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Research Article
Ameliorative Effect of *Zygophyllum album* Extract Against Hepatotoxicity Induced by Doxorubicin in Male Mice

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Abstract

Background and Objective: Treatment of tumor with chemotherapeutic drugs is the most widely tool to prevent cancer progression. However, hepatotoxicity is one of the major side effects resulted by using these drugs. So, using natural products isolated from medicinal plants is one of the promising methods to reduce the side effects of the chemotherapy. In Egypt there are several medicinal plants which were already used in therapy of many diseases. Thus, the goal of the current study was to evaluate the protective role of *Zygophyllum album* (*Z. album*) against hepatotoxicity resulted from doxorubicin (DOX) induced genetic and biochemical alterations in liver of male mice. **Materials and Methods:** Male mice (n = 70) were allocated in several groups treated with different doses of *Z. album* extract alone or in combination with DOX. **Results:** The results showed that male mice treated with DOX showed significant increase in the chromosomal aberrations either in the bone marrow or in spermatocyte cells, increase the rate of DNA fragmentation and increase the micronucleus formation as well as increase the MDA activity. In contrast, treatment of DOX-exposed mice with *Z. album* extract decreased these harmful effects. **Conclusion:** The results of this study indicated that the protective action of *Z. album* extract could be attributed to inhibition the ROS generation induced by DOX. This protective action of *Z. album* may be resulted by the presence of the phenolic compounds which have repair actions of the DNA structure in several mammalian cell types.

Key words: Doxorubicin, hepatic toxicity, genetic and biochemical alterations, *Zygophyllum album*, liver protection

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Several drugs are widely used in tumor treatment in which doxorubicin is one of the most drugs used widely because it is fighting different types of tumors such as hematological cancers, sarcomas and carcinomas[1,2]. Despite its efficacy in fighting a variety of tumors it has been reported that doxorubicin has side effects on several organs such as heart, brain, kidney and liver causing high toxicity[3-6].

It has been reported that most of patients having liver cancer and treated with doxorubicin, are suffering from liver injury due to inducing oxidative stress and arresting of cell cycle[7,8]. Although the biological mechanism of these negative actions of doxorubicin on liver cells was not fully understood.

Therefore, reducing the toxicity of doxorubicin through using antioxidant products, chelating agents and cytokines is one of the important roles of drug discovery biologists[9-11]. Additionally, one of the most challenges for the pharmacologists is discovering new products reducing doxorubicin side effects promoting its therapeutic actions.

Treatment of liver tumor is complicating procedure, because the cancer cell is coupled with numerous biological alterations leads to difficult understanding how the drug is acting with the cancer cells[12].Therefore, several biological models such as *in vitro* and *in vivo* models were used to investigate the therapeutic strategy against tumor progression[12]. These models aimed to discover the genetic codes which enhance the biological pathways such as gene regulation, transcription and DNA repair which are responsible for different diseases including cancer[12].

Natural compounds are recently more attractive products in the pharmaceutical engineering because they have very low toxicity producing from its supplementation. Natural products have effective components including flavonoids with very high concentration of antioxidants which have the capability of tumor inhibition[13].

Traditional medicines resulting mostly from plant products have been found to play main function in the management of several diseases[14-16]. Recently, the role of alternate therapeutic regimes has been became very important, because an one plant may has many therapeutic indexes[17] (such as stress, anti-oxidant, anti-diabetic and anti-tumor activity).

Zygophyllum album is one of the important plants which have been found to have expectorant and anti-inflammatory activities[18]. The main components extracted from *Zygophyllum* species are glycosides, quinovic acid and zygophyllin, which have been reported to own anti-pyretic and anti-inflammatory activities.

Therefore, the present study evaluated the Egyptian species of *Zygophyllum album* as anti-hepatotoxicity agent. Thus, anti-genotoxicity effect of *Zygophyllum album* extracts against doxorubicin induced liver toxicity in male mice was investigated.

MATERIALS AND METHODS

Animals: About 70 adult Swiss albino male mice (22-27 g), purchased from the Animal House Colony, Giza, Egypt, were maintained on standard laboratory diet (protein: 16.04%, fat: 3.63%, fiber: 4.1% and metabolic energy: 0.012 MJ) and water *ad libitum* at the Animal House Laboratory, National Research Centre, Dokki, Giza, Egypt. After an acclimation period of 1 week, animals were divided into groups (10 mice/group) and housed individually in filter-top polycarbonate cages, housed in a temperature-controlled (23±1°C) and artificially illuminated (12 h dark/light cycle) room free from any source of chemical contamination. All animals received humane care in compliance with the guidelines of the Animal Care and Use Committee of National Research Centre, Egypt.

Chemicals: Doxorubicin (DOX) was purchased from Sigma-Aldrich (Saint Louis, MO 63103, USA). Kits and reagents for cytogenetic and biochemical analyses were purchased from Invitrogen (Germany).

Plant materials: *Zygophyllum album* samples were collected from South of Sinai, Egypt (March, 2014). This halophyte was identified at desert research center (DRC). A voucher specimen was deposited at the Herbarium of the Plants at the DRC.

Plant extracts preparation: The methods and protocol according to El Hawary *et al.*[19] have been used with minor modification such as fresh whole plants were collected and plant materials were dried at ambient temperature and stored in a dry place prior to use. The plant was washed well with water, dried at room temperature in the dark and then ground in an electric grinder to give a coarse powder.

The powder (150 mL methanol for 50 g powder) was soaked in solvent at room temperature for 24 h. The plant extract was collected drop wise and filtered using whatman No.1 filter paper. The powder (150 mL methanol for 50 g powder) was soaked in methanol at room temperature for 24 h. Plant extract was collected drop wise and filtered using Whatman No.1 filter paper. The residues soaked at 150 mL of methanol for 28 h and filtered it again. Extract was then dried and finally placed in glass vials and stored frozen at -20°C and the residue was re-suspended in dimethyl sulfoxide (DMSO) 1% before testing.

Experimental design: Animals were divided into 7 groups (10 animals each) as follow: Group 1: Control animals treated with physiological saline. Group 2: Animals were treated with DMSO. Group 3: Animals were injected i.p. with doxorubicin (DOX, 10 mg kg^{-1}) twice per week for 1 month. Group 4: Animal were treated with low dose of *Zygophyllum album* extract (100 mg kg^{-1} b.wt.) for 1 month. Group 5: Animals treated with high dose of *Zygophyllum album* extract (300 mg kg^{-1} b.wt.) for 1 month. Group 6: Animals were treated with DOX+low dose of *Zygophyllum album* extract (100 mg kg^{-1} b.wt.) for 1 month. Group 7: Animals were treated with DOX+high dose of *Zygophyllum album* extract (300 mg kg^{-1} b.wt.) for 1 month. The selected doses of doxorubicin and *Zygophyllum album* extract were used according to Mitry and Edwards[20] and El Ghoul *et al.*[21]. Mice were sacrificed and subjected to chromosomal aberration analysis in both somatic (bone marrow cells) and germ cells (spermatocyte cells), DNA fragmentation analysis and micronucleus test. For biochemical analysis, MDA levels were measured as liver function indicators.

Chromosomal aberrations analysis: Mice were sacrificed 24 h after treatment termination for chromosome aberration analysis. Cytogenetic analysis was performed on tibia bone marrow cells according to the recommendations of Adler[22], with slight modifications. Experimental animals were injected (i.p.) with colchicines (4 mg kg^{-1}) 1.5 h before sacrifice. Both tibia were dissected out and cleaned of any adhering muscle. Bone marrow cells were collected from both tibias by flushing in KCl (0.075 M, at 37°C, 5 mL) and incubated at 37°C for 25 min. Materials were centrifuged at 2000 rpm for 10 min, fixed in methanol: Acetic acid (Carnoy's fixative, 3:1 v/v). Centrifugation and fixation (in the cold) were repeated 5 times at least, at an intervals of 20 min. The material was re-suspended in a little volume of fixative, dropped onto chilled slides, flame-dried and stained in 5% Sorenson buffered Giemsa (pH: 6.8). At least 75 good metaphases containing 42 chromosomes were examined per animal to score different types of aberrations.

For spermatocyte cells, chromosomal preparations were made according to the air- drying method Evans *et al.*[23]. Mice were injected (i.p.) with colchicines (0.1%) 2 h before killing by cervical dislocation. The testes were transferred to 2.5 mL of a 2.2% citrate solution in petri dishes and the tunica removed. The contents of the tubules were gently teased out with curved forceps. The cell suspension produced was aspirated well and centrifuged at 1000 rpm for 10 min the supernatant was discarded and the pellet was re-suspended in 2 mL of hypotonic solution (1% sodium citrate) at 37°C. After 12 min, the suspension was centrifuged for 10 min at 1000 rpm. Then the supernatant was removed. The cells were fixed 3 times with cold fixative solution (3:1 of methanol and glacial acetic acid). Slides were stained with Giemsa in phosphate buffer (pH 6.8) for 8 min. Fifty primary spermatocytes/mouse at diakinesis-metaphase I were scored. Abnormalities recorded included univalents (x-y univalent and autosomal univalent), chains, rings, N±1 and polyploidy.

DNA fragmentation: Blood samples from the treated animals were aspirated for DNA extraction. Peripheral blood leukocyte cells were harvested by centrifugation and lysed by 600 mL lysing buffer (50 mM NaCl, 1 mM Na_2 EDTA, 05% SDS, pH 8.3) (Sigma). The cell suspension was shaken gently and kept overnight at 37°C. DNA was extracted using the method of Aljanabi and Martinez[24].

Micronucleus test by acridine orange fluorescent staining: Acridine orange staining of erythrocytes was performed according to the protocol of Ueda *et al.*[25]. To assess this assay, 5 animals from each treatment were sacrificed after exposure period. The bone marrow cells were collected from both femur and resuspended in a small volume of fetal calf serum (FBS, Sigma) on a 0.003% acridine orange-coated glass slide. The slide was then covered with a cover glass to prepare bone marrow specimens. Slides were dried overnight and fixed with methanol for 10 min. Bone marrow specimens were examined in a blinded manner using fluorescence microscopy at 600X or higher magnification with a blue excitation wavelength (e.g,. 488 nm) and yellow to orange barrier filter (e.g., 515 nm long pass). Two slides per animal were observed once by one observer who has sufficient experience of micronucleus test. The number of micronucleated polychromatic erythrocytes (% MnPCEs) was measured at a rate of 3000 polychromatic erythrocytes (PCEs) per animal.

Determination of malondialdehyde (MDA) level: Liver tissues were homogenized and the supernatant was chemically treated and centrifuged at 10000 rpm for 3 min for quantitative measurement of lipid peroxidase malondialdehyde (MDA) according to the method of Ohkawa *et al.*[26].

Statistical analysis: All data were analyzed using the general liner models (GLM) procedure of statistical analysis system (SAS)[27] followed by Scheffé-test to assess significant differences between groups. The values are expressed as mean±SEM. All statements of significant were based on probability of $p<0.05$.

RESULTS

Effect of *Zygophyllum album* extract on the chromosomal aberrations: This study indicated that DOX induced highly significant increase in chromosome aberrations in both bone marrow (Table 1) and spermatocyte (Table 2) cells. The DOX treatment induced high percentage of cells contained chromosome aberrations. The gap and break aberrations were the main types of chromosomal aberrations in bone marrow cells. Since DNA is considered as constant genetic component of every cell in all organs, the decrease of DNA content may be due to the genotoxicity of DOX. On the other hand, X-Y and autosomal univalents are the main types of chromosomal aberrations in spermatocyte cells treated by DOX. In contrary, the treatments with *Zygophyllum album* extract either alone of combined with DOX significantly decreased all types of chromosomal aberrations in both bone marrow and spermatocyte cells.

Effects of *Zygophyllum album* extract on the DNA fragmentation and MDA levels: The effect of *Zygophyllum album* extract and/or DOX on DNA fragmentation and MDA levels in mice were summarized in Table 3. The results revealed that treatment of male mice with DOX increased the DNA fragmentation and MDA levels in peripheral blood leukocyte cells and liver tissues of male mice, respectively.

On the other hand, the rates of DNA fragmentation and MDA levels in male mice treated with different doses of *Zygophyllum album* extract exhibited low levels in which they were relatively similar to that in control group. Moreover, treatment of male mice exposed to DOX combined with *Zygophyllum album* extract decreased significantly the rate of DNA fragmentation and MDA levels compared with those in DOX group.

Effect of *Zygophyllum album* extract on the micronucleus formation in male mice: Effect of *Zygophyllum album* extract on MnPCEs formation in the bone marrow cells of male mice is showed in Fig. 1. The results indicated that following treatment of male mice with different doses of *Zygophyllum album* extract MnPCEs formation was similar with those in control group. Treatment of male mice with Dox exhibited that formation of MnPCEs increased significantly compared with control group. In contrast, exposure of male

Table 1: Mean percentages ±SEM of chromosomal aberration in bone marrow cells of male mice treated with DOX and/or *Zygophyllum album* extract

	Structural aberrations							Numerical variation		
Groups	Gap	Break	Chromatid break	Deletions	Fragments	End-to-end	Total structural aberrations	N-1	N+1	Total
Control	0.60±0.02^{c}	0.40±0.03^{c}	0.40±0.04^{d}	0.20±0.03^{c}	0.60±0.02^{d}	0.40±0.03^{c}	2.60±0.04^{d}	0.40±0.11^{c}	0.40±0.14^{c}	0.80±0.12^{c}
DMSO	1.50±0.04^{c}	0.70±0.02^{c}	0.40±0.03^{d}	0.60±0.03^{c}	0.60±0.02^{d}	0.60±0.24^{c}	3.40±0.04^{d}	0.50±0.17^{c}	0.60±0.13^{c}	1.10±0.11^{c}
LD	1.00±0.12^{c}	0.80±0.15^{c}	1.20±0.14^{c}	0.60±0.12^{c}	0.40±0.11^{d}	0.80±0.14^{c}	4.80±0.24^{d}	0.60±0.12^{c}	0.40±0.11^{c}	1.00±0.14^{c}
HD	1.00±0.11^{c}	1.60±0.14^{bc}	1.60±0.20^{c}	1.60±0.15^{b}	1.20±0.13^{c}	1.40±0.17^{bc}	8.40±0.24^{cd}	0.60±0.13^{c}	1.20±0.11^{b}	1.80±0.15^{bc}
DOX	6.00±0.14^{a}	5.60±0.17^{a}	5.20±0.13^{a}	4.40±0.16^{a}	6.60±0.19^{a}	6.00±0.14^{a}	33.80±0.50^{a}	3.20±0.13^{a}	3.60±0.13^{a}	6.80±0.17^{a}
LD+DOX	3.50±0.14^{b}	2.20±0.17^{b}	3.80±0.13^{ab}	1.80±0.18^{b}	6.50±0.19^{a}	2.00±0.13^{b}	19.8±0.31^{b}	1.40±0.17^{b}	1.80±0.13^{b}	3.20±0.14^{b}
HD+DOX	1.20±0.14^{c}	0.80±0.13^{c}	2.70±0.12^{b}	0.70±0.06^{c}	3.70±0.09^{b}	2.00±0.14^{b}	11.10±0.50^{c}	1.40±0.12^{b}	1.20±0.11^{b}	2.60±0.23^{b}

DOX: Doxorubicin, LD: Low dose of *Zygophyllum album* extract, HD: High dose of *Zygophyllum album* extract. ^{a,b,c}Mean values within columns with unlike superscript letters were significantly different ($p<0.05$, Scheffé-test)

Table 2: Mean parentages of chromosomal aberration in spermatocyte cells (n = 250 per a group) of male mice treated with DOX and/or *Zygophyllum album* extract

	Structural aberrations									Numerical variation				
	Chain		Ring		Autosomal univalent		x-y univalent		Total aberrations	N-1		N+1		Total number variation
Groups	No	%	No	%	No	%	No	%	No	No	%	No	%	No
Control	1	0.30	1	0.30	0	0.00	1	0.30	3	1	0.50	1	0.50	2
DMSO	2	0.40	1	0.20	1	0.20	1	0.20	5	2	0.70	1	0.30	3
LD	1	0.25	1	0.25	1	0.25	1	0.25	4	2	0.50	2	0.50	4
HD	2	0.30	2	0.30	1	1.60	1	1.60	6	2	0.40	3	0.60	5
DOX	16	0.30	11	0.23	9	0.19	12	0.25	48	15	0.52	14	0.48	29
LD+DOX	11	0.34	8	0.25	6	0.19	7	0.22	32	11	0.60	9	0.40	20
HD+DOX	5	0.26	7	0.37	3	0.16	4	0.21	19	6	0.60	5	0.40	11

DOX: Doxorubicin, LD: Low dose of *Zygophyllum album* extract, HD: High dose of *Zygophyllum album* extract

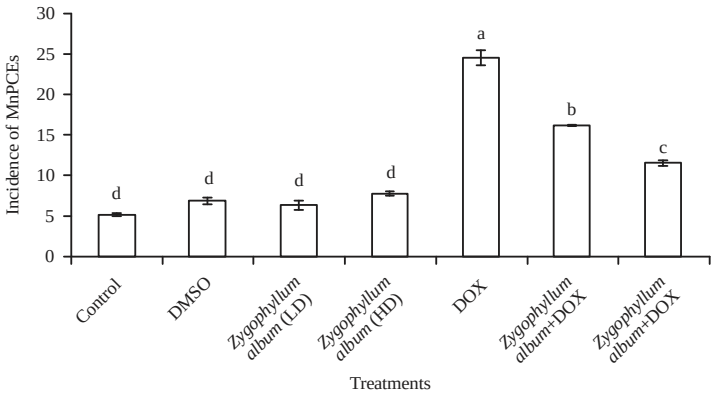

Fig. 1: Micronucleated polychromatic erythrocytes (MnPCEs) of male mice exposed to doxorubicin and/or *Zygophyllum album* extract. [a,b,c]Mean values within tissue with unlike superscript letters were significantly different ($p<0.05$, Scheffé-test)

Table 3: Effect of *Zygophyllum album* extract and/or DOX on DNA fragmentation and MDA levels in mice

Treatments	DNA fragmentation	MDA (nmol MDA g^{-1} wet weight)
Control (-ve)	4.51±0.47[c]	1.30±0.64[d]
DMSO	6.86±0.43[cd]	3.98±0.12[cd]
Z. album (LD)	6.26±0.55[d]	3.22±0.23[cd]
Z. album (HD)	8.11±0.40[d]	4.15±0.75[c]
DOX (+ve)	46.60±1.20[a]	16.53±0.82[a]
Z. album (LD)+DOX	26.84±0.41[b]	9.24±0.92b
Z. album (HD)+DOX	18.70±0.44[c]	6.18±0.13[bc]

MDA: Malondialdehyde, DMSO: Dimethyl sulfoxide, Data are presented as mean±SEM. [a,b,c]Mean values within tissue with unlike superscript letters were significantly different ($p<0.05$)

mice with low and high doses of *Zygophyllum album* extracts decreased significantly the incidence of MnPCEs compared with DOX treated group.

DISCUSSION

The protective effect of *Zygophyllum album* extracts against doxorubicin inducing toxicity (including chromosomal aberration, DNA fragmentation, micronucleus formation and MDA activity alterations) in male mice was studied.

The current study has confirmed that doxorubicin induced several of negative effects including decline in the weight of the body and liver as well as increase in the activity levels of MDA. Moreover, doxorubicin was able to induce chromosome aberrations in both bone marrow and spermatocyte cells, increase the micronucleus formation and DNA fragmentation in liver cells. These results were in same line with Zhao *et al.*[28], that doxorubicin decreased the liver weight and increase the serum AST and ALT as well as increase the inflammation and necrosis in liver cells. In addition, it was previously found that doxorubicin (DOX) is used in the chemotherapy as anticancer drugs and also used in the animal model experiments for various experimental animals as hepatotoxic drug[29,30].

These biological changes in mouse model induced by doxorubicin seem to be as acute liver injury in human cells. Because the increase in the activity levels of AST, ALT and MDA could be attributed to damaged cell membranes of liver cells[31].

The toxicity mechanism of DOX on hepatic cells was reported in several studies[5,32,33]. It has been reported that during DOX pathway some byproducts are resulted in which semiquinone is one of them which produced by several enzymes such as oxido-reductases[32]. DOX-semiquinone radical is suggested to be responsible for hepatotoxicity. DOX semiquinone radical is generating free radicals by interaction with oxygen molecule[33]. So, the free radical species resulting from DOX semiquinone radical are attacking the hepatocytes inducing cell damage and liver failure[5].

The findings of the present study showed that *Zygophyllum album* extract indicated protective impact toward DOX drug induced genetic toxicity. Our results indicated that treatment of male mice with different doses of *Zygophyllum album* extracts decreased significantly the chromosomal aberration, DNA fragmentation and MnPCEs formation as well as MDA levels induced by DOX administration. For our knowledge no data has been published regarding the protective role of *Zygophyllum album* against genetic toxicity. However, other species of album have been investigated such as *Viscum album* (*V. album*). Onay-Ucar *et al.*[34] reported that methanolic extract of *V. album* has been used to protect HeLa cells against DNA damage. In addition, antioxidant activity of *V. album* extract has been investigated[35,36]. They indicated that methanolic extract of *V. album* inhibited the generation of free radicals

species induced by H_2O_2. Moreover, methanolic extract of *V. album* was able to prevent ROS formation in heart and kidney tissues of rats and protecting them from the oxidative stress[37].

The natural bioactive plant compounds have high antioxidant activity. This antioxidant activity is associated with several bioactive compounds such as phenolics which have scavenged free radicals ability. Onay-Ucar *et al.*[34] reported that methanolic extract of *V. album* is rich in the phenolic compound namely quercetin. They also suggested that the high antioxidant activity of methanolic extract of *V. album* is attributed to quercetin content protecting the DNA damage in HeLa cells.

CONCLUSION

The current findings indicated that *Zygophyllum album* extract exhibited protective impact toward DOX drug induced genetic toxicity in hepatic cells of male mice. The protective action of *Zygophyllum album* extract could be attributed to inhibit the ROS generation induced by DOX. This protective action of *Zygophyllum album* may be due to the presence of the phenolic compound (quercetin) which is highly recommended for repairing the DNA damage in several cell types either *in vitro* or *in vivo* studies.

SIGNIFICANCE STATEMENTS

The current study represents a novel plant extract *(Zygophyllum album*) which has the potentiality to prevent the hepatotoxicity induced by doxorubicin in male mice. This study will help the scientists in pharmacy to find out natural products extracted from *Zygophyllum album*. These compounds could be as promising drugs which protect the patients from the side effects induced by using doxorubicin. Therefore, novel active ingredients from *Zygophyllum album* may be considered at new composition of drug discovery.

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