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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Protection by wheat germ oil against doxorubicin-induced pathological changes and apoptosis in the kidney of male mice

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Abstract

Doxorubicin is a highly effective antineoplastic agent, but it is well known for its oxidative damage to various body organs such as nephrotoxicity. The present study aimed to investigate the possible protective role of the natural antioxidant wheat germ oil on Doxorubicin -induced kidney toxicity. Studies were performed on four groups of mice. Control group, wheat germ oil group (100 mg/kg b.w.), Doxorubicin group (2.5 mg/kg/day) for seven days, and doxorubicin plus wheat germ oil group. Histopathological examination of kidney sections revealed that doxorubicin caused glomerular congestion with wide Bowman's space and widened tubular lumen. Immunohistochemical localization of Caspase-3 for apoptosis was performed. Doxorubicin treated animals showed positive reaction to Caspase 3 in glomerulii and renal tubules as compared with controls. Administration of wheat germ oil reversed kidney damage with a marked reduction in tubular damage and apoptosis induced by doxorubicin. These results have suggested that wheat germ oil ameliorated doxorubicin -induced nephrotoxicity in male mice.

Keywords: Doxorubicin, wheat germ oil, histopathology, kidney, mice

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Introduction

Cancer is a global health problem with approximately 12.7 million diagnosed cases and 7.6 million cancers-related deaths per year. Doxorubicin, an anthracycline antibiotic, is a broad-spectrum Antineoplastic agent, which is commonly used in the treatment of uterine, ovarian, breast and lung cancers and as in several other cancer types. Doxorubicin has been widely used over the past several decades to treat patients with various cancers, including hepatocellular carcinoma. The kidneys, brain, liver and the skeletal muscles are affected by Doxorubicin (Santos et al., 2007). Doxorubicin -induced changes in the kidneys of rats include increased glomerular capillary permeability and tubular atrophy (Wapstra, et al., 1999). It is believed that oxidative stress and the formation of free radicals play a crucial role in the mechanism of Dox toxicity by reacting with oxygen (Kalender, 2005; Yagmurca et al., 2007). Doxorubicin produces hydroxy radical which destroys DNA primarily in cancerous cells. In addition to hepatotoxicity, excessive exposure also causes nephrotoxicity (Saad et al., 2001; Ray, 2003; El-Shitany et al., 2008). Liver damage is a relatively common adverse effect in patients with other cancers who are treated with doxorubicin (Cainelli and Vallone, 2009). Doxorubicin hepatotoxicity has been reported in a number of animal studies (Ray et al., 2000a&b; Ray and Mehendale, 2000; Ray, 2003). Several mechanisms for the doxorubicin-induced cardiotoxicity have been proposed, including membrane lipid peroxidation and free radical formation (Li et al., 2002).

Natural products are valued for their ability to protect against all types of diseases, drug and chemically induced (Ray et al., 2004; Wambi et al., 2009). Several natural and synthetic antioxidants have been suggested to protect against doxorubicin-derived

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cardiotoxicity (Asensio-Lopez et al., 2012; El-Bakly et al., 2012). Wheat germ is a rich source of B complex vitamins, the richest source of tocopherols. Wheat germ oil also contains alpha-and gamma- tocotrienols (Leenhardt et al., 2008; Hassanein and Abedel-Razek, 2009). These nutrients and phytochemicals may have significant implications in chemoprevention, (Jensen et al., 2004). This oil is a source of easily assailable vitamin E which acts as inhibitor of oxidation processes in body tissues. It protects cells against the effects of free radicals, which can cause tissues damage. Wheat germ oil contains unsaturated fatty acids which enhances the antioxidant activity and generates DNA-protective properties (Krings et al., 2006).

The present study aimed to investigate the possible adverse pathological effects of doxorubicin in experimental animals and the protective role of wheat germ oil supplementation in alleviation of the toxic effects of doxorubicin.

Materials and Methods

Animals and experimental design

Sexually mature male Swiss albino mice weighing 35 ± 5 g were used. The animals were housed in cages, fed a standard laboratory diet and water *ad libitum*. The animals were exposed to a 12 h light/dark cycle at a room temperature and left to acclimatize for one week before the experiments.

Mice were divided into four groups, six for each:
Group I (control group): treated with saline for seven days orally once a day.
Group II (wheat germ oil group): received orally oil (100 mg/kg b.w).
Group III (Doxorubicin group): doxorubicin was injected intraperitoneally (2.5 mg/kg/day), for consecutive seven days.
Group IV (Doxorubicin plus wheat germ oil group): received doxorubicin intraperitoneally (2.5 mg/kg/day), for consecutive seven days plus wheat germ oil (100 mg/kg b.w).

Histopathological examinations

At the end of the experiment, animals from each group were killed by cervical dislocation. Kidneys from animals was carefully separated and cut into small pieces. Tissue immediately fixed in 10% neutral buffered formalin for histopathological and immune-histochemical studies. For histopathological, samples were dehydrated in ascending grades of ethanol, cleared in xylene and embedded in paraffin. Sections (6 μm thickness) were cut and stained with hematoxylin and eosin.

Immunohistochemistry

For the detection of caspase-3 protein, immune-histochemical staining was performed. Slides were deparaffinized, dehydrated, washed in phosphate buffer saline then covered with peroxide block staining and incubated at room temperature in humidity chamber for 10 min. Monoclonal caspase-3 antibody was applied on the tissue sections then incubated horizontally in humidity chamber for an hour, at room temperature. After removal of excess buffer, the sections were incubated in preformed strept avidin peroxidase. DAB substrate-chromogen (3.3-Diaminobenzidine tetrahy-drochloride) was applied on slides for 5-15 min until the desired brown colour was obtained. Sections were counterstained Mayer's hematoxylin.

Results

Histological changes in the kidney

Microscopic examination of kidney sections after the end of the experiment revealed that sections stained with H&E are in the normal structure of renal tissue in the control and wheat germ oil treated animals. Renal corpuscles surrounded by sections of proximal and distal convoluted tubules are normal histological structure (Figs. 1A&B). In case of doxorubicin group, there were glomerular congestion with wide Bowman's space and widened tubular lumen. Some of the renal tubules were degenerated and others were dilated (Fig. 2C). Also, haemorrhage and necrosis were observed in kidney tissues. There are improvements in both glomerular and renal tubules changes in case of doxorubicin plus wheat germ oil group (Fig. 2D).

Morphometrical measurments

Data obtained from kidney histomorphometrical measurements (Table 1) demonstrated statistically significant decreased ($P<0.05$) in the mean values of glomerular area and glomerular diameter of doxorubicin-treated group in comparison with the control. The mean values of glomerular area, glomerular diameter significantly increased ($P<0.05$) in doxorubicin plus wheat germ oil-treated animals. Renal tubules lumen area showed statistically significant increased ($P<0.05$) in doxorubicin-treated animals in comparison with the control group, while in case of doxorubicin plus wheat germ oil-treated animals, renal tubules lumen area showed statistically significant decrease ($P<0.05$).

Immunohistochemical observations (Table 2 &Fig. 2)

Expression of renal tissue caspase-3 (apoptotic index)

In this study, apoptotic immunopositive reactions in the kidney were investigated with caspase-3. The expression of renal caspase-3 was weak in glomerular epithelial cells in control and wheat germ oil treated

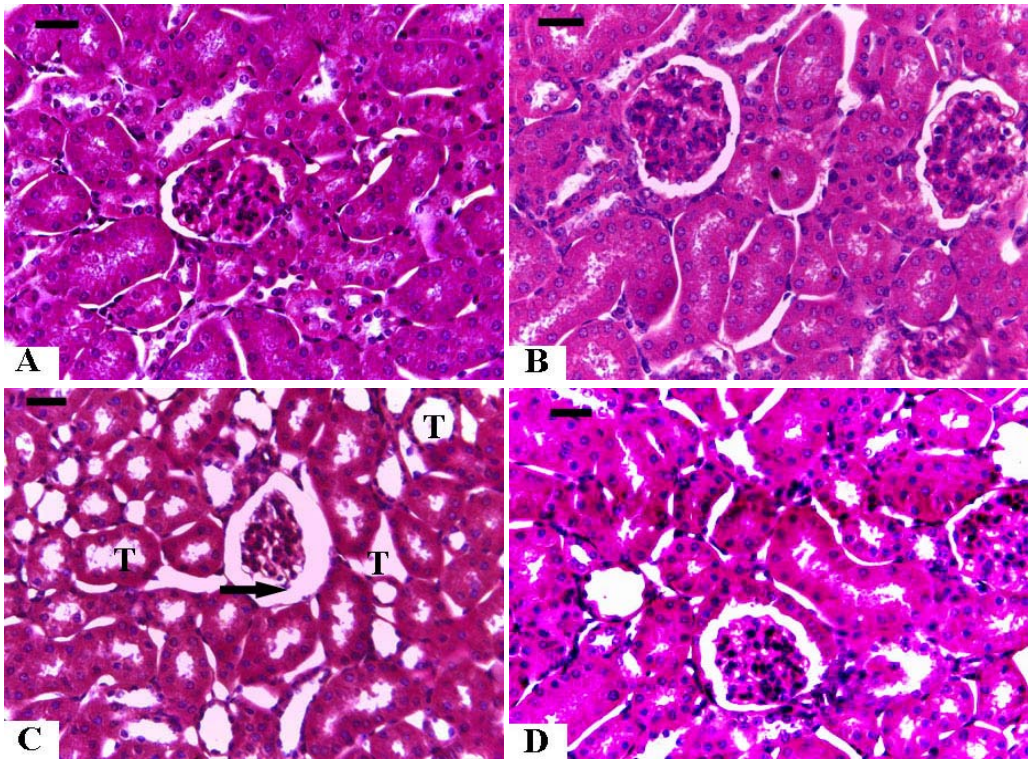

Fig. 1: A&B: Kidney section of control and germ oil treated mice showing normal glomeruli (G) and tubules (T). (C) Doxorubicin treatment illustrating wide Bowman's space and (arrow) widened tubular lumen (T). (D) Doxorubicin plus wheat germ oil treatment. Scale Bar = 25 um.

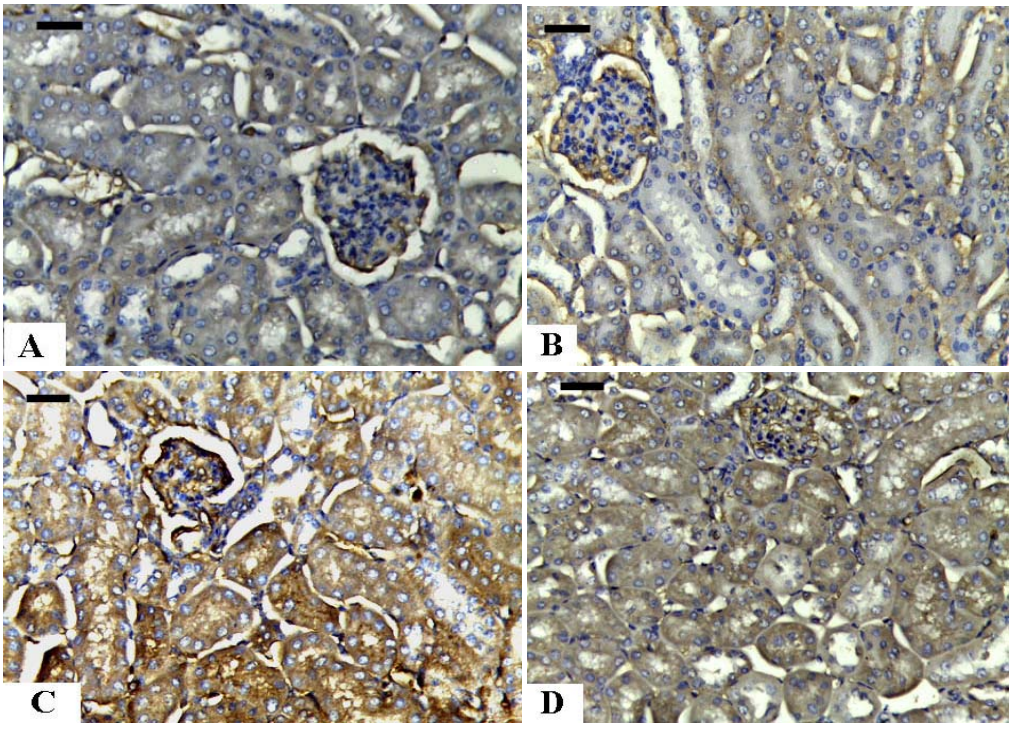

Fig. 2: Immunohistochemical localization of Caspase3 in the renal tissue of mice. (A&B) Control and wheat germ oil treated animals kidney sections. C: Mice treated with doxorubicin, sections appeared with staining of Caspase3. D: Mice treated with doxorubicin plus wheat germ oil. Scale Bar = 25 um.

Table 1: Glomerular area and diameter, tubules lumen area in different experimental groups

Groups	Glomerulus area (sq pixel)	Glomerulus diameter (pixel)	Tubules lumen area (sq pixel)
Control	25387±1587	278±12.6	1486±55.9
Wheat germ oil	25894±1593	286±11.8	1502±57.7
Doxorubicin	23683±1399*	237±9.87*	1373±53.6*
Doxorubicin &wheat germ oil	24961±1533**	263±10.6**	1432±51.4**

*Indicates significant difference between doxorubicin group and control group (P<0.05); **Indicates significant difference between Doxorubicin and wheat germ oil group and Doxorubicin group (P<0.05)

Table 2: Semiquantitative analysis of caspase-3 Immunstaining density in kidney

	Control	Germ oil	Doxorubicin	Doxorubicin &wheat germ oil
(Caspase-3)	+	+	+++	++

Caspase-3 reactivity was estimated as follows: weak: + , moderate: ++, strong: +++.

animals (Figs. 2A & 2B). The expression of caspase-3 was positive in glomerular epithelial cells, renal tubular epithelial cells in case of doxorubicin treated animals as compared with controls (Fig. 2C) Doxorubicin plus wheat germ oil treated animals showed less reaction to Caspase3 immunoreactivity (Fig. 2D).

Discussion

In the present study, the histological examination of control mice showed normal architecture when viewed under the microscope. Doxorubicin treatment induced kidney histopathological changese such as glomerular congestion with wide Bowman's space and widened tubular lumen. It is well known that oxidative stress and free radicals production are involved in doxorubicin action, in relation to its anticancer and toxic effect. Thus, it has been reported that doxorubicin leads to direct oxidative injury to DNA and generates lipid peroxidation (Mataix et al., 1997). Our results are similar to those obtained by Deman et al. (2001) and Yagmurca et al. (2004). They demonstrated that treatment with doxorubicin significantly reduces antioxidant capacity in kidney which leads to kidney damage. The present results are consistent with the previous report done by Yagmurca et al. (2004) demonstrated that glomerular sclerosis was seen 10 days after doxorubicin injection in rats. They have demonstrated structural changes in renal tissue of doxorubicin-treated animals and the protective effect of various agents. Apoptosis is an important mechanism regulating cell number and their development in different organs and tissues, as well as in removing harmful and useless cells from the body (Francesch, 1986). The apoptotic caspase enzymes are named as initiator caspases and effector caspases. Caspase-3 has been identified as being a key mediator of apoptosis in mammalian cells the effector caspases act via the activation of initiator caspases which trigger the apoptotic process (Salvesen and Dixit, 1997). Apoptosis is a common feature of renal toxicity induced by chemicals or drugs. In the present study, the number of apoptotic cells was increased in the kidney of doxorubicin group when compared with control group, but, in the doxorubicin plus germ oil group, apoptotic cell numbers (immunopositive reactions) decreased. Our results in which Dox cause apoptic renal cells are in accordance with Cummings and Schnellmann (2002) who stated that renal cell death is a consequence of chemotherapy treatment and is one of the major factors cause, renal tubules histological changes such as apoptosis. Recent studies stated that using plant-derived chemopreventive agents in combination with chemotherapy can enhance the efficacy of chemotherapeutic agents and lower their toxicity to normal tissues (Gasic et al., 2007; Cayir et al., 2009; Silici et al., 2010).

Wheat is an important source of vitamins, minerals, dietary fibre and phyto-chemicals. The oil is a rich source of toco-pherols and toco-trienols. Wheat germ oil not only prevents autoxidation of unsaturated fatty acids but also generates DNA protective properties (Gelmeza et al., 2009). In this study, wheat grem oil improves kidney histopathological and apoptotic index when compared to doxorubicin group. This is in agreement with some previous reports (Paranich et al., 2000; Leenhardt et al., 2008) who stated that germ oil containing compounds have antioxidant activity, owing to its composition, and has the potential of inducing positive effects on the antioxidant defence system. Niu et al. (2011) and Zhu et al. (2011) stated that wheat germ oil/extract has phenolic compounds which can scavenge free radicals.

In conclusion, the present study illustrated that injection of doxorubicin to mice caused renal injury 7 days after injection. Pretreatment with wheat germ oil protected renal tissues against doxorubicin induced nephrotoxicity and apoptosis.

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