



Research Article

Effects of *Zataria multiflora* Extract and Carvacrol on Doxorubicin-Induced Oxidative Stress in Rat Brain

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ABSTRACT

Background: Due to the antioxidant effects of *Zataria multiflora* (ZM) and Carvacrol (CAR) in various problems and the prominent role of the ROS in neurotoxicity induced by Doxorubicin (DOX), this study was designed to investigate the effects of ZM hydroalcoholic extract and CAR on DOX-induced oxidative stress in rat brain

Methods: 24 male rats were randomly divided into four groups including: 1)Control, 2)Doxorubicin (DOX) that received DOX via a tail vein on the first day of the study, 3,4) ZM+DOX and CAR+DOX which received ZM and CAR by gavage for 28 consecutive days. Brain tissue removed for redox markers evaluation.

Results: MDA level in the DOX group was significantly increased compared to control group while in treated groups did not show any significant changes in comparison with the DOX group. Also, Thiol content in DOX group showed significant reduction compared to control group. Thiol contents in treated groups showed no significant difference compared to DOX group. Catalase (CAT) activity, an antioxidant enzyme, in the DOX group were significantly decreased compared to control group and increased in treated rats in comparison with the DOX group. Activity of Superoxide dismutase (SOD), an antioxidant enzyme, in the DOX group was significantly reduced compared to control group and increased in treated rats in comparison with the DOX group.

Conclusion: The present study showed that ZM hydroalcoholic extract and CAR could inhibit DOX induced oxidative stress of the brain mainly with effect on the enzymatic antioxidant defense system.

Introduction

Doxorubicin (DOX) called adriamycin, a commercial name, is a chemotherapeutic agent that is used to treatment of many cancers. DOX is known to generate Reactive Oxygen Species (ROS) in many organs including kidney,¹ liver,² heart and brain³ that limit the effective usage of this drug in cancer treatment. One of the important side effects of DOX is neurotoxicity⁴ which is being used in various studies as animal models for neurotoxicity induction.

Since DOX is used commonly in the treatment of various tumors, it is very good if it did not have any harmful effects on healthy cells or even neurons. Although DOX does not pass the blood-brain barrier but after intravenous injection of DOX, it can pass into area which are outside the blood-brain barrier so-called circumventricular organs⁵ and directly traced into the nuclei in the brain.⁵ However, administration of DOX cause the oxidative stress in brain via increasing the lipid peroxidation and protein oxidation as well as increasing the multidrug resistance-associated protein1 (MRP1) expression.³

Zataria multiflora (ZM) (Figure 1) belonging to the Labiateae family grows in Iran, Afghanistan and Pakistan.



Figure 1. *Zataria multiflora*

The herd named of the plant is Avishan Shirazi which has various traditional usages such as anesthetic and antispasmodic.⁶ Studies on ZM reported different effective phytochemicals including components of the plant essential oil such as *p*-cymene (10%), Carvacrol

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(CAR) (52%) and thymol (16%).^{7,8} CAR (2-methyl-5-(1-methylethyl)-phenol) is a potent monoterpenic phenol.⁹ ZM and CAR had an ameliorating effect on many conditions such as anti-inflammatory effect on ulcer,¹⁰ cytotoxic effect on cancer¹¹ and antioxidant effect.¹² Due to the antioxidant effects of ZM and CAR in various problems and the prominent role of the ROS in neurotoxicity induced by DOX, this study was designed to investigate the effects of ZM hydroalcoholic extract and CAR on DOX-induced oxidative stress in rat brain.

Materials and Methods

Extract preparation

ZM was purchased from a herbal shop in Mashhad, Khorasan Razavi, Iran and identified by a Eng Joharchi in the Ferdowsi University of Mashhad herbarium (ID:35314).

ZM aerial parts, about 100 gr were dried, grounded, weighed, and homogenized in 70% ethanol at a ratio of 1:10 of plant to ethanol 70% and were macerated for 72 hr at 37°C with occasional shaking. The mixture was then filtered and the resultant extract was concentrated under decreased pressure at 45°C in a rotary evaporator (EYELA CO, Japan). The concentrated extract was then kept in the incubator at 45°C for 72 hr to evaporate the ethanol residue yielding the crude extract. Consequently, the extract was dissolved in saline before being gavaged to rats.

Animals and treatments

Type of study is experimental (interventional) by randomized sampling and approved by ethics committee of Mashhad University of Medical Sciences (ethical code: IR.MUMS.fm.REC.1396.470). Twenty four male Wistar rats were kept on standard laboratory condition. They were randomly divided into four groups (n = 6 in each group) including:

1. Control group (Co) that received saline via the tail vein on the first day of the study.
2. Doxorubicin group (DOX) that received DOX (5mg/kg)² via the tail vein on the first day of the study.
3. ZM extract plus DOX group (ZM+DOX) which received ZM (200mg/kg)¹³ by gavage for 28 consecutive days.
4. CAR extract plus DOX group (CAR+DOX) which received CAR (20 mg/kg)¹⁴ by gavage for 28 consecutive days.

Preparation of samples

Weighting the rats was performed at the beginning and the end of the experimental period. On the final day of the experiment the rats were deeply anesthetized by urethane and the brain tissues were rapidly excised and after weighing, stored at -80 °C for oxidative markers evaluation.

Total thiol contents were evaluated by using DTNB (2, 2'-dinitro- 5, 5'-dithiodibenzoic acid), a reagent that reacts with the SH groups and produces a yellow colored complex that has a peak absorbance at 412 nm.¹⁵

Generally, 1 ml Tris-EDTA buffer (pH=8.6) was added to 50 µl of brain homogenate in 1 ml cuvettes and the absorbance was read at 412 nm versus Tris-EDTA buffer (A1). Then 20 µl of DTNB reagents (10 mM in methanol) were added to the mixture and after 15 min incubation in room temperature, the absorbance was read again (A2). Also, the absorbance of DTNB reagent was read as a blank (B). A total thiol content (mM) was calculated based on an equation described by Hosseini et al.¹⁶ Malondialdehyde (MDA) concentrations as an index of lipid peroxidation were evaluated in the brain tissue. MDA reacts with thiobarbituric acid (TBA) as a thiobarbituric acid reactive substance (TBARS) and produces a red colored complex which has a peak absorbance at 535 nm. Briefly, 2 ml TBA/trichloroacetic acid (TCA)/ hydrochloric acid (HCL) reagent was added to 1 ml of homogenate and the solution was incubated in a boiling water bath for 40 min. After cooling, the whole solutions were centrifuged at 1000 g for 10 min. The absorbance of supernatant was read at 535 nm. The MDA concentration (C) was calculated as follows.^{16,17}

$$C (m) = \text{Absorbance} / (1.65 \times 10^5)$$

Catalase (CAT) activity was estimated by using the Aebi method.¹⁸ The principle of the assay is based on determination of the rate constant, k, (dimension: s-1, k) of hydrogen peroxide decomposition. By measuring the reduction in absorbance at 240 nm per minute, the rate constant of the enzyme was evaluated. Activities were expressed as k (rate constant) per liter.

Superoxide dismutase (SOD) activity was evaluated by the procedure explained by Madesh and Balasubramanian.¹⁹ A colorimetric assay involving production of superoxide by pyrogallol auto-oxidation and the inhibition of superoxide-dependent reduction of the tetrazolium dye, MTT (3-(4, 5-dimethylthiazol-2-yl) 2, 5-diphenyltetrazolium bromide) to its formazan by SOD was determined at 570 nm. One unit of SOD activity was defined as the amount of enzyme causing 50% inhibition in the MTT reduction rate.

Brain index percentage was calculated according to the following formula:

$$\% \text{ brain index} = \text{brain weight} / \text{final body weight}$$

Statistical analysis

All data were expressed as mean±SEM. Normality test (Kolmogorov–Smirnov) was performed. Different groups were compared by one way ANOVA followed by tukey's Post Hoc comparison test using the SPSS software 11.5. Differences were considered statistically significant when p<0.05.

Results

Results showed that MDA level in the brain tissue in DOX group was significantly increased compared to control group (p<0.05) while in treated groups it did not show any significant difference in comparison with the DOX group (Figure 2). Also, Thiol contents in DOX

group showed significant reduction compared to control group ($P < 0.05$).

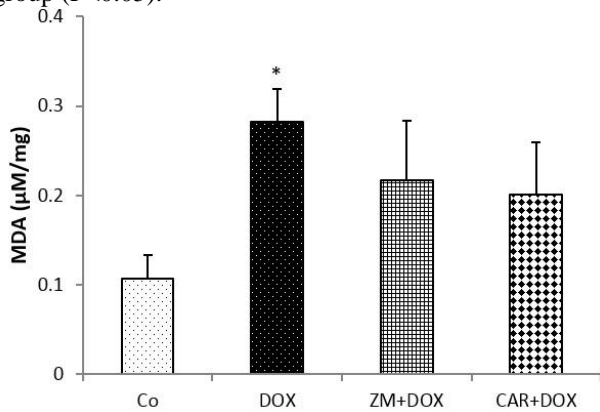


Figure 2. Comparison of the MDA concentration in brain tissue of four groups. Data are presented as Mean \pm SEM (n= 6 in each group). * $P < 0.05$ compared with Co group. Co: Control, DOX: Doxorubicin, Z.M: *Zataria multiflora*, CAR: Carvacrol

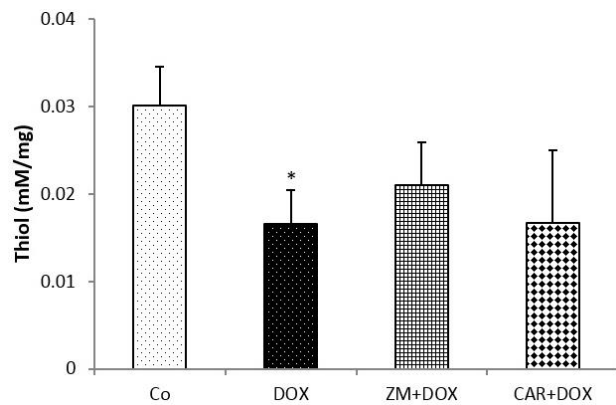


Figure 3. Comparison of the Thiol concentration in brain tissue of four groups. Data are presented as Mean \pm SEM (n= 6 in each group). * $P < 0.05$ compared with Co group. Co: Control, DOX: Doxorubicin, Z.M: *Zataria multiflora*, CAR: Carvacrol

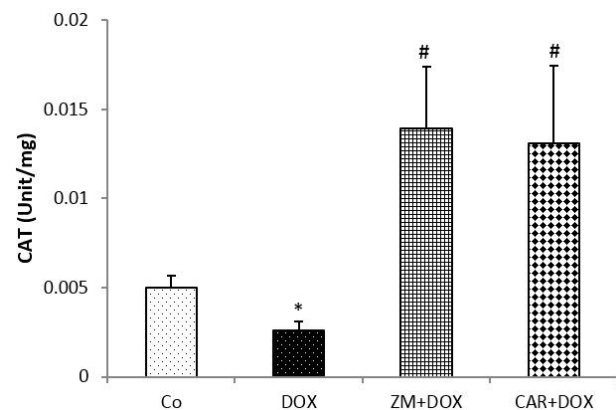


Figure 4. Comparison of the CAT activity in brain tissue of four groups. Data are presented as Mean \pm SEM (n= 6 in each group). * $P < 0.05$ compared with Co group. # $P < 0.05$ compared with DOX group. Co: Control, DOX: Doxorubicin, Z.M: *Zataria multiflora*, CAR: Carvacrol

Thiol contents in treated groups showed no significant difference compared to DOX group (Figure 3).

CAT activity in the brain tissue of the DOX group were significantly decreased compared to control group ($P < 0.05$) and increased in treated rats in comparison with the DOX group ($p < 0.05$) (Figure 4).

Activity of SOD enzyme in the DOX group were significantly reduced compared to control group ($P < 0.001$) and increased in treated rats in comparison with the DOX group ($p < 0.001$) (Figure 5).

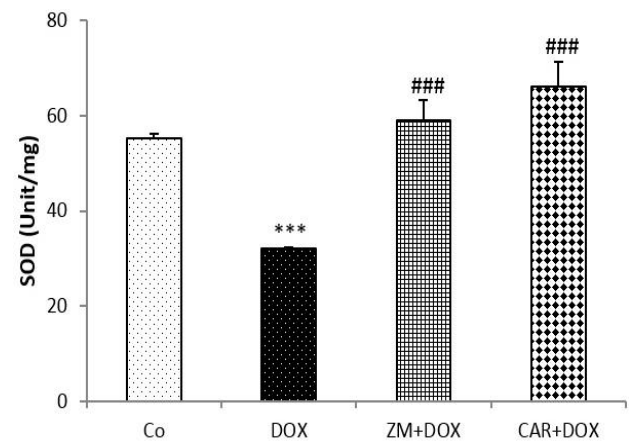


Figure 5. Comparison of the SOD activity in brain tissue of four groups. Data are presented as Mean \pm SEM (n= 6 in each group). *** $P < 0.001$ compared with Co group. ### $P < 0.001$ compared with DOX group. Co: Control, DOX: Doxorubicin, Z.M: *Zataria multiflora*, CAR: Carvacrol

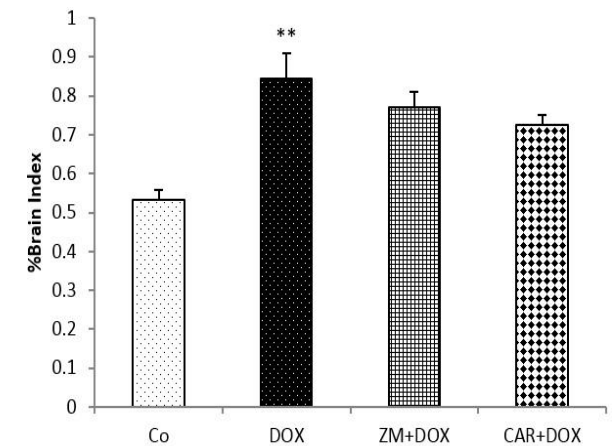


Figure 6. Comparison of the %Brain index in different groups. Values are mean \pm SEM (n= 6). ** $p < 0.01$ compared to Co group. Statistical analyses were made using the one-way ANOVA followed by the Tukey's test POST HOC.

Table 1. Weight changes in different groups.

Group	Weight changes (g)
Co	18.0 \pm 4.6
DOX	-49.4 \pm 17.6**
ZM+DOX	-36.0 \pm 9.3
CAR+DOX	1.0 \pm 10.8##

Values are mean \pm SEM (n= 6). ** $p < 0.01$ compared to Co group, ## $p < 0.01$ compared to DOX group. Statistical analyses were made using the one-way ANOVA followed by the Tukey's test POST HOC.

The weight changes in the DOX group were significantly reduced compared to Co group ($p < 0.01$) while in the CAR+DOX group was significantly increased compared to DOX group ($p < 0.01$) (Table 1). Also, the brain index percentage in the DOX group was significantly increased compared to Co group ($p < 0.01$) while in treated rats no significant difference were observed compared to DOX group (Figure 6).

Discussion

Briefly, our results showed that intravenous injection of DOX induced the oxidative stress in brain tissue after 28 days and treated rats with ZM and CAR have been improved. Previous studies have shown that treated rats with DOX showed significant oxidative stress and lipid peroxidation in renal and hepatic tissues.^{1,2} Jortner et al, showed that neurotoxic effect of DOX in rats has been induced following an intravenous injection of DOX as single dose (5 mg/kg).²⁰ Deliconstantinos et al have shown that DOX increased membrane fluidity conditions for neurotoxicity induction.²¹ The DOX could potentially cause neurotoxic effects as was shown in studies performed by Kondo et al. In their studies DOX induced severe neurotoxicity following the blood brain barrier disruption via administration of mannitol as a hyperosmotic agent.^{22,23} In another study Kondo et al. showed that administration of DOX with dose of 0.05mg/kg into sciatic nerve via retrograde axoplasmic transport caused the neurotoxicity.²⁴ Also, Fronzo et al. showed that DOX did not pass the blood-brain barrier²⁵ while recent studies indicated that the drug, after intravenous injection, could pass into area which were outside the blood brain barrier so-called circumventricular organs (CVO).⁵ CVOs had specific feature of the vascular architecture. It contained extensive vasculature and fenestrated capillaries which lead to a leaky blood brain barrier at the site of the organs and this specific alignment allows direct exchange between the blood and the nervous tissue of these organs.²⁶ By using the fluorescence-microscopic method it was shown that DOX directly traced into the nuclei and glial cells in the brain.⁵

In our recent study, the effects of peripheral administration of doxorubicin on oxidative brain damage was investigated, and the results showed that after 10 days of single dose of doxorubicin, markers of oxidative brain damage were increased and anti-oxidants had declined, indicating the oxidative effects of this drug on the brain and central nervous system.²⁷ In this regard, our study also showed that after four weeks of single dose administration of doxorubicin, oxidative markers were increased in the doxorubicin group and were decreased antioxidant enzymes. There is also an evidence that administration of Z.M and carvacrol for one month has a good response to systemic and local oxidative damage.

Some medicinal plants such as ZM can have beneficial effects on the neurotoxicity of drugs. This plant could improve the toxicity problems by having plenty of antioxidants, such as CAR. CAR had antioxidant effect

both in vivo and in vitro conditions.²⁸ In a study performed by Mandegary et al. anticonvulsant effect of the ZM methanolic extract and its essential oil on electrical and chemically seizures were investigated.²⁹ This study showed that essential oil of ZM has better effect than ZM methanolic extract in convulsion. ZM like other medicinal plants were effective in oxidant-related diseases decreasing lipid peroxidation and improving total antioxidant power of body.³⁰ CAR was the main component of the ZM essential oil. Kavooosi et al showed that the IC₅₀ for reactive oxygen scavenging and reactive nitrogen scavenging was 4.2 µg/ml and 6.6 µg/ml for CAR, respectively. In this study, ZM essential oil and CAR significantly decreased H₂O₂ generation as well as NADH oxidase activity. Therefore, ZM essential oil and CAR could be used in the oxidative damage treatment.⁸ Another study showed that CAR administration with doses of 25, 50 and 100 mg/kg for 46 days, significantly reduced the MDA level and increased the activities of antioxidant enzymes, including SOD, CAT and glutathione peroxidase as well as decreased the levels of the oxidative parameters, 8-isoprotane, following spinal cord injury in rats. Administration of CAR with three doses improves oxidative damage induced by chronic stress in rat brain²⁸. The neuroprotective effect of ZM and CAR may be related to inhibiting oxidative stress following neurotoxicity in rats.

Conclusion

The present study showed that ZM hydroalcoholic extract and CAR could inhibit DOX induced oxidative stress of the brain mainly with effect on the enzymatic antioxidant defense system.

Acknowledgments

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Conflict of Interests

The authors claim that there is no conflict of interest.

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