

## Assessment of the chemo-preventive effects of various plant constituents against doxorubicin-induced toxicity in rats

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**Abstract:** Doxorubicin (DXR) is a very potent drug with broad spectrum of biological activity, used to treat a wide variety of human malignancies and many solid tumors. The Clinical efficacy of drug in a wide range of malignant disorders is hampered by its dose limiting side effects such as cardiotoxicity, hepatotoxicity, nephrotoxicity and skin toxicity. Due to its great importance in chemotherapy for the treatment of many types of cancer, researchers have expended great efforts trying to prevent or attenuate the side effects of doxorubicin administration. The most immediate approach has been the combination of the drug delivery together with an antioxidant in order to reduce oxidative stress. The effect of doxorubicin intraperitoneal injection at dose level of (cumulative dose, 12 mg/kg body weight) without or with oral administration of wheat germ oil at dose level 1.5 ml / kg.bw /day (~1400 mg/kg.bw/day) and phytic acid at dose level of (20 mg/kg b. w./day) for 6 weeks was evaluated in adult male rats. Serum Alanine aminotransferase, Aspartate aminotransferase, and Alkaline Phosphatase activities as well as total bilirubin, total protein, albumin, urea, uric acid, and creatinine levels were assayed. Lipid peroxidation (indexed by MDA) and antioxidants of liver, kidney, and spleen like glutathione content and glutathione transferase, peroxidase, superoxide dismutase and catalase activities were assessed. In doxorubicin - administered rats, there was an increase in serum ALT, AST, and ALP activities and total bilirubin concentration reflecting liver dysfunction. On the other hand, these serum total protein, albumin and globulin levels were decreased. While serum creatinine, urea and uric acid concentrations were increased reflecting kidney injury. With regards oxidative stress, glutathione content and glutathione-S-transferase, peroxidase, SOD and CAT activities were decreased while lipid peroxidation was increased in liver, kidney, and spleen. Co-administration of wheat germ oil and phytic acid successfully improved the adverse changes in liver, kidney, and spleen functions with an increase in antioxidants activities and reduction of lipid peroxidation. In conclusion, it can be supposed that dietary wheat germ oil and phytic acid supplementation may provide a cushion for a prolonged therapeutic option against DXR toxicity without harmful side effects. However, further clinical studies are required to assess the safety and efficacy of these extract in human beings. [Hanan A.E. Soliman; Rasha R. Ahmed and Asmaa T. Ali. **Assessment of the chemo-preventive effects of various plant constituents against doxorubicin-induced toxicity in rats.** *J Am Sci* 2014;10(9):153-164]. (ISSN: 1545-1003). <http://www.jofamericanscience.org>. 21

**Key words:** Doxorubicin; wheat germ oil; phytic acid; toxicity; antioxidants; oxidative stress.

### 1. Introduction

Chemotherapy is considered to be a major treatment at late stages and to avoid the recurrence of cancer after surgery (Poornima *et al.*, 2014) It is a common method of treatment for many types of cancer (Sahin *et al.*, 2010). Chemotherapy drugs are effective against cancer cells because they are designed to interfere with rapidly dividing cells. Doxorubicin (adriamycin) is one of the most effective antitumor antibiotics belonging to the class of anthracyclines used as a chemotherapeutic agent against a variety of human malignancies (Hortobagyi, 1997). DXR is isolated from cultures of *Streptomyces peucetius* and is used in the management of various hematological malignancies and neoplastic diseases such as leukemia, breast cancer and AIDS (acquired immune deficiency syndrome)

related Kaposi's sarcoma, lung, testes, prostate, cervix, bladder and Ewing's sarcoma (Tan *et al.*, 1967) but its use is limited by high incidence of cardiac toxicity (Kelishomi *et al.* 2008). Acute administration of high DOX doses causes severe multiple organ damage, including that of the heart, liver and kidney, thus limiting its clinical use. Although inflammation, apoptosis, mitochondrial DNA damage, impairment of calcium metabolism, iron and free radicals may all contribute to a variable extent to the deterioration of organ function; the exact mechanism of DXR-mediated multiple organ toxicity remains largely unknown (Zhang *et al.*, 2014). Unfortunately doxorubicin is not specifically targeted to the tumour, and it can affect the growth of many other cell types in the body (Tacar *et al.* 2012). This results in the immune system becoming

depressed, and as the numbers of immune cells reduce, the patient becomes more susceptible to microbial infections, fatigue and healing time decreases. The severity of these effects and their occurrence depends on the dosage of doxorubicin and the regeneration capacity of the patient's bone marrow (**Chatterjee 2010**). The main toxic effects on hepatocytes include: arrest cell cycle of hepatocytes, (**Kassner et al. 2008**)oxidative stress and disruption of electron transport, generation of free radicals, and activation of the nuclear factor-kappa B(**Zhao et al., 2012**). But the exact mechanism of hepatotoxicity of doxorubicin is not fully clarified. Due to these serious side effects, the clinical use of doxorubicin has been restricted (**Zhao et al. 2012**).

There are several concepts that provide the evidence of DXR-induced hepatotoxicity. It was found that the drug causes disruption in basal metabolism by showing toxic effect especially in liver (**Durak et al., 1998; Kalender et al., 2001; Kalender et al., 2005; Kwiecień et al., 2006**). Among them, the same mechanism as free radical generation is the most thoroughly investigated. DXR not only increases hepatic free radical production, but also decreases its ability to detoxify reactive oxygen species. Increased superoxide dismutase (SOD), catalase activity (CAT) and glutathione peroxidase (GPx) activities in liver tissues indicate that DXR have hepatotoxic effects. It has also reported that malondialdehyde (MDA) level increased in DXR-treated rats which act as an indicator of DXR-induced hepatic injury(**Grafs et al., 1987;Durak et al., 1998; Kalender et al., 2001; Kalender et al., 2005; Kwicien et al., 2006**). Doxorubicin is known to cause cardiotoxicity through multiple routes including the build-up of reactive oxygen species and disruption of the calcium homeostasis in cardiac myocytes, but the effect of drug treatment on the associated biomechanics of cardiac injury remains unclear (**Gharanei et al., 2014**). Leakage of intravenous infusions from the intravascular region into the interstitial space is called extravasations (**Yılmaz et al., 2001**).

DXR causes cellular death with direct toxic effects to living cells and results in the release of a DXR-DNA complex into the intercellular space and this process prevents the release of the cytokines and growth factors that participate in wound healing and results in skin necrosis. It has also reported that DXR increased the mononuclear cells infiltration and congestion of blood vessel which cause necrosis (**Dorr et al., 1980; Gokcimen et al., 2007; Amandeep et al., 2012**). Among the different hypothesis, DXR-induced cardiotoxicity has been attributed to increased oxidative stress that leads to damage of macromolecules, membranes and DNA, thereby contributing to cellular damage, energetic deficit and

acceleration of cell death through apoptosis and necrosis (**Amandeep et al., 2012**). Further, membrane lipid per oxidation, mitochondrial damage, iron-dependent oxidative damage to macromolecules, histamine release and disruption of calcium homeostasis are also implicated in the mechanism of drug related side effects(**Amandeep et al., 2012**).

A number of studies were conducted for antioxidants screening from the natural medicine aiming to minimize oxidative injury by DOX. Several studies suggest that dietary supplementation with antioxidants can influence the response to chemotherapy as well as the development of adverse side effects that results from treatment with antineoplastic agents (**Conklin 2000**).

Wheat germ oil is extracted from the germ of the wheat kernel, wheat germ oil is a valuable source of essential fatty acids, including linolenic, palmitic and oleic, protein, minerals, it is naturally rich in vitamins A, D and E, and also, contains vitamins B1, B2, B3, B6, policosanal and octacosanols, and dietary fibers, phytochemicals and antioxidant properties (**Abdel Fattah et al., 2011**). Wheat germ is a rich source of B complex vitamins, with wheat germ oil being the richest source of tocopherols. These nutrients and phytochemicals may have significant implications in chemoprevention (**Jensen et al., 2004;Lui, 2007**). Wheat germ is also rich in unsaturated fatty acids, mainly oleic, and  $\alpha$ -linoleic acids (**Sjovall et al., 2000**), and in functional phytochemicals, mainly flavonoids, sterols, octacosanols and glutathione (**Zhu et al., 2006**).

The germ is the most nutritious portion of the wheat and it makes up about 2.5 % of the weight (**Abdel Fattah et al., 2011**). During the milling process the germ is separated from the bran and starch (**Jensen et al., 2004; Lui, 2007**). Vitamins are ideal antioxidants to increase tissue protection from oxidative stress due to their easy, effective and safe dietary administration in a large range of concentrations (**Al-Attar, 2011**). Wheat germ oil is a source of easily assailable vitamin E which acts as inhibitor of oxidation processes in body tissues; it is an important antioxidant factor. It is known to possess various physiological functions. A major contributor to non-enzymatic protection against lipid peroxidation is vitamin E, a known free radical scavenger (**Fraga et al., 1987;Rikans et al., 1991**).

Phytic acid (inositol hexaphosphate, IP6) is a naturally occurring poly-phosphorylated carbohydrate, abundantly present in many plant sources and in certain high-fiber diets, such as cereal grains and most legumes including corn, soy beans, wheat bran, nuts oilseeds, tubers, pollen, spores, and organic soils (**Graf et al., 1987; Tsao et al., 1997;Barrett et al., 1998; Vucenic and Shamsuddin, 2009**) and it is also found in some edible vegetables (**Weglarz et al., 2007**).

Multiple mechanisms of action, including gene alteration, cell cycle inhibition, increased natural killer (NK) cell activity, and antioxidant functions, have been proposed for phytic acid's antineoplastic abilities. However, the exact mechanism by which it exerts these effects has yet to be elucidated (**Fox and Eberl, 2002**). After rapid intake and de-phosphorylation, IP6 enters the pool of inositol phosphates and acts as a strong antioxidant, enhances immune function, elicits anti-inflammatory activity, modifies phase I and II metabolizing enzymes, modulates oncogene expression, normalizes abnormal cell proliferation, induces cell differentiation, induces apoptosis, and inhibits angiogenesis. In addition, IP6 prevents tumor metastasis formation (**Vucenik and Shamsuddin, 2009**). Based on these issues and concerns, the present study was designed to investigate the preventive effect of wheat germ oil and phytic acid on liver dysfunction and oxidative stress in doxorubicin-administered rats.

## 2. Materials and methods

### 2.1. Experimental animals:

Male Wistar albino rats weighing about 180-220 g were used as experimental animals in the present investigation. They were obtained from the animal house of Research Institute of Ophthalmology, El-Giza, Egypt. They were kept under observation for about 15 days before the onset of the experiment to exclude any intercurrent infection. The chosen animals were housed in plastic cages with good aerated covers at normal atmospheric temperature ( $25\pm 5^{\circ}\text{C}$ ) as well as 12 hours daily normal light periods. Moreover, they were given access of water and supplied daily with standard pellet diet *ad libitum*. All animal procedures are in accordance with the recommendations of the Canadian Committee for Care and Use of animals (**Canadian council on Animal care [CCAC], 1993**).

### 2.2. Chemicals and drugs

Doxorubicin was purchased from Pharmacia-Italia S.P.A (Italy). Wheat germ oil was purchased from El-Captain Company (CAP PHARMA), 6th October City, Egypt. Phytic acid IP6 (sodium salt from rice) was purchased from Sigma company. Total bilirubin and ALP (alkaline phosphatase) kits were obtained from SCICO Diagnostic Company and ALT (alanine aminotransferase), AST (aspartate aminotransferase) and GGT (gamma-glutamyltransferase) kits were purchased from QuimicaClinicaAplicada S.A. Company (Spain). Albumin and total protein kits were obtained from Diamond Diagnostics, Egypt. Chemicals used in measurement of antioxidants were obtained from Sigma Chemical Company, USA.

### 2.3. Experimental Animal grouping and experimental design:

The animals of the present experiment were allocated into 6 groups:

1-Normal control: The rats of this group were given the equivalent volume of vehicle (0.9% NaCl) for 45 days

3-Wheat germ oil control: The rats of this group were given the equivalent volume of vehicle (wheat germ oil) for 45 days.

4-Phytic acid control: The rats of this group were given the equivalent volume of vehicle (phytic acid) for 45 days; IP6 was freshly prepared in distilled water each time before use, pH adjusted to 7.4.

2-Doxorubicin-administered control: (cumulative dose, 12 mg/kg body weight) was administered to rats in six equal (intra-peritoneal) injections over a period of 6 weeks (**Mohamed et al., 2004**).

5-Doxorubicin-administered group treated with wheat germ oil: This group was intraperitoneally administered doxorubicin at a dose of (cumulative dose, 12 mg/kg body weight) for 6 weeks and was orally treated (by oral gavage) in one daily dose with wheat germ oil at dose level of 1.5 ml / kg.bw /day (1400 mg/kg.bw/day) for a period of 30 days (**Karabacak et al., 2011**).

6-Doxorubicin-administered group treated with phytic acid: This group was intraperitoneally administered doxorubicin at a dose of (cumulative dose, 12 mg/kg body weight) for 6 weeks and was orally treated (by oral gavage) in one daily dose with phytic acid at dose level of (20 mg/kg) for a period of 30 days (**Vucenik and Shamsuddin, 2009**).

### 2.4. Preparation of blood and tissue homogenates

By the end of the experimental periods (6 weeks), rats were sacrificed under mild diethyl ether anesthesia at fasting state. Blood samples were collected and allowed to coagulate at room temperature. The clear, non-haemolysed supernatant sera were quickly removed and divided into four portions for each individual, and stored at  $-20^{\circ}\text{C}$  for subsequent analysis. Liver, kidney, spleen was quickly excised, weighed and homogenized in a saline solution (0.9% NaCl) (10% w/v) using Teflon homogenizer (Glas-Col, Terre Haute, USA). The homogenates were centrifuged at 3000 r.p.m. for 15 minute and the supernatants were kept at  $-20^{\circ}\text{C}$  for the assay of biochemical parameters related to oxidative stress and antioxidant defense system.

### 2.5. Assay of liver function parameters:

ALT and AST activities in serum were determined according to the method of **Reitman and Frankel (1957)** using reagent kits purchased from Quimica Clinica Aplicada S. A. Company (Spain). ALP activity was measured according to the method of **Kind and King (1954)** by using reagent kits obtained from SCICO Diagnostic Company, Egypt. Total

bilirubin concentration in serum was determined in serum according to the method of **Walter and Gerade (1970)** using the reagent kits purchased from SCICO Diagnostic Company, Egypt. Direct Bilirubin concentration was also determined in serum according to the method of **Walter and Gerade (1970)**, using the reagent kits purchased from SCICO Diagnostic Company, Egypt. Albumin concentration was determined in serum according to the method of **Doumas et al. (1971)**, using the reagent kits purchased from Diamond Diagnostics, Egypt. Serum total proteins concentration was determined according to the method of **Henry (1964)**, using reagent kits purchased from Diamond Diagnostics, Egypt.

Urea concentration was determined in serum according to the Urease-modified Berthelot reaction, **Patton and Crouch, (1997)** using the reagent kits purchased from Diamond Diagnostics, Egypt. Creatinine concentration was determined in serum according to the method of **Larsen(1972)** by using reagent kits obtained from Biodiagnostic (Egypt). Uric acid concentration was determined in serum according to the method of **Barham and Trinder (1972)** using the reagent kits purchased from Biodiagnostic Company, Egypt.

## 2.6. Assay of Lipid peroxidation and antioxidant parameters

Tissues oxidative stress and antioxidant defense parameters were estimated using chemicals purchased from Sigma Chemical Company (USA) and using Jenway Spectrophotometer (Germany), Glutathione activity in homogenates was determined according to the chemical method of **Beutler et al. (1963)** with little modification. Lipid peroxidation in homogenates was determined according to the chemical method of **Preuss et al. (1998)**. Peroxidase (POX, EC 1.11.1.7) activity in homogenates was estimated according to the modified chemical method of **Kar and Mishra (1976)**. Superoxide dismutase (SOD EC 1.15.1.1) activity in homogenates was determined according to the chemical method of **Marklund and Marklin (1974)**. Glutathione-S-transferase (GST, EC 2.5.1.18) concentration in homogenates was determined according to the chemical method of **Mannervik and Guthenberg (1981)**. Catalase (CAT, EC 1.11.1.6) activity in homogenates was assayed according to the chemical method of **Cohen et al. (1970)**.

## 2.7. Statistical analysis

The statistical package for the social sciences (SPSS for WINDOWS, version 11.0; SPSS Inc., Chicago) was used for the statistical analyses. Comparative analyses were conducted by using the general linear models procedure (SPSS Inc.). Values of ( $P > 0.05$ ) were considered statistically insignificant, while values of ( $P < 0.05$ ) were considered statistically

significant, values of ( $P < 0.01$ ) were considered statistically highly significant and ( $P < 0.001$ ) were considered statistically very highly significant.

## 3. Results

### 3.1. Biochemical effects

The doxorubicin-administered rats showed a very highly significant increase ( $P < 0.001$ ) in serum ALT, AST, and ALP activities as compared to normal control group. The treatment of doxorubicin-administered rats with wheat germ oil and phytic acid induced a very highly significant decrease of the elevated serum of ALT, AST, and ALP ( $P < 0.001$ ) activities as compared to doxorubicin-administered rats (Table 1).

The doxorubicin-administered rats showed a very highly significant decrease ( $P < 0.001$ ) in serum level of total protein, albumin and globulin levels as compared to normal control group. The treatment of doxorubicin injected rats with wheat germ oil and phytic acid induced a very highly significant increase of the serum total protein, albumin and globulin ( $P < 0.001$ ) level. The treatment of doxorubicin-administered rats with wheat germ oil and phytic acid induced a highly significant increase ( $P < 0.01$ ) of serum total protein, albumin and globulin as compared to doxorubicin-administered rats (Table 2).

The doxorubicin-administered rats showed a very highly significant increase ( $P < 0.001$ ) in serum level of total bilirubin concentration as compared to normal control group. The treatment of doxorubicin-administered rats with wheat germ oil and phytic acid induced a very highly significant decrease of the elevated serum total bilirubin ( $P < 0.001$ ) level as compared to doxorubicin-administered rats (Table 3).

The doxorubicin-administered rats showed a very highly significant decrease ( $P < 0.001$ ) in serum level of urea, creatinine and uric acid 1 as compared to normal control group. The treatment of doxorubicin-administered rats with wheat germ oil and phytic acid induced a highly significant increase ( $P < 0.01$ ) of serum level of urea, creatinine and uric acid as compared to doxorubicin-administered rats (Table 4).

In liver organ, administration of wheat germ oil and phytic acid to normal rats induced a non-significant ( $P > 0.05$ ) change of liver lipid peroxidation products as compared with their corresponding control value. Lipid peroxidation exhibited a very highly significant increase in doxorubicin-injected rats as compared to normal rats; the recorded percentage increase was 89.95. Liver peroxidation was profoundly ( $P < 0.01$ ) improved in doxorubicin-administered rats treated with wheat germ oil and phytic acid recording percentage decreases of -32.621 and -18.545 respectively. The liver antioxidants levels of glutathione, catalase, SOD, peroxidase and glutathione-S-transferase in doxorubicin-administered rats showed a highly significant decrease

( $P < 0.01$ ) recording percentage decreases of -72.97, -75.22, -51.298, -74.72 and -64.27% respectively as compared to normal control group. The treatment of doxorubicin-administered rats with wheat germ oil induced a highly significant increase of the serum glutathione, catalase, SOD, peroxidase and glutathione-S-transferase levels ( $P < 0.01$ ); the recorded percentage increases were 150, 132.14, 61.33, 182.21 and 87.17% respectively as compared to doxorubicin-administered rats. The treatment of doxorubicin-administered rats with wheat germ oil and phytic acid induced a highly

significant increase ( $P < 0.01$ ) in serum glutathione, catalase, SOD, peroxidase and glutathione-S-transferase activities recording percentage increases of 60, 75, 32, 138.04 and 41.77% respectively as compared to doxorubicin-administered rats (Table 5). Same behavior of DXR-induced biochemical alterations and oxidative stress in liver are also recorded in kidney and spleen (Table 6&7 respectively). In general, both of wheat germ oil and phytic acid seemed to be potent and has antioxidant activities against DXR-induced toxicity and oxidative stress in liver, kidney, and spleen.

**Table (1) Effect of wheat germ oil and phytic acid on serum aspartate transaminase (AST) activity, alanine transaminase (ALT) activity, and alkaline phosphatase (ALP) activity (U/L) of rats treated with doxorubicin.**

Groups	Serum AST Activity		Serum ALT Activity		Serum ALP Activity	
	(U/L)	% of change	(U/L)	% of change	(U/L)	% of change
Normal control	33.73 ±0.44	–	14.78 ±0.30	–	33.15 0.36±	–
Wheat germ oil control	34.20 ±0.35	1.4	15.05 ±0.37	1.82	32.98 0.23±	- 0.51
Phytic acid control	33.48 ±0.45	-0.74	14.72 ±0.51	-0.40	33.87 0.55±	2.17
Doxorubicin administered control	90.50 <sup>+++</sup> ±1.18	168.30	45.63 <sup>+++</sup> ±1.34	208.72	57.97 <sup>+++</sup> 1.59±	74.87
Doxorubicin+ Wheat germ oil	51.23 <sup>***</sup> ±1.01	- 43.4	25.55 <sup>***</sup> ±0.27	- 44.00	40.80 <sup>***</sup> 1.18±	-29.61
Doxorubicin+ Phytic acid	65.65 <sup>**</sup> ±1.39	-11.53	36.65 <sup>**</sup> ±2.06	- 19.68	47.23 <sup>***</sup> 0.61±	-18.52

-Values significantly different compared to normal: <sup>+++</sup> $P < 0.001$ .

-Values significantly different compared to intoxicated control: <sup>\*\*</sup> $P < 0.01$ , <sup>\*\*\*</sup> $P < 0.001$ . -Data are expressed as Mean ±S.D.

-The number of animals in each group is six (N=6).

-Percentage changes (%) were calculated by comparing with their corresponding control ones.

**Table (2) Effect of wheat germ oil and phytic acid on serum total protein concentration (TP), Albumin concentration, and Globulin concentration (gm/dl) of rats treated with doxorubicin.**

Groups	Serum Total Protein		Serum Albumin concentration		Serum Globulin concentration	
	(gm/dl)	% of change	(gm/dl)	% of change	(gm/dl)	% of change
Normal control	6.53 ±0.13	–	4.37 ± 0.07	–	2.20 0.08±	–
Wheat germ oil control	6.62 ± 0.12	1.37	4.45 ± 0.10	1.83	2.18 0.07±	- 0.90
Phytic acid control	6.72 ± 0.11	2.90	4.38 ± 0.05	0.22	2.23 ±0.08	1.36
Doxorubicin administered control	3.13 <sup>+++</sup> ±0.10	-52.06	2.40 <sup>+++</sup> ±0.08	-45.08	1.23 <sup>+++</sup> 0.04±	- 44.09
Doxorubicin+ Wheat germ oil	5.38 <sup>***</sup> ±0.09	71.88	3.97 <sup>***</sup> ±0.08	65.41	1.73 <sup>***</sup> 0.06±	40.65
Doxorubicin+ Phytic acid	4.28 <sup>***</sup> ±0.09	36.74	3.05 <sup>***</sup> ±0.12	27.08	1.48 <sup>***</sup> 0.05±	20.32

Values significantly different compared to normal: <sup>+++</sup> $P < 0.001$ . -Values significantly different compared to intoxicated control: <sup>\*\*\*</sup> $P < 0.001$

-Data are expressed as Mean ±S.D. -The number of animals in each group is six (N=6).

--Percentage changes (%) were calculated by comparing control groups with normal and treated groups with their corresponding control ones

**Table (3): Effect of wheat germ oil and phytic acid on plasma total bilirubin, direct bilirubin, and indirect bilirubin ( $\mu\text{mol/L}$ ) of rats treated with doxorubicin.**

Groups	Plasma Total Bilirubin		Plasma Direct Bilirubin		Plasma Indirect Bilirubin	
	$\mu\text{mol/L}$	% of change	$\mu\text{mol/L}$	% of change	$\mu\text{mol/L}$	% of change
Normal control	0.60 $\pm 0.02$	–	0.23 $\pm 0.02$	–	0.37 $\pm 0.01$	–
Wheat germ oil control	0.60 $\pm 0.02$	0	0.24 $\pm 0.01$	4.34	0.37 $\pm 0.01$	0
Phytic acid control	0.61 $\pm 0.00$	1.66	0.23 $\pm 0.00$	0	0.38 $\pm 0.01$	2.70
Doxorubicin administered control	1.73 <sup>+++</sup> $\pm 0.05$	188.33	0.81 <sup>+++</sup> $\pm 0.03$	252.17	0.92 <sup>+++</sup> $\pm 0.02$	148.64
Doxorubicin+ Wheat germ oil	1.19 <sup>***</sup> $\pm 0.03$	-31.21	0.48 <sup>***</sup> $\pm 0.02$	-40.74	0.71 <sup>***</sup> $\pm 0.02$	-22.82
Doxorubicin+ Phytic acid	1.37 <sup>***</sup> $\pm 0.03$	-20.80	0.58 <sup>***</sup> $\pm 0.02$	-28.39	0.79 <sup>***</sup> $\pm 0.01$	-14.13

Values significantly different compared to normal: <sup>+++</sup>P<0.001.

-Values significantly different compared to intoxicated control: <sup>\*\*\*</sup>P<0.001

-Data are expressed as Mean  $\pm$  S.D.

-The number of animals in each group is six (N=6).

--Percentage changes (%) were calculated by comparing control groups with normal and treated groups with their corresponding control ones

**Table (4): Effect of wheat germ oil and phytic acid on serum urea, creatinine and uric acid level (mg/dl) of rats treated with doxorubicin**

Groups	Serum Urea (mg/dl)	% of change	Serum Creatinine (mg/dl)	% of change	Serum Uric Acid (mg/dl)	% of change
Normal control	17.17 $\pm 0.60$	-	0.81 $\pm 0.03$	-	4.74 $\pm 0.34$	-
Wheat germ oil control	18.33 $\pm 0.67$	6.75	0.84 $\pm 0.04$	3.70	4.75 $\pm 0.22$	0.21
Phytic acid control	17.83 $\pm 0.60$	3.84	0.82 $\pm 0.04$	1.23	4.79 $\pm 0.16$	1.05
Doxorubicin administered control	55.50 <sup>+++</sup> $\pm 1.15$	223.23	1.82 <sup>+++</sup> $\pm 0.09$	124.7	13.05 <sup>+++</sup> $\pm 0.56$	175.31
Doxorubicin+ Wheat germ oil	41.83 <sup>***</sup> $\pm 1.35$	-24.63	1.18 <sup>***</sup> $\pm 0.03$	-35.16	8.69 <sup>***</sup> $\pm 0.53$	-33.40
Doxorubicin+ Phytic acid	48.83 <sup>***</sup> $\pm 0.60$	-12.01	1.43 <sup>**</sup> $\pm 0.05$	-21.42	10.94 <sup>**</sup> $\pm 0.40$	-16.16

Values significantly different compared to normal: <sup>+++</sup>P<0.001.

-Values significantly different compared to intoxicated control: <sup>\*\*</sup>P<0.01; <sup>\*\*\*</sup>P<0.001

-Data are expressed as Mean  $\pm$  S.D.

-The number of animals in each group is six (N=6).

--Percentage changes (%) were calculated by comparing control groups with normal and treated groups with their corresponding control ones

**Table (5): Effect of wheat germ oil and phytic acid on hepatic tissue lipid peroxidation and levels of antioxidants of doxorubicin - administrated rats.**

Groups	Lipid peroxidation products		superoxide dismutase activity		Liver glutathione content		Liver glutathione-S-transferase Activity		Catalase Activity		peroxidase Activity	
	nmol/mg tissue	% of change	U/gm tissue.10 <sup>3</sup>	% of change	nmol/100 mg tissue	% of change	Mu/100g/tissue .10 <sup>2</sup>	% of change	K. 10 <sup>3</sup>	% of change	U/gm. tissue	% of change
Normal control	172.67 ±5.94	-	1.54 ±0.08	-	0.037 ±0.004	-	0.851 ±0.033	-	1.13 ±0.18	-	229.55 ±11.11	-
Wheat germ oil control	176.50 ±7.95	2.218	1.47 ±0.07	- 4.545	0.036 ±0.004	- 2.70	0.804 ±0.033	- 5.52	1.05 ±0.14	-7.07	223.21 ±10.71	- 2.761
Phytic acid control	167.67 ±8.91	- 2.895	1.59 ±0.07	3.246	0.034 ±0.003	- 8.10	0.810 ±0.025	- 4.81	1.01 ±0.14	- 10.61	239.28 ±3.34	4.23
Doxorubicin administered control	328.00 <sup>++</sup> ±16.12	89.95	0.75 <sup>+++</sup> ±0.04	- 51.298	0.010 <sup>+++</sup> ±0.001	- 72.97	0.304 <sup>+++</sup> ±0.044	- 64.27	0.28 <sup>+</sup> ±0.05	- 75.22	58.03 <sup>++</sup> ±6.52	- 74.72
Doxorubicin + Wheat germ oil	221.00 <sup>*</sup> ±13.26	- 32.621	1.21 <sup>***</sup> ±0.05	61.333	0.025 <sup>***</sup> ±0.003	150	0.569 <sup>***</sup> ±0.024	87.17	0.65 <sup>*</sup> ±0.04	32.14	163.77 <sup>***</sup> ±2.87	182.21
Doxorubicin + Phytic acid	267.17 <sup>*</sup> ±11.83	- 18.545	0.99 <sup>***</sup> ±0.03	32	0.016 <sup>**</sup> ±0.003	60	0.431 <sup>**</sup> ±0.011	41.77	0.49 <sup>*</sup> ±0.06	75	138.14 <sup>***</sup> ±10.53	138.04

Values significantly different compared to normal: <sup>+++</sup>P<0.001.

-Values significantly different compared to intoxicated control: <sup>\*\*</sup>P<0.01; <sup>\*\*\*</sup>P<0.001 -Data are expressed as Mean ±S.D.

-The number of animals in each group is six (N=6).

--Percentage changes (%) were calculated by comparing control groups with normal and treated groups with their corresponding control ones

**Table (6): Effect of wheat germ oil and phytic acid on kidney tissue lipid peroxidation and levels of antioxidants of doxorubicin - administrated rats.**

Groups	kidney MDA		kidney SOD Activity		kidney GSH Activity		kidney GSH reductase Activity		kidney Catalase Activity		kidney peroxidase Activity	
	nmol/mg tissue	% of change	U/gm tissue. 10 <sup>3</sup>	% of change	(nmol/100mg tissue)	% of change	(Mu/100g/tissue .10 <sup>2</sup> )	% of change	K x 10 <sup>3</sup>	% of change	U/gm. tissue	% of change
Normal control	135.17 ±5.50	-	1.39 ±0.08	-	0.094 ±0.002	-	2.442 ±0.084	-	0.97 ±0.21	-	203.86 ±7.52	-
Wheat germ oil control	141.67 ±5.90	4.808	1.32 ±0.07	- 5.03	0.088 ±0.005	- 6.38	2.291 ±0.100	- 6.2	0.86 ±0.08	- 11.34	194.78 ±8.44	- 4.45
Phytic acid control	143.33 ±4.85	6.036	1.45 ±0.07	4.316	0.089 ±0.003	- 5.31	2.322 ±0.087	- 4.91	0.93 ±0.07	- 4.12	188.84 ±7.40	- 7.36
Doxorubicin-administered control	292.17 <sup>+++</sup> ±11.88	116.15	0.60 <sup>+++</sup> ±0.03	- 56.834	0.033 <sup>+++</sup> ±0.001	- 64.9	1.203 <sup>+++</sup> ±0.094	- 50.73	0.11 <sup>+</sup> ±0.02	- 88.65	112.93 <sup>+++</sup> ±14.64	- 44.60
Doxorubicin + Wheat germ oil	195.67 <sup>***</sup> ±12.92	- 33.02	1.05 <sup>***</sup> ±0.04	75	0.070 <sup>***</sup> ±0.003	112.12	1.614 <sup>***</sup> ±0.062	34.16	0.55 <sup>+</sup> ±0.01	400	162.88 <sup>***</sup> ±6.23	44.23
Doxorubicin + Phytic acid	244.33 <sup>***</sup> ±9.74	- 16.37	0.85 <sup>***</sup> ±0.03	41.666	0.051 <sup>***</sup> ±0.002	54.54	1.940 <sup>***</sup> ±0.022	61.26	0.37 <sup>*</sup> ±0.06	236.36	136.48 <sup>**</sup> ±3.85	20.85

Values significantly different compared to normal: <sup>+++</sup>P<0.001.

-Values significantly different compared to intoxicated control: <sup>\*\*\*</sup>P<0.001

-Data are expressed as Mean ±S.D.

-The number of animals in each group is six (N=6).

--Percentage changes (%) were calculated by comparing control groups with normal and treated groups with their corresponding control ones

**Table (7): Effect of wheat germ oil and phytic acid on kidney tissue lipid peroxidation and levels of antioxidants of doxorubicin - administrated rats.**

Groups	Spleen MDA Activity		spleen SOD Activity		spleen total glutathione content		Spleen reduced glutathione content		Spleen Catalase Activity		Spleen peroxidase Activity	
	nmol/mg tissue	% of change	U/gm tissue.10 <sup>3</sup>	% of change	nmol/100 mg. tissue	% of change	nmol/100 mg. tissue	% of change	K x 10 <sup>3</sup>	% of change	U/gm. tissue	% of change
Normal control	155.50 ±6.61	-	1.64 ±0.07	-	0.042 ±0.003	-	0.042 ±0.003	-	1.02 ±0.16	-	164.14 ±2.18	-
Wheat germ oil control	161.17 ±6.72	3.646	1.56 ±0.07	- 4.87	0.045 ±0.001	7.14	0.045 ±0.001	7.14	0.92 ±0.07	- 9.80	160.90 ±4.33	-1.97
Phytic acid control	164.33 ±10.31	5.678	1.70 ±0.07	3.65	0.042 ±0.001	0	0.042 ±0.001	0	0.97 ±0.17	- 4.90	155.73 ±5.63	- 5.12
Doxorubicin-administered control	318.33 <sup>+</sup> ±13.03	104.71	0.82 <sup>+++</sup> ±0.03	- 50	0.012 <sup>+++</sup> ±0.001	- 71.42	0.012 <sup>+++</sup> ±0.001	- 71.42	0.25 <sup>++</sup> ±0.01	- 75.5	59.36 <sup>++</sup> ±5.71	- 63.83
Doxorubicin + Wheat germ oil	201.50 <sup>+</sup> ±14.42	- 36.70	1.33 <sup>***</sup> ±0.03	62.195	0.029 <sup>***</sup> ±0.001	141.66	0.029 <sup>***</sup> ±0.001	141.66	0.66 <sup>**</sup> ±0.07	164	129.89 <sup>+</sup> ±4.59	118.81
Doxorubicin + Phytic acid	247.67 <sup>+</sup> ±8.20	- 22.19	1.11 <sup>***</sup> ±0.03	35.36	0.023 <sup>***</sup> ±0.001	91.66	0.023 <sup>***</sup> ±0.001	91.66	0.48 <sup>**</sup> ±0.06	92	81.52 <sup>**</sup> ±2.50	37.33 <sup>*</sup>

Values significantly different compared to normal: <sup>+++</sup>P<0.001.

-Values significantly different compared to intoxicated control: <sup>\*\*</sup>P<0.01; <sup>\*\*\*</sup>P<0.001 -Data are expressed as Mean ±S.D.

-The number of animals in each group is six (N=6).

--Percentage changes (%) were calculated by comparing control groups with normal and treated groups with their corresponding control ones

#### 4. Discussion

Doxorubicin, a quinone-containing anthracycline antibiotic, is an important agent against a wide spectrum of human neoplasms. However, its toxicity limits usage in cancer chemotherapy (Singal *et al.*, 1987). It has been shown that free radicals are involved in doxorubicin-induced toxicities (Yagmurca *et al.*, 2004). It has been reported that doxorubicin caused severe damage in some organs like liver, heart and kidneys (Gokcimen *et al.*, 2007). The chemical structure of doxorubicin causes the generation of free radicals and the induction of oxidative stress that correlates with cellular injury (Saad *et al.*, 2001). Doxorubicin causes an imbalance between free oxygen radicals (ROS) and antioxidants. The disturbance in oxidant-antioxidant systems results in tissue injury that is demonstrated with lipid peroxidation and protein oxidation in tissue (Karaman *et al.*, 2006).

The present study revealed that intraperitoneal injection of (cumulative dose, 12 mg/kg body weight) doxorubicin for 6 weeks induced hepatotoxicity manifested biochemically by a significant increase of serum ALT, AST, and ALP activities and bilirubin concentration in addition to a significant decrease in serum total protein, albumin and globulin levels. These

results are in accordance with Injac *et al.* (2008) who attributed the increase in the serum enzyme levels to their increased leakage from damaged and necrotic hepatocytes as a result of toxicity. El-Maraghy *et al.* (2009) attributed alteration in serum protein to changes in protein and free amino acids and their synthesis in the injured liver cells and/ or increased protein degradation. The increase in serum total bilirubin may be owing to blockage of bile ductless as a result of the inflammation and fibrosis in the portal triads and/ or due to regurgitation of conjugated bilirubin from the necrotic hepatocytes to sinusoids (Ahmed, 2001).

The elevated activities of these enzymes seen in the current study run in parallel with those of Sakr and Abo-El-Yazid (2012) who stated that Liver of DXR-treated animals showed biochemical alterations, The liver enzymes, were increased in the sera of treated rats. Ultimately this injury was indicated by elevation of transaminases (ALT, AST) in the sera of these animals.

In the present study, our results elucidated a very highly significant increase in the level of urea, uric acid and creatinine levels in the serum of DXR-intoxicated rats when compared to normal rats. The elevated serum levels of urea and creatinine indicate reduced ability of

the kidney to eliminate toxic metabolic substances. This is in accordance with **Injac et al. (2008)** who investigated that DOX-induced nephrotoxicity causes increased capillary permeability and glomerular atrophy.

Moreover, these results are in agreement with previously published reports **El-Moselhy and El-Sheikh (2014)** who demonstrated that DXR caused deterioration in renal function as it significantly increased blood urea nitrogen (BUN), creatinine, compared to control, with distortion in normal renal histology. Also the elevated levels of these parameters seen in the current study run in parallel with those of **Ayla et al. (2011)**, and **Abou Seif (2012)** showed that DXR caused a marked rise in serum urea, creatinine, sodium and potassium levels. Concomitant to these biochemical changes in kidney were observed in DXR-administered animals.

The previous deleterious biochemical alterations of the present study were associated with a marked elevation of liver, kidney, and spleen lipid peroxidation and a significant decrease of non-enzymatic antioxidant (glutathione) content and enzymatic antioxidants (catalase, superoxide dismutase, peroxidase and glutathione-S-transferase) enzyme activities. These results are in agreement with many other authors (**Abd El-Aziz et al., 2001; Kalender et al., 2005; Yagmurca et al., 2007**) who stated that one of the most prevailing hypothesis of hepatic damage from doxorubicin administration is the ability of the drug to produce reactive oxygen species (ROS) and suppress antioxidant defense mechanism. They also revealed that the increased lipid peroxidation play a critical role in liver injury. **Yeh et al. (2009)** reported that rats administered with DXR showed increase in the level of lipid peroxidation (MDA) and depressed antioxidant enzymes activities (SOD, glutathione peroxidase and glutathione) and elevate apoptotic index. **Mohan et al. (2006)** reported that DXR might cause excessive consumption, reduced production or chemical deactivation of these enzymes. The putative role of oxidative stress in the induction of DOX nephrosis may be supported by the protective effect of the activation of superoxide dismutase on the development of DOX nephropathy (**Okasora et al. 1992**).

DXR-induced renal toxicity is well documented (**Yilmaz et al., 2006; Mohan et al., 2010; Injac et al., 2008**). DXR induced a severe nephrotic syndrome with massive albuminuria, proteinuria, hyperlipidemia, hypo-albuminemia and hypoproteinemia. These changes were associated with a marked decrease in the antioxidant defense of the kidney as manifested by the significant increase in lipid peroxides, NPSH depletion and a significant decrease in CAT activity. The changes reflect many functional alterations such as a drop in glomerular filtration rate, glomerular capillary

damage and tubulotoxicity; thus approved that DXR induced pathogenesis of nephropathy through involvement of free radicals (**Badary et al., 2000**).

The treatment of doxorubicin-administered animals with wheat germ oil and phytic acid successfully improved the elevated serum ALT, AST, ALP and GGT activities and serum bilirubin concentration. The lowered serum total protein, albumin and globulin levels were potentially ameliorated in doxorubicin-administered rats treated with both plant constituents. A markedly decrease in the levels of urea, creatinine, and uric acid as compares with DXR-intoxicated rats had been recorded in the present study. The results of the present study are in agreement with previously published authors **Gokcimen et al. (2007)**, and **Khalifa et al. (2011)** who concluded that vit. E may inhibit general toxic and hepatotoxic effects of doxorubicin. Restoration in the levels of lipid peroxidation after administration of wheat germ oil and phytic acid could be related to its ability to scavenge reactive oxygen species, thus preventing further damage to membrane lipids. Our results are in line with previous studies by **Kalender et al. (2005)** who have shown that vitamin E (a major constituents of wheat germ oil) exhibits excellent antioxidant property.

In many studies, vitamin E neutralizes lipid peroxidation and unsaturated membrane lipids because of its oxygen scavenging effect (**Quiles et al., 2002**). **Kalender et al., (2005)** concluded that vitamin E treatment has protective effect against doxorubicin induced hepatotoxicity.

The previous results of current study are in agreement with previously published reports of (**Zajdel et al., 2013; Verghese et al., 2006; Midorikawa et al., 2001**) who concluded that phytic acid may inhibit the generation of highly reactive species from H<sub>2</sub>O<sub>2</sub> by chelating transition metal ions, resulting in chemoprevention of cancer. Biological activity of phytic acid in vivo in most cases seems to be associated with its antioxidant activity, chelation of Fe (III), and suppression of hydroxyl radical formation (**Zajdel et al., 2013**). phytic acid is an antioxidant acting as a potent inhibitor of iron-catalyzed radical formation by chelating free iron and blocking its coordination sites. Moreover, **Sakac et al. (2010)** observed the inhibition of hydroxyl radical generation over a wide range of phytate/iron ratio (1: 5.5–1: 22) and found that one phytate molecule could bind up to 6 divalent cations.

The improvement of liver, kidney, and spleen integrity and function may be mediated via the antioxidant activity of wheat germ oil and phytic acid. This is confirmed by the current study which revealed a significant decrease of lipid peroxidation and increase in CAT, SOD, peroxidase and glutathione-S-transferase activities and glutathione levels.

## Conclusion

The co-administration of wheat germ oil and phytic acid potentially prevented the deleterious effects of doxorubicin-induced toxicity in liver, kidney, spleen. This improvement effect in organs injury may be mediated *via* enhancement of the antioxidant defense system. However, further clinical studies on human beings are required to assess the efficacy and safety of the wheat germ oil and phytic acid.

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