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The protective role of garlic oil against doxorubicin-induced hepatotoxicity in male mice

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Abstract

Doxorubicin, also known as Adriamycin, is a highly effective antineoplastic agent, but it is well known for its oxidative damage to various body organs, such as hepatotoxicity. The present study aimed to investigate the possible protective role of the natural antioxidant garlic oil on Doxorubicin-induced liver toxicity. Studies were performed on four groups of mice. Control group, garlic oil group, doxorubicin group for seven doses, and doxorubicin plus garlic oil group. Histological examination of liver sections revealed that doxorubicin caused pathological changes in liver cells as well as the reduction in the liver nuclear area and nuclear volume. Administration of garlic oil plus doxorubicin showed a reduction in liver damage induced by DOX.

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Introduction

The liver is one of the largest and most important organs in the body and has diverse functions in metabolism, detoxification and synthesis of many biomolecules and it is the first organ affected by DOX treatment (Prasanna *et al.,* 2020). Doxorubicin (DOX is one of the most effective anthracycline antibiotics and anti-tumour agents used to treat a variety of human malignancies, including hepatocellular carcinoma (Osama and Suzan, 2008). Doxorubicin has a greater effect on cells that are multiplying rapidly, but it can kill both healthy and cancerous cells (Saalu *et al.,* 2009.). Doxorubicin has many side effects, including hepatotoxic, cardiotoxic, nephrotoxic, and immunosuppression (Chaudhary *et al.*, 2016). Liver damage is a relatively common adverse effect in patients with other cancers who are treated with doxorubicin (Cainelli and Vallone, 2009). Oxidative stress is generally considered a major cause of DOX hepatotoxicity. Dox produces hydroxy radical, which destroys DNA primarily in cancerous cells. Doxorubicin can cause an imbalance of oxygen free radicals and antioxidants which causes damage to liver tissue (Mohan *et al.,* 2014). Natural products, including plants, have been used by humans in traditional medicines to treat and alleviate different diseases and are valued for their ability to protect against all types of diseases (Wambi *et al.,* 2009). Several natural and synthetic antioxidants have been suggested to protect against DOX-derived cardiotoxicity (Asensio-Lopez *et al.,* 2012; El-Bakly *et al.*, 2012). Plant phenolic compounds such as flavonoids and isoflavones have an important role in the treatment of many diseases and some of them induce a potent antioxidant and hepatoprotective effect (Seif, 2016). Garlic (*Alium sativum*) is a bulbous plant whose bulb has a strong taste and characteristic odor. Garlic has been well known for its medicinal use since ancient times and has been reported to have antioxidant properties *in vitro* (Reitz *et al.,* 1995). Garlic is used as hypolipidemic and cardiotonic. Some garlic preparations also appear to possess hepatoprotective, immune-enhancing, anticancer, chemopreventive and antioxidant activities (Harunobu *et al.,* 2001). In mammals, garlic has effects of hypolipidaemia, hypoglycemia, hypotriglyceridaemia and hypocholesterolaemia (Ali *et al.*, 2000).

The aim of the present study is to investigate the potential beneficial role of garlic administration against histomorphometrical alterations induced in the liver by doxorubicin in male mice

Materials and methods

Animals and experimental groups

Thirty-two mature male mice weighing 35±5 g were used in the present study. Animals were placed in cages at room temperature in which a 12 h light cycle was maintained and left to acclimatize for 1 week before the start of the experiment. A standard diet and tap water were provided to animals *ad libitum*. Animals were divided into four groups, 8 in each group:

Group I (control): Received saline for 14 days orally.
Group II: Received orally garlic oil (20 mg/kg b. w. /day) for 14 days by gavage.

Group III: Animals were injected intraperitoneally with seven doses of DOX (2.5 mg/kg b.w/day), on alternative days for a period of 14 days.

Group IV: Animals were injected intraperitoneally with DOX (2.5 mg/kg/day for each dose) and received garlic oil (20 mg/kg b. w. /day) 1 hour before DOX dose on alternative days for a period of 14 days.

One day after the last dose, animals were anaesthetized and rapidly dissected. Samples of the liver were removed immediately and kept in a fixative.

Histological examinations

At the end of the experiment, animals from each group were killed by cervical dislocation. The liver from animals was carefully separated and cut into small pieces. Samples were placed in a 10% buffered formalin solution for tissue fixation. Samples were dehydrated in ascending grades of ethanol, cleared in

xylene and embedded in paraffin. Sections (5 µm thick) were cut, stained with hematoxylin and eosin and examined under a microscope (Leica system, Germany).

Karyometry

In the stained paraffin sections, the nuclear area, nuclear volume and ratios were evaluated. SigmaScan Pro (version 5.0, Jandel Scientific, SPSS Inc., Chicago, USA) was used for image analysis and morphometric data acquisition. One hundred cells /animal were measured in the liver of control and treated rats.

Statistics

Quantitative results were expressed as means±S.E. Differences between means were tested by One Way Analysis of Variance followed by Mann–Whitney Rank Sum Test.

The inhibition or stimulation percentage of the measured parameters was calculated as follows:

$$(\%) = \frac{\text{mean control value} - \text{mean DoX treated value}}{\text{mean control value}} \times 100$$

Results

Histological examination

Light microscopic examination of liver sections of control and garlic-treated animals showed that the hepatic lobules appeared to be formed of hepatocytes arranged in cords radiating from the central veins. The hepatocytes with well-preserved cytoplasm and visible rounded nuclei with the fine arrangement of Kupffer cells (Fig.1A). In contrast, animals receiving DOX revealed obvious changes relative to control animals. The most obvious pathological abnormalities include the destruction of hepatic architecture; Many of the hepatocytes were destroyed, forming degeneration areas. There were increasing numbers of inflammatory cells and necrotic hepatocytes. The blood sinusoids were dilated and some of the hepatocytes showed cytoplasmic vacuoles with degenerated nuclei (Fig.1B). Doxorubicin plus garlic oil-treated animals showed an improvement of the liver tissue (Fig. 1C) and morphometry (Table 1).

Table 1. Mean nuclear area and nuclear volume, volume ± S.E. in control, DOX-treated and DOX-treated plus garlic oil animals.

Measurements	control	DOX - treated	DOX % lower than control	DOX treated plus garlic oil	DOX + garlic oil % stimulation than DOX
Nuclear area (sq pixel)	354 ± 23	265 ± 22*	25 %	298 ± 21**	11%
Nuclear volume (cubic pixel)	3850 ± 312	3140 ±286*	18%	3465 ± 295**	9%

* Significantly different from the control at *P< 0.05*.

**Significantly different from DOX - treated group at P< 0.05.

Karyometry

The quantitative data of the nuclear area, the nuclear volume of the liver of control, DOX-administered and DOX-administered plus garlic oil animals are summarized in Table 1. DOX administration reduced the mean value of Nuclear area (265 ± 22) sq pixel compared to those of nuclear area control (354 ± 23), 25 % lower than control. Statistically, the inhibition was significant (p < 0.05). DOX administration reduced nuclear volume (3140 ± 286), compared to those of nuclear volume control (3850 ± 312),18% cubic pixel. Statistically, the inhibition was significant (p < 0.05). Garlic oil treatment to the DOX-administered animals restored the Nuclear area to (298 ± 21) and nuclear volume to (3465 ± 295) stimulation than DOX 11% and 9%, respectively.

Discussion

Doxorubicin is an anthracycline antibiotic whose mechanism of action is to treat both solid and hematological cancers and can cause oxidative damage associated with acute toxicity (Bilgic *et al.,*

2019). The present study was designed to demonstrate pretreatment with garlic oil, which would have a hepatoprotective effect on doxorubicin-induced liver damage as a side effect. The liver is one of the largest and most important organs in our body, which plays an important role in regulating different biological processes. The liver is the main site of DOX metabolism. Oxidative stress plays an important role in DOX-induced damage of normal cells or tissues (Agapito *et al.*, 2001; Kolarovic *et al.,* 2010). Side effects of the DOX in the present study exhibit structural changes in the liver tissue. These changes include a marked disruption of hepatic cords, hepatocyte degeneration, dilated blood sinusoids, inflammatory infiltration and necrosis. The toxic action produced by DOX might be attributed to its ability to generate reactive oxygen species (ROS), which induce oxidative damage in several tissues. Doxorubicin is known to kill cells primarily by fragmenting DNA and causing cell death by apoptosis (Ray and Jena, 2000; Patel *et al.,* 2010). Liver damage is a relatively common adverse effect in patients with other cancers who are treated with Dox (Cainelli and Vallone, 2009). Pathological changes in this study due to exposure to DOX are in agreement with previous reports in rats (Kalender and Kalender, 2005; El- Sayyad *et al.,* 2009). Results of the present study reveal a significant decrease in the mean values of the nuclear area and nuclear volume of the liver of DOX-treated animals versus those of controls. Many studies dealt with the nuclear volume and nuclear size is very important for cellular function and cellular activity. In contrast, decreased cellular function is associated with the diminution of the volume of the nucleus (Watanabe and Tanaka, 1982). Vidal *et al.,*2004) found a significant reduction in the nuclear volume of relay neurons as a result of aging. The reduction in the liver nuclear area and nuclear volume due to DOX- toxicity is in agreement with the previous studies.

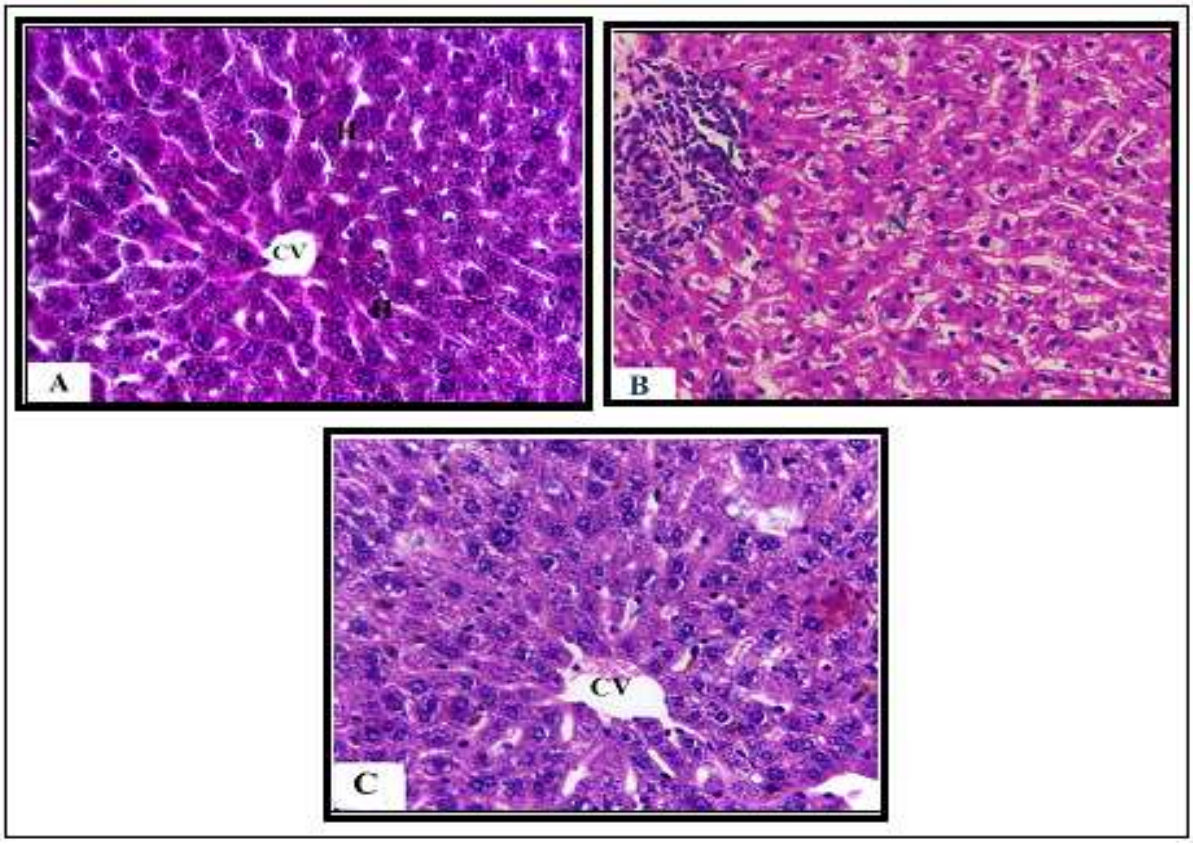

Fig. 1. A: liver section of control animals showing normal structure, central vein (CV) and hepatocytes (H). B: DOX treated animals showing disturbance of hepatic cords, hepatocytes degeneration, inflammatory infiltration (I), swallow sinusoids and necrosis (N). C: DOX plus garlic oil illustrating improvement of the liver tissue (H&E, X400).

Garlic (*Allium sativum*) is one of the most multipurpose medicinal plants used as a traditional herbal medicine to prevent and treat many diseases (Anwar and Younus, 2017). Contents of garlic oil, particularly E-ajoene and organosulfur compounds, have shown a broad activity against several DNA and RNA viruses and are known as hepatoprotective agents (Sheen *et al.,* 1999) and reduce serum cholesterol and triglycerides' levels (Fenwick and Hanley 1985). Garlic is a good source of natural antioxidants and it has a protective effect against chemicals (Naji, 2017). In the present work, pretreatment with garlic oil decreases Dox-injured liver and restores normal liver tissues and are in agreement with those of (Sutejo and Erfan, 2017).

Conclusion

The results of the present investigation suggest that the administration of garlic extract improves the hepatocytes architecture through the maintenance of histopathological changes and has the potential to protect against Dox-induced liver damage. So, the consumption of garlic can be a potential therapeutic against Dox side effects.

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