer growth <sup>[18]</sup>. Curcumin, the main effective component of *C. longa*, is as powerful and antioxidant as vitamins C, E and  $\beta$ -Carotene, making *C. longa* usage a consumer choice for cancer prevention, premature aging and liver protection. The ample published reports also show that *C. longa* inhibits the growth of several different types of cancer cells and significantly decreased liver injury in experimental models of animals<sup>[19, 20]</sup>.

Administration of ethanolic extracts of *N. sativa* and *C. longa* to patients that suffer from viral hepatitis C exhibited powerful therapeutic benefits through decreasing viral load and attenuate the altered liver function, with more potent effect offered by the mixture <sup>[17]</sup>. In pervious study we show that combination therapy of *C. longa* and *N. sativa* has been synergic antioxidant effect on oxidative stress in rat kidney <sup>[21]</sup>.

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

## Materials and methods

### *Plant material and preparation of the extract*

*C. longa* rhizomes and *N. sativa* seeds were purchased from local herbal shop in Mashhad, Khorasan province, Iran and identified by botanists in the herbarium of Ferdowsi University of Mashhad.

*C. longa* rhizomes (100 g) and *N. Sativa* seeds (100 g) were cleaned, dried, ground, weighed, and homogenized in 70% ethanol at a ratio of 1:10 of plant to ethanol and left to soak 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. [22] Extracts were then dissolved in 96% ethanol (0.5%, w/w) before being orally administrated to animals [21].

### **Chemicals and drugs**

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

### **Animals and treatments**

Sixty four male Wistar rats (220 - 250 g, 10 weeks old) were kept on a 12 h light-dark cycle, under constant temperature ( $22 \pm 2$  °C), and were allowed free access to standard laboratory diet and drinking water. All experiments were performed under license from the Animal Experimentation Ethics Committee of Mashhad University of Medical Sciences (MUMS).

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

1. Control group (CO), which received normal saline via a tail vein on the 1th day of the study.
2. Adriamycin group (ADR), which received Adriamycin (5mg/kg) [23] via a tail vein on the 1th day of the study.
3. Vitamin C group (Vit C), which received Vitamin C (100 mg/kg) [24] in drinking water for 28 consecutive days.
4. Vitamin C plus Adriamycin group (ADR+VitC), which received Adriamycin (5mg/kg) via a tail vein on the 1th day of the study and received Vitamin C (100 mg/kg) in drinking water for 28 consecutive days.
5. *Nigella sativa* extract without Adriamycin group (NS-ADR) which received *Nigella sativa* extract (200 mg/kg) [25] in drinking water for 28 consecutive days.
6. *Nigella sativa* extract plus Adriamycin group (NS+ADR) which received Adriamycin (5mg/kg) via a tail vein on the 1th day of the study and received *Nigella sativa* extract (200 mg/kg) in drinking water for 28 consecutive days.
7. *C. longa* extract plus *Nigella sativa* extract without Adriamycin group (NS+CL-ADR) which

received *Nigella Sativa* (200mg/kg) and *C. longa* (1000 mg/kg) [26] in drinking water for 28 consecutive days.

8. *C. longa* extract plus *Nigella sativa* extract plus Adriamycin group (NS+CL+ADR) which received Adriamycin (5mg/kg) via a tail vein on the 1th day of the study and received *Nigella Sativa* (200mg/kg) and *C. longa* (1000mg/kg) in drinking water for 28 consecutive days.

### **Preparation of rat liver tissue**

At the end of the treatment period, the animals were anesthetized deeply with ether and euthanized by decapitation with a guillotine. The liver tissue was rapidly dissected out and after washing, stored at -80 °C.

### **Malondialdehyde (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 as 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. After reaching to the room temperature, the solution was centrifuged at 1000 g for 10 minutes. The absorbance was read at 535 nm. [27] The MDA concentration was calculated according to follow equation.

$$\text{MDA concentration (M)} = \text{Absorbance} / (1.56 \times 105 \text{ cm}^{-1} \text{ M}^{-1})$$

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 total thiol groups. The produced yellow complex has a peak absorbance at 412 nm. Briefly, 50  $\mu$ L of tissue homogenates was added to 1 ml Tris-EDTA (ethylenediaminetetraacetic acid) buffer (pH = 8.6) and the absorbance was read at 412 nm against Tris-EDTA buffer alone (A1). Then, 20  $\mu$ L of 10 mM solution of DTNB was mixed with the solution and it was stored in room temperature for 15 minutes and the absorbance was read again (A2). The absorbance of DTNB reagent was also read as blank (B). [28] The thiol levels were determined by a spectrophotometric method based on the use of Ellman's reagent and the results are expressed as per gram of tissue.

Total thiol concentration (mM) =  $(A2 - A1 - B) \times 1.07 / (0.05 \times 14,150)$

### Determination of catalase (CAT) activity

Catalase activity was measured according to the Aebi method. Measurements of enzyme activity at substrate saturation or determination of the Ks is therefore impossible. In contrast to reactions proceeding at substrate saturation, the enzymic decomposition of H<sub>2</sub>O<sub>2</sub> is a first-order reaction, the rate of which is always proportional to the peroxide concentration present. Consequently, to avoid a rapid decrease in the initial rate of the reaction, the assay must be carried out with

relatively low concentrations of H<sub>2</sub>O<sub>2</sub> (about 0.01 M). The principle of the assay is based on the determination of the rate constant, k, (dimension: s<sup>-1</sup>, 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 as k (rate constant) per 100 g tissue. [29]

### Statistical analysis

All data were expressed as means  $\pm$  SEM. Normality test (Kolmogorov-Smirnov) was done. Different groups were compared by one way ANOVA followed by tukey's Post Hoc comparison test with SPSS software 11.5. Differences were considered statistically significant when  $p < 0.05$ .

### Results and Discussion

The weight changes in different groups were lower than control groups. The percent of weight changes in VitC, NS+ADR and NS+CL+ ADR were decreased compared to control group ( $p < 0.05$ ). In addition, the percent of weight changes in ADR and NS+Cl-ADR groups ( $p < 0.01$ ) and ADR+VitC compared to control group decreased significantly ( $p < 0.001$ ), (Table1).

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

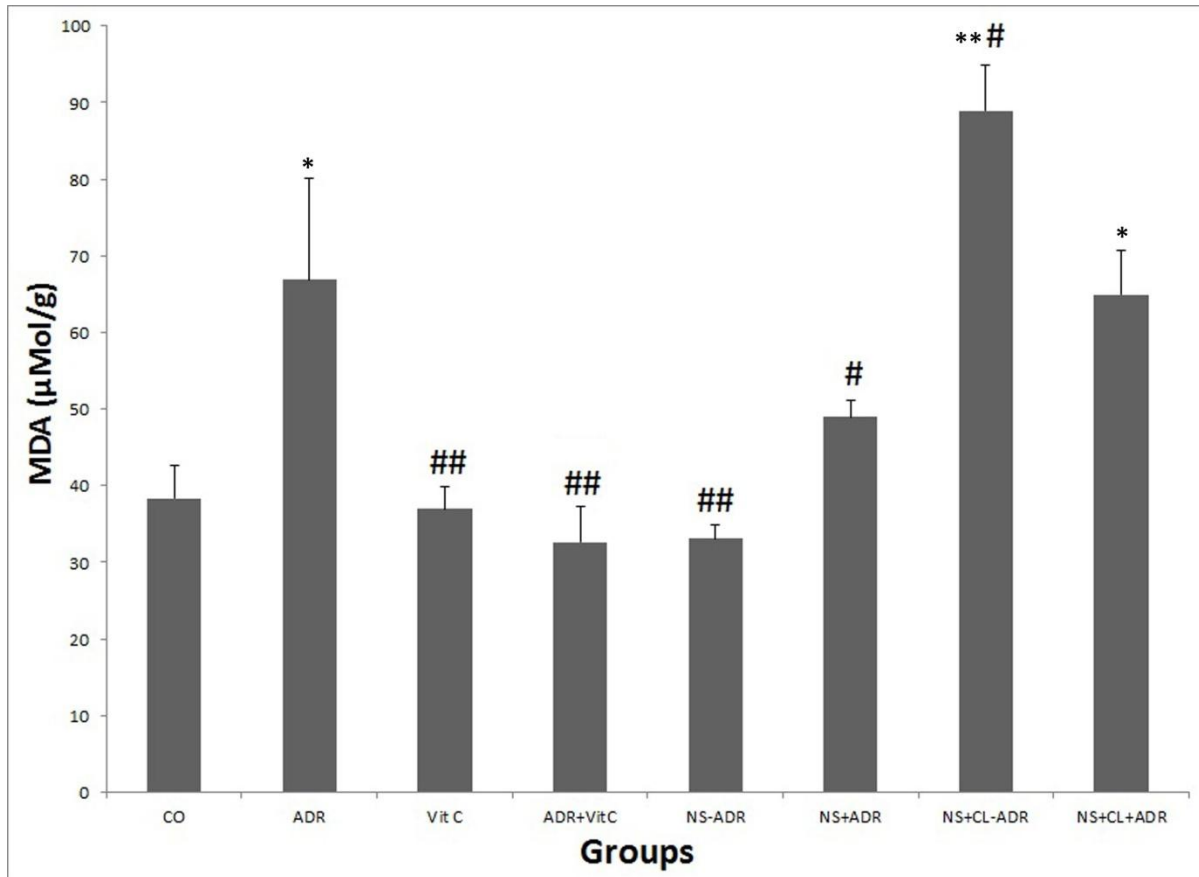
Group	Weight changes (%)
CO	33.75 $\pm$ 6.13
ADR	13.75 $\pm$ 0.78**
Vit C	23.75 $\pm$ 1.26*
ADR+VitC	0.62 $\pm$ 2.03***
NS-ADR	39.28 $\pm$ 0.24
NS+ADR	23.57 $\pm$ 3.03*
NS+CL-ADR	13.75 $\pm$ 2.97**
NS+CL+ADR	23.75 $\pm$ 5.06*

Values are means  $\pm$  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 malondialdehyde levels of liver tissue

MDA level in the liver tissue in ADR and NS+CL+ADR groups ( $p < 0.05$ ) and NS+CL-ADR group ( $p < 0.01$ ) were increased compared to CO

group. The MDA level in NS+CL-ADR group was increased ( $p < 0.05$ ) but in NS+ADR group ( $p < 0.05$ ), ADR+VitC and NS-ADR groups decreased compared to ADR group ( $p < 0.01$ ) (Figure 1).

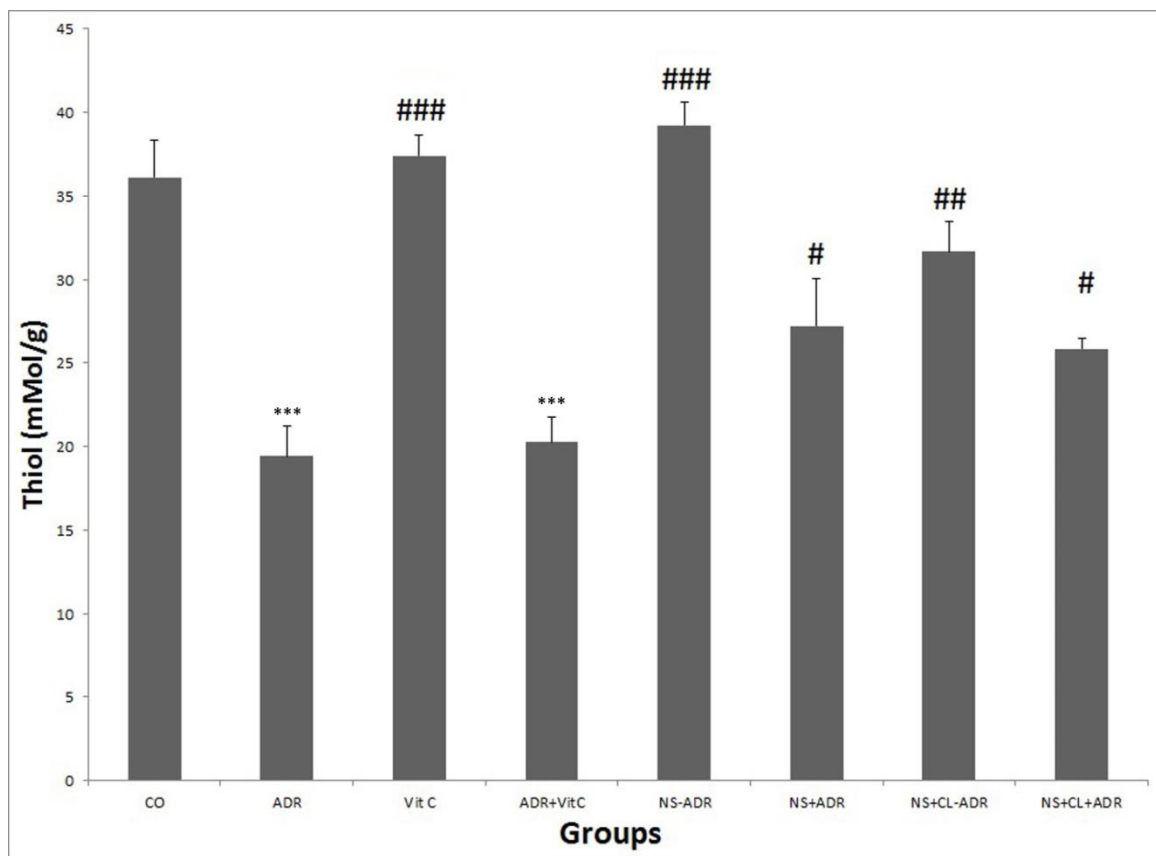


**Fig. 1.** The malondialdehyde (MDA) level of liver tissue in CO: control, ADR: Adriamycin, ADR+VitC: Adriamycin with Vitamin C, Vit C: Vitamin C, NS-ADR: Nigella sativa without Adriamycin, NS+ADR: Nigella sativa with Adriamycin, NS+CL-ADR: Curcuma longa + Nigella sativa without Adriamycin, NS+CL+ADR: Curcuma longa + Nigella sativa with Adriamycin. Values are means  $\pm$  SEM ( $n = 8$ ). \*  $p < 0.05$ , \*\*  $p < 0.01$  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.

### The thiol levels in liver tissue

Thiol levels in ADR and ADR+VitC groups were decreased compared to CO group ( $p < 0.001$ ). Thiol levels in NS+ADR and NS+CL+ADR groups were

increased compared to ADR group ( $p < 0.05$ ). Thiol levels in NS+CL-ADR and VitC groups ( $p < 0.01$ ) and NS-ADR group were also significantly increased compared to ADR group ( $p < 0.001$ ) (Figure 2).

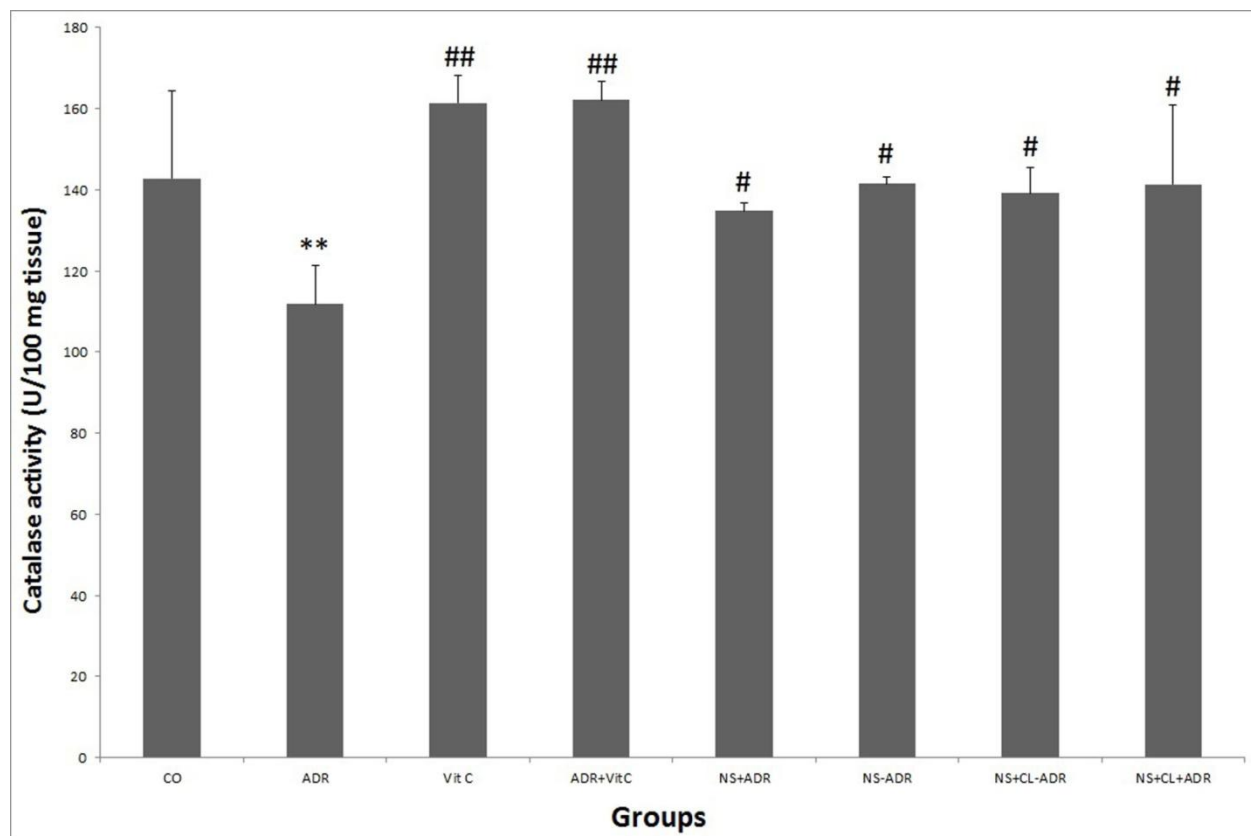


**Fig. 2.** The thiol level of liver tissue in CO: control, ADR: Adriamycin, ADR+VitC: Adriamycin with Vitamin C, Vit C: Vitamin C, NS-ADR: Nigella sativa without Adriamycin, NS+ADR: Nigella sativa with Adriamycin, NS+CL-ADR: Curcuma longa + Nigella sativa without Adriamycin, NS+CL+ADR: Curcuma longa + Nigella sativa with Adriamycin. Values are means  $\pm$  SEM (n = 8). \*\*\* p < 0.001 compared to control group. # p < 0.05, ##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.

### ***The catalase activity in liver tissue***

The activities of CAT in liver tissue of ADR group were lower than CO group ( $p < 0.01$ ), and

increased in treatment groups comparison with ADR group ( $p < 0.05$ -  $p < 0.01$ ) (Figure 3).



**Fig. 3.** The catalase (CAT) activity (U/100 mg tissue) CO: control, ADR: Adriamycin, ADR+VitC: Adriamycin with Vitamin C, Vit C: Vitamin C, NS-ADR: Nigella sativa without Adriamycin, NS+ADR: Nigella sativa with Adriamycin, NS+CL-ADR: Curcuma longa + Nigella sativa without Adriamycin, NS+CL+ADR: Curcuma longa + Nigella sativa with Adriamycin. Values are means  $\pm$  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.

The results of the present study indicate that administration of Adriamycin caused oxidative excess in liver tissue of rats. In the Adriamycin group, malondialdehyde level was increased and total thiol level and catalase activity was decreased compared to control group in liver tissue. Several reports 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.<sup>[30]</sup> Adriamycin enhances NADPH-dependent lipid peroxidation in liver microsomal via the formation

of superoxide anion radicals (O<sub>2</sub><sup>-</sup>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). So, Adriamycin stimulated malondialdehyde production.<sup>[31]</sup> Chemotherapy with Adriamycin increased the malondialdehyde level, nitrogen oxide and decreased the activities of total superoxide dismutase, manganese superoxide dismutase, glutathione, catalase and total antioxidant capacity.<sup>[30]</sup> In one of the study, liver injury was induced by the ethanol. According to this study also some experimental studies have reported that hepatic superoxide formation, MDA, and protein carbonyl levels increased, and GSH levels decreased, in mice after binge ethanol administration.<sup>[32, 33]</sup>

It has been reported that malondialdehyde level significantly decreased glutathione level in

Adriamycin treated rat liver tissue compared to control group.<sup>[34]</sup>

The results of present study shown that the antioxidant effect of Vit C, *C. longa* and *N. sativa* in ADR-induced hepatotoxic rats were increased compared to ADR treated rats. Vitamin C influenced mitochondrial function by decreasing of 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).<sup>[35]</sup>

Vitamin C is a potential antioxidant that has been hypothesized to antagonize the effects of ROS-generating antineoplastic drugs including of methotrexate, vincristine, cisplatin and Adriamycin.<sup>[36]</sup>

*N. sativa* has been considered as a powerful hepatoprotective plant medicine. Treatment with *N. sativa* and its products has been reported to attenuate CCl<sub>4</sub>- induced liver injury. There are reports referring to TQ, one of its antioxidant and anti-inflammatory constituents, as the exhibitor of these properties<sup>[37, 38]</sup>. *N. sativa* seeds appeared to be safe and possibly protective against CCl<sub>4</sub>-induced hepatotoxicity.<sup>[39]</sup> The protective effects of *N. sativa* and TQ (its main constituent) on induced neurotoxicity have been shown<sup>[40]</sup>.

In the present study, in addition to ADR group, in NS+CL- ADR group MDA level was increased in the liver tissue compared to CO group. Then, in NS+CL+ADR group the weight decreased compared to control group.

Hydro-alcoholic extracts of turmeric and curcumin exhibit potent 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. The animal studies have been shown that turmeric's hepatoprotective effects from a variety of hepatotoxic insults, including carbon tetrachloride (CCl<sub>4</sub>), acetaminophen, galactosamine and Aspergillus aflatoxin. 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- $\alpha$  and IL1 $\beta$ . In rats with CCl<sub>4</sub>-induced acute liver injury,

curcumin administration significantly decreased liver injury in treated animals compared to controls<sup>[19]</sup>. In human hepatocyte L02 cell line curcumin was able to avoid the ROS formation by increasing SOD activity and reducing glutathione levels after treatment with the antimicrobial feed additive quinocetone as a generator of free radicals<sup>[41]</sup>.

Thymoquinon and curcumin as effective compounds of *N. sativa* and *C. longa*, respectively have an antioxidant effect, these compounds may be show pro-oxidative properties in high doses. MDA as a one oxidative marker evaluated and has been shown insignificant decrease compared to ADR group while activity of CAT and thiol concentration have a significant increase compared to ADR group. Therefore, we suggest that insignificantly decreasing in MDA in NS+CL+ADR group compare to ADR is reason of hyperlipidemia effect of ADR and increasing of lipid peroxidation chance.

The other studies confirmed that dietary polyphenols possess protective and therapeutic potential in peptic ulcer and Inflammatory bowel disease mediated by suppressing oxidative damage of mucus and amplifying antioxidant performance <sup>[42, 43]</sup>.

Also a review focuses on the most efficacious medicinal plants and their phytochemical and antioxidant agents, which have been consumed for the management of Osteoarthritis <sup>[44]</sup>. In the current study also confirmed that the *C. longa* and *N. sativa* contain polyphenols that have antioxidant effect <sup>[45, 46]</sup>.

## Conclusion

The results showed that chronic administration of *N. sativa* hydroalcoholic extracts could decrease the oxidative stress injuries, whereas administration of combination of *C. longa* with *N. sativa* extracts has been showed the increase of MDA and decrease the antioxidant enzymes such as thiol and CAT in Adriamycin-induced oxidative stress in liver tissue of rat.



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## Conflict of Interests

Authors certify that there is no actual or potential conflict of interest in relation to this article.

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