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RESEARCH ARTICLE

Potential Protective Effect of *Atriplex halimus* Extract toward the Doxorubicin - Induced Apoptotic Gene Expression, Genetic and Hepatic Toxicity in Mice

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Abstract

Objective: The anthracycline doxorubicin (DOX) is widely used in chemotherapy as an anticancer drug due to its efficacy in fighting a wide range of cancers such as carcinomas, sarcomas and hematological cancers. However, it can induce injury to non-targeted organs after cancer treatment. Thus, exploration of effective drug targets or active lead compounds against DOX-induced organ damage is necessary. Method: In this study the protective effect of *Atriplex halimus* (Ah) extract towards DOX-induced genetic and hepatic toxicity and apoptosis in male mice was investigated. Animals were divided into 7 groups (10 animals each): 1- Control group treated with physiological saline. 2- Animals treated with DMSO. 3- Low dose of Ah (0.5 mg/kg bw) extracts. 4-high dose of Ah (5mg/kg bw) extracts. 5- Animals were injected i.p with DOX. 6- DOX + low dose of Ah (0.5 mg/kg bw) extracts for 1 week. 7- DOX + high dose of Ah extracts for 1 month. Results: DOX treatment induced a significant increase in the chromosomal aberrations either in the bone marrow or in the spermatocyte cells, increase the DNA fragmentation, malondialdehyde (MDA) level and the expression of apoptosis-related genes, Bax and caspase 3 genes. However, the treatment with Ah declined these negative effects. Conclusion: The findings demonstrated that Ah extract had protected the mice against the negative effects of DOX-induced genetic, hepatic toxicity and apoptosis and could be used as natural antioxidant product for scavenging the reactive oxygen species and to attenuate DOX-induced genetic and hepatic toxicity.

Keywords: *Doxorubicin (DOX), Atriplex halimus (Ah), Apoptosis, Gene expression, Genotoxicity, Hepatic, mice.*

Introduction

Doxorubicin (DOX) is an anthracycline, antibiotic drug effective against human malignancies such as leukemia, lymphoma, and other solid tumors the introduction of antineoplastic antibiotics like DOX to the chemotherapy of malignant neoplasms has been one of the major successes of cancer medicine [1].

Several mechanisms have been proposed for the antitumor effects of DOX, such as DNA synthesis inhibition, DNA binding and alkylation, DNA crosslinking, interference with DNA strand separation, inhibition of topoisomerase II and free radical induction and lipid peroxidation in solid tumors [2]. A significant increase in DNA damage in both liver and kidney as measured by DNA fragmentation was reported in Wistar rats administered an acute dose of DOX (15 mg/kg) compared to control, which suggested that DOX-induced genotoxicity through induction of DNA strand breaks [3], and that frequently leads to genotoxicity in various organs [4] which may be induced primarily via the production of reactive oxygen species (ROS) [5].

DOX was found to induce a high frequency of chromosomal aberrations in bone marrow cells of mice and the chromatid breaks were the most frequent chromosomal aberrations, as well in all types of aberrations in spermatocyte cells, DNA damage, level of lipid peroxidation and apoptosis indicating its genotoxicity [6].

Moreover, DOX-treatment was found to induce a highly significant percentage of chromosomal aberrations, micro nucleated and apoptotic cells in rat liver ($p<0.001$) and the more frequent aberrations were gaps, breaks and /or fragments and deletions [7].

Another study by Hajra et al [8] and Boriollo et al [9]. Showed that DOX-induced toxicities represented in chromosomal aberrations, micronuclei formation, DNA damage, lipid peroxidation and up-regulation of Bax and caspase-3 expression and apoptosis in Swiss albino mice. Genotoxic stress by DOX was found to trigger the activation of caspases 3 and 9, gene expression and apoptosis in human proliferating endothelial cells [10] and in a number of different cell types [11]. The same findings were reported by Song et al [12].

Who concluded that DOX-induced liver damage via oxidative stress, inflammation, and apoptosis in mice. Medicinal plants are rich with many natural compounds which have antioxidant properties such as phytochemicals (which include phenolic acids, flavonoids, and stilbenes/lignans), are promise to prevent or reduce the toxic effects of doxorubicin without decreasing its anticancer effect [13].

Some medicinal plant compounds which contains a bioactive flavonoid, having antioxidant and anti-inflammatory potential to mitigate DOX-induced hepatotoxicity and a significant increase in MDA level, probably through diminishing the oxidative stress and inflammation in male albino Wistar rats Wali et al [14]. *Atriples halimus* L. (Ah) is a medicinal plant and belongs to family Amaranthaceae (formerly Chenopodiaceae), a shrub species, woody stems and triangular leaves. It is growing naturally in Mediterranean region, Sinai (Egypt), Saudi Arabia and East Africa [15] and has many medicinal uses [16] and antioxidant activity [17].

The protective effect of its extract can be attributed to the activity of its antioxidants and the ROS scavenging capacity. The aqueous extract of Ah contains higher levels of total phenolic and condensed tannin [18]. Many researchers reported a significantly high correlation between the high antioxidant activity of plant extracts and its high total phenolic compounds. Further, Ah contain a wide range of bioactive metabolites. It is a source of Vitamins A, C, and D, polyphenols, flavonoids, tannins, alkaloids, saponins, and resins. The hepatoprotective activity of flavonoids is due to their ability to scavenge and reduce cellular free radicals [19, 20]. The combination of Ah and DOX suggested antigenotoxic effects, indicating that Ah reduced genotoxic effects induced by chemotherapeutic agents in mouse bone marrow [9].

Ah was found to improve biochemical parameters (MDA level) and oxidative stress and toxicity induced in rat [21]. The apoptotic process triggered by *A. halimus* involved the down regulation or inactivation of apoptosis-related genes, Bcl-2 and caspase-3 and upregulation or activation of p53 expression [20, 22, 23] supporting its anti-apoptotic effect.

The antioxidative, antimutagenic and hepatoprotective properties of this medicinal plant may be attributed to the presence of important phytochemicals which have been responsible for its broad pharmacological effects including cancer prevention [20] & Chen et al [24]. Therefore, the aim of the present study was to investigate the protective effects of Ah extract against DXO-induced genetic and hepatic toxicity and apoptosis gene expression in mice.

Materials and Methods

Animals

Swiss albino male mice were purchased from the animal colony of the Animal House of the National Research Center, Dokki, Cairo, Egypt and were kept for 1 week for acclimatization and supplied with feed and water *ad libitum*.

Chemicals

Doxorubicin (DOX) was purchased from Sigma-Aldrish (3050 Spruce Street, Saint Louis, MO 63103, USA). Kits and reagents for biochemistry and molecular biology were purchased from Invitrogen (Germany).

Plant Extracts Preparation

Samples of Ah were purchased commercially and then powdered. Extract was obtained from (500 g) via extraction with 4 L of water at room temperature over the course of 3 days. The resulting solution was centrifuged and filtered and the supernatant removed, evaporated, and freeze-dried under a

vacuum. The residue (100 mg) was then dissolved in 1 ml of water. The extracted material was stored at -20º C up to use [25].

Experimental Design

Animals were divided into 7 groups (10 animals each) as follow: 1- Control group treated with physiological saline. 2- Animals treated with DMSO. 3- Low dose of Ah (0.5 mg/kg bw) extract. 4-high dose of Ah (5mg/kg bw) extract. 5- Animals were injected i.p with DOX. 6- DOX + low dose of Ah (0.5 mg/kg bw) extract for 1 week. 7- DOX + high dose of Ah extract for 1 month. Mice were sacrificed and subjected to DNA fragmentation, chromosomal aberration analysis in both somatic (bone marrow cells) and germ cells (spermatocyte cells). For biochemical analysis, MDA level was measured as liver function indicator. As well, gene expression of Bax and caspase 3, as apoptotic related genes was evaluated.

Chromosomal Aberrations Analysis

Mice were sacrificed 24 h after administration of the last treatment for chromosome aberration analysis. Cytogenetic analysis was performed in tibia bone marrow cells according to the method of Adler ID [26], with slight modifications.

Experimental animals were injected (i.p.) with colchicines (4 mg/kg) 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 ºC, 5 mL) and incubated at 37 ºC for 25 min. Material was 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 five times at least at intervals of 20 min.

The material was resuspended 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 42chromosomes 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. [27]. 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 resuspended 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/rat at diakinesis-metaphase I were scored. Abnormalities recorded included univalents (x–y univalent and autosomal univalent), chains, rings, N±1 and polyploidy.

DNA Fragmentation

Peripheral blood leukocytic cells were harvested, followed by centrifugation and lysed by 600 ml lysing buffer (50 mM NaCl, 1mM Na2 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 [28]. The DNA samples were mixed with 6x loading buffer and analyzed on a 2% agarose gel stained with 1 mg/ml ethidium bromide [29].

Determination of malondialdehyde (MDA) level

Liver was 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. [30].

Molecular Genetics Assay

Real-time PCR for Determination of Apoptotic Related Genes

Total RNA was isolated from the liver tissue using TRIzol Reagent (Invitrogen Life Technologies, Carlsbad, CA, USA), following manufacturer's instructions. One microgram total RNA and random primers were used for cDNA synthesis using the RevertAid H minus Reverse Transcriptase (Fermentas, Thermo Fisher Scientific Inc., Canada). For real time PCR analysis, the cDNA samples are run in triplicate and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is used

as reference gene. Each PCR amplification includes non-template control containing all reagents except cDNA. Real time PCR reactions were performed using Power SYBR Green (Life Technologies, CA) and was conducted on the Applied Biosystems 7500 Instrument. The primers used in this study are listed in Table (1). The thermal profile is initial denaturation at 95◦C for 3min, followed by 35 cycles with an annealing temperature at 58°C for GAPDH for 30 seconds, followed by extension at 72°C for 1 minute and a final extension at 72°C for 8 minutes. For Bax gene, 30 cycles of amplification with annealing at 56°C for 30 seconds, followed by extension at 72°C for 1 minute and a final extension at 72 °C for 8 minutes. For Caspase-3 gene, 28 cycles of amplification with annealing for 15 sec at 55 °C, and primer extension for 45 sec at 72°C and a final extension at 72 °C for 8 minutes.. Data from the real-time PCR were analyzed using the $2^{-\Delta\Delta Ct}$ method. Statistical significance was evaluated using the Student's t-test (Microsoft Excel). A P value < 0.05 was considered statistically significant.

Table 1: Sequence of the primers used in the RT-PCR analysis

Gene Name	Sequence (5'-3')	Reference
Bax	F: AGGATGATTGCTGATGTGGATAC R: CACAAAGATGGTCACTGTCTGC	van der Hoeven *et al* [31]
Caspase -3	F: AAATTCAAGGGACGGGTCAT R: ATTGACACAATACACGGGATCTGT	Liu et al. [32]
GADPH	F: CAAGGTCATCCATGACAACTTTG R: GTCCACCACCCTGTTGCTGTAG	Ahmed et al. [33]

Statistical Analysis

All Data are expressed as mean ± SE. Statistical analyses were performed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison of means test. Different small superscript letters indicate significant correlation at *P* value of 0.05 or less.

Results

Effect of *Atriplex halimus* extract on the DNA Fragmentation and MDA Levels in Male Mice

Table (2) shows Effect of Ah extract and /or DOX on DNA fragmentation and MDA levels in mice. The results revealed that treatment of male mice with DOX increased the DNA fragmentation and MDA levels in liver tissues of male mice. However, the rate of DNA fragmentation and MDA levels in male mice treated with different doses of Ah extract were low and relatively similar to that in control group. Additionally, treatment of male mice exposed to DOX with Ah extract decreased significantly the rate of DNA fragmentation and MDA levels compared with those in DOX group.

Table 2: Effect of Ah extract and /or DOX on DNA fragmentation and MDA levels in mice.

Treatment	DNA fragmentation	MDA
Control (-Ve)	5.40 ± 0.24^{a}	2.72 ± 0.36^{a}
DMSO	7.40 ± 0.92^{b}	5.10 ± 0.42^{b}
Ah (LD)	10.20 ± 0.37^{c}	9.25 ± 0.35^{c}
Ah (HD)	12.40 ± 0.50^{d}	11.40 ± 0.24^{d}
DOX (+Ve)	40.40 ± 0.74^{g}	46.20 ± 0.48^{g}
Ah(LD +DOX)	26.70 ± 0.43^{f}	24.70 ± 0.30^{f}
Ah(HD +DOX)	18.90 ± 0.33^{e}	17.80 ± 0.37^{e}

N.B; DOX: Doxorubicin; Ah (LD): low dose of Atriplex halimus extract; Ah (HD): high dose of Atriplex halimus extract. Different small superscript letters are differing significantly. Data are expressed as mean ± SE

Effect of Ah Extract on the Chromosomal Aberrations in Bone Marrow and Spermatocyte Cells

The results revealed that DOX induced highly significant increase in chromosome aberration in both bone marrow (Table 3) and spermatocyte (Table 4) cells. The DOX treatment caused high percentage of cells contained chromosome aberrations. The break aberration is the main type 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 MMC. X-Y and autosomal univalents are the main types of chromosomal aberrations in spermatocyte cells treated by DOX. However, the treatment with Ah extracts plus DOX significantly decreased all types of chromosomal aberrations in both bone marrow and spermatocyte cells.

Table 3: Mean parentages ± SE of chromosomal aberration in male mice bone marrow cells treated with DOX and/or Ah extract

Treatment	Structural aberrations						Total structural aberrations	Numerical variation		Total
	Gap	Break	Chromatid gap	Fragment	Deletions	Endowments		N-1	N+1	
Control	0.60 ± 0.24^{b}	0.20 ± 0.20^{a}	0.40 ± 0.24^{a}	0.60 ± 0.24^{b}	0.40 ± 0.24^{b}	0.20 ± 0.20^{a}	2.20 ± 0.20^{a}	0.20 ± 0.20^{a}	0.40 ± 0.24^{a}	0.60 ± 0.40^{a}
DMSO	0.60 ± 0.24^{b}	0.60 ± 0.24^{f}	1.00 ± 0. 44^{f}	0.20 ± 0.20^{a}	0.80 ± 0.20^{e}	0.60 ± 0.24^{e}	4.60 ± 0.24^{b}	0.40 ± 0.24^{e}	0.60 ± 0.24^{e}	1.00 ± 0.31^{e}
Ah (LD)	1.00 ± 0.00^{d}	0.80 ± 0.20^{f}	1.20 ± 0.37^{f}	1.20 ± 0.20^{d}	0.60 ± 0.24^{e}	0.80 ± 0.20^{e}	6.80 ± 0.37^{e}	1.00 ± 0.31^{e}	1.00 ± 0.31^{e}	2.00 ± 0.00^{e}
Ah (HD)	1.40 ± 0.24^{a}	1.20 ± 0.20^{b}	2.00 ± 0.54^{b}	1.40 ± 0.24^{b}	1.40 ± 0.24^{b}	1.40 ± 0.24^{b}	8.40 ± 0.24^{c}	1.40 ± 0.24^{b}	1.60 ± 0.40^{b}	3.00 ± 0.31^{b}
DOX	4.20 ± 0.37^{a}	5.20 ± 0.73^{c}	3.80 ± 0.58^{c}	4.80 ± 0.48^{b}	4.40 ± 0.40^{b}	4.20 ± 0.20^{b}	26.40 ± 0.60^{d}	2.40 ± 0.87^{b}	2.60 ± 0.40^{b}	5.00 ± 0.70^{b}
Ah(LD +DOX)	3.40 ± 0.24^{c}	4.20 ± 0.37^{e}	3.40 ± 0.24^{e}	2.80 ± 0.20^{e}	2.80 ± 0.20^{d}	3.20 ± 0.20^{d}	17.80 ± 0.37^{g}	1.80 ± 0.37^{d}	2.60 ± 0.40^{b}	4.20 ± 0.37^{d}
Ah(HD +DOX)	2.60 ± 0.50^{b}	3.00 ± 0.31^{d}	2.80 ± 0.37^{d}	2.40 ± 0.24^{c}	2.20 ± 0.20^{c}	2.60 ± 0.24^{c}	14.60 ± 0.24^{e}	1.60 ± 0.40^{c}	1.80 ± 0.37^{c}	3.60 ± 0.50^{c}

N.B; DOX: Doxorubicin; Ah (LD): low dose of *Atriplex halimus* extract; Ah (HD): high dose of *Atriplex halimus* extract.
Different small superscript letters are differing significantly

Table 4: Mean parentages of chromosomal aberration in male spermatocyte cells treated with DOX and/or Ah extract

Treatment	No. of examined cells	Structural aberrations								Total aberration		Numerical variation				Total numerical variation	
		x-y univalent		Autosomal univalent		Chain		Ring				N-1		N+1			
		No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Control	250	1	0.4	1	0.4	0	0.00	1	0.4	3	1.2	0	0.0	1	0.4	1	0.4
DMSO	250	1	0.4	2	0.8	1	0.40	2	0.8	6	2.4	3	1.2	2	0.8	5	2.0
Ah (LD)	250	5	2.0	2	0.8	4	1.6	2	0.8	13	5.2	5	2.0	3	1.2	8	3.2
Ah(HD)	250	4	1.6	6	2.4	4	1.6	2	0.8	16	6.4	7	2.8	4	1.6	11	4.4
DOX	250	15	6.0	14	5.6	13	5.2	12	4.8	54	21.6	15	6.0	14	5.6	29	11.6

Ah(LD +DOX)	250	9	3.2	8	3.2	10	4.2	6	2.4	33	13.2	13	5.2	11	4.4	24	9.6
Ah(HD +DOX)	250	6	2.4	7	2.8	6	2.4	4	1.6	23	9.2	8	3.2	9	3.6	17	6.8

N.B; DOX: Doxorubicin; Ah (LD): low dose of *Atriplex halimus* extract; Ah (HD): high dose of *Atriplex halimus* extract.
Different small superscript letters are differing significantly

Effect of *Atriplex halimus* Extracts on the Gene Expression of Apoptotic Related Genes (Caspase-3 and Bax)

Figure (1) showed up-regulation of *Caspase-3* gene level in liver tissues in DOX- treated group that significantly increased to 3.3 ± 0.125 fold when compared to the control group. The expression of *Caspase-3* gene was down regulated significantly in mice treated with the Ah extract in low concentration to 2.8±0.4 fold and in high concentration to 2.6±0.45 fold as compared to DOX- treated group.

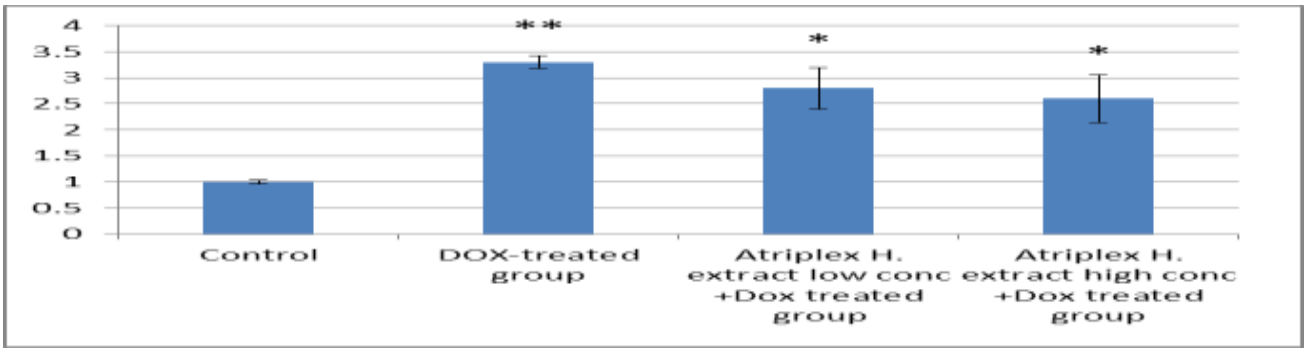

Fig. 1: The expression levels of Caspase-3 gene in liver tissues of male mice treated with DOX and/or Ah extract

DOX treatment significantly increased the hepatic Bax mRNA expression (2.7±0.35) compared with that of the control group. Treatment with Ah extract markedly regulated the expressions of Bax mRNA, which becomes 2.17±0.10 fold with the low concentration and 2.05 ± 0.4 fold in the high concentration (Fig 2).

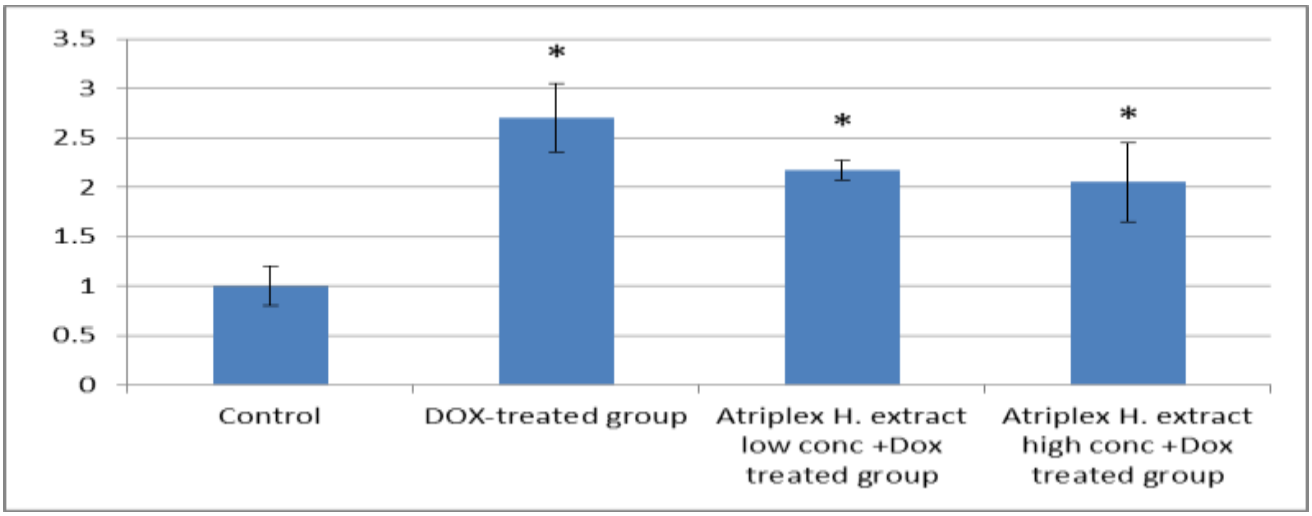

Fig. 2: The expression levels of Bax gene in liver tissues of male mice treated with DOX and/or Ah extract

Discussion

DOX is a chemotherapeutic drug widely used in the treatment of a variety of cancers, including leukemias, sarcomas, and breast cancer [34]. However, DOX can cause a range of significant side effects in normal tissues. Thus, its use is severely limited for its genetic, hepatic toxicity and apoptosis, a frequent side effects produced by DOX, which have been documented in a variety of animal models [35, 36]. The mechanisms responsible for DOX-induced toxicity are complex.

In recent studies, oxidative stress has been considered as one major mechanism of oxidation-induced toxicity. DOX can induce oxidative stress, which is characterized by

ROS accumulation and the decrease of antioxidant defense on oxygen imbalance, culminating in attacking and oxidizing DNA and then inducing cell toxicity and apoptosis [37]. In the present study, the protective effect of Ah extract on the genetic alterations including chromosomal aberration, DNA fragmentation, MDA level and gene expression changes in male mice exposed to the DOX as anti-tumor drug used in the chemotherapy was investigated. Ah extract exhibited protective effect against the DOX drug toxicity. MDA is used as a marker for lipid peroxidation of the cell membrane, which may cause cell damage [38].

The level of MDA was reduced in Ah treated mice demonstrating its protective activity and that in accordance with [14], and Wali et al [20].Who proved that some medicinal plant compounds which contains a bioactive flavonoid, having antioxidant and anti-inflammatory potential to mitigate DOX-induced hepatotoxicity and a significant increase in MDA level.

DNA damage as measured by DNA fragmentations as a genotoxic effect of DOX in our study coincide with those of [3], which may be induced primarily via oxidative stress and the production of reactive oxygen species (ROS) [5]. Song et al [12]. Ah extract may has the ability to protect against DOX-induced lipid peroxidation and cellular DNA damages due to its antioxidative and antimutagenic activity [2, 21]. Genotoxic effect of DOX represented in high frequency of chromosomal aberrations in somatic (specially breaks, fragment and deletion) and germ cells (all types of aberrations) in mice [6, 7].

In the same line with our findings. However, Ah was found to improve biochemical parameters (MDA level) and oxidative stress and genotoxicity induced by DOX [21]. Apoptosis induced by DOX is associated with two distinct pathways: the death receptor pathway and the mitochondrial pathway.

The mitochondrial apoptotic pathway is regulated by various apoptosis-related genes, such as Bax and Caspase-3. Bax is a pro-apoptotic protein residing in the cytosol but translocates to the mitochondria upon the induction of apoptosis [23]. Caspase-3 is an apoptotic protein and is the critical effectors' caspase of the both pathways.

Apoptotic related genes, Bax and Caspase-3 mRNA expression in liver tissues of DOX-treated group was significantly higher (up-regulated) than the control group. However, the pre-administration of Ah (both low and high doses) extract decreased the level of apoptotic genes, Bax and Caspase-3 expression in mice liver cells as compared to the DOX-treated group, specially with caspase 3 gene expression. Ah extract may have the ability to protect against DOX-induced apoptosis through their direct effects on the levels of apoptotic gene expression Bax and Caspase-3.

That in consistent with Youle and Strasser 2008 [20] Al-Senosy et al., 2018 [22] Parvez et al. [23]. Supporting its anti-apoptotic effect. Many previous studies have proved that Ah extract exhibit antigenotoxic and antioxidant activity. That refers to contain certain flavonoids which have protective effect on liver due its antioxidant properties and useful in the treatment of liver damage [39].

Also, Ah was characterized by the presence of the flavonoids, the tannins, the alkaloids and the saponins. This molecule was known to show medicinal activity as well as exhibiting physiological activity. This antioxidant property may explain its action against DOX toxicity.

Conclusion

This study has been conducted to study the protective effect of Ah extract on the genetic and hepatic toxicity as well apoptosis in male mice exposed to DOX as anti-cancer drug used in chemotherapy. The combination of Ah and DOX suggested antigenotoxic effects, indicating that Ah reduced genotoxic and hepatotoxic effects induced by chemotherapeutic agents. That due to they have important antioxidant properties, allowing it to reduce the harmful effects of free radicals that are highly generated during treatment.

Hence, it can be concluded that the aqueous extract of Ah has antioxidant properties. Finally, it has became clear that some medicinal plants and herbs extracts such as Ah extract might had a protective effect when provided in combination with anti-cancer therapeutic DOX and should be considered as a good source for drug discovery [40-54].

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