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Research Article

MYRTUS SPECIES PREVENTS REPRODUCTIVE TOXICITY INDUCED BY DOXORUBICIN IN MALE MICE

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ABSTRACT

Objective: *Myrtus* sp. is one of the natural products being used in Unani system of medicine. Its leaves are frequently used for various ailments like diarrhea, dysentery, vomiting, and osmotic purposes. The main goal of the present work was to investigate the protective effect of *Myrtus* sp. extract against doxorubicin (DOX) induced sperm abnormalities, genetic toxicity, and gene expression alterations.

Methods: Plant samples were used to obtain plant extraction. Eighty male albino mice were allocated in several groups and treated with DOX alone, DOX plus *Myrtus* sp. extracts for 30 days starting from 24 or 48 hrs after DOX treatment or supplemented with *Myrtus* sp. extracts for 30 days then treated with DOX treatment.

Results: The results revealed that treatment of male mice with DOX then *Myrtus* sp. for 1-month was the best treatment strategy for protection against DOX induced toxicity. Whereas, *Myrtus* sp. extract significantly preserved male mice from sperm abnormalities induced by DOX treatment, genetic toxicity, and gene expression alterations.

Conclusion: The results suggested that phenolic compounds exist in the *Myrtus* sp. extract might be contributed to the prevention of the reproductive disorders and genotoxicity.

Keywords: *Myrtus* sp., Doxorubicin, Sperm abnormalities, Genetic toxicity, Reproductive genes.

INTRODUCTION

Stress agents and compounds induced toxicity are compounds causing chemical or physical alterations in many of biomolecules including antioxidant enzymes and DNA structure leading to inaccurate replication and alteration in the gene expression of specific regions of the genome [1]. Approximately, 30% of all marketed drugs exhibit genotoxic effect when tested by the standard genetic toxicology tests [2]. Doxorubicin (DOX) is used in the treatment of several types of human malignancies. However, it has a wide variety of toxic effects, including cardiotoxicity, induction of chromosomal aberrations (CAs), and cytotoxicity [3].

In the last two decades, much emphasis has been placed on natural antioxidants. Therefore, both the naturally occurring nutritive and non/nutritive antioxidants have become a major area of scientific research [4]. The protection provided by fruits and vegetables against mutagenicity and cytotoxicity have been attributed to the various natural antioxidants they contain [5,6]. Among them phenolic substances such as tocopherols (vitamin E and related compounds), various classes of flavonoids, phenolic acid, tannins, lignans, etc., are of special significance [7].

Myrtus sp. is an evergreen shrub belonging to the Myrtaceae family. It grows spontaneously throughout the Mediterranean area and has been used for medicinal, food, and spice purposes since ancient times. The leaves and fruit are traditionally used as antiseptic, disinfectant, and hypoglycemic agents [8]. In folk medicine, the fruit of the plant is used in the treatment of various infectious diseases, including diarrhea, and dysentery, whereas the leaves are used as antiseptic and anti-inflammatory agents, as a mouthwash, for treatments of candidiasis, for healing wounds, as well as in the therapy of urinary diseases [9,10].

Until now several studies have indicated that myrtle herbs could be used as a source of antioxidant and antimutagenic agents [11]. In general, these studies were mainly focused toward the phenolic compounds in myrtle extracts [12]. Nevertheless, no reports on the comparative *in vivo* protection of *Myrtus* sp. on reproductive disorders induced by DOX in male mice have been available up to this moment. In the present study, screening of protective effect of *Myrtus* sp. on the sperm abnormalities, genotoxicity, and gene expression alterations induced by DOX in male mice was investigated.

MATERIA AND METHODS

Drugs and reagents

DOX was purchased from Sigma (St. Louis, MO, USA). Trizol was bought from Invitrogen (Carlsbad, CA, USA). The reverse transcription (RT) and polymerase chain reaction (PCR) kits were obtained from Fermentas (Glen Burnie, MD, USA). SYBR Green Mix was purchased from Stratagene (La Jolla, CA, USA).

Plant collection and extraction

The leaves of *Myrtus* species (*Myrtus communis*) were collected from a local market in Cairo Governorate, Egypt. The plants were identified by Department of Therapeutical Chemistry, Pharmaceutical, and Drug Industries Division, National Research, Center, Giza, Egypt.

A 500 g of the shade-dried powdered leaves of SO were extracted separately with 70% ethanol by maceration and percolation for 24 hrs. The process of extraction was repeated twice. The alcohol extract of each plant was pooled together and evaporated under reduced pressure at 45°C till free from solvent. The alcohol-free residue of each extract was weighted to give 9.66 g of salvia. Preliminary phytochemical tests were carried out to identify the main constituents of each extract [13].

Experimental animals

Eighty adult male albino mice purchased from the Animal House Colony, National Research Centre, 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, Egypt. After an acclimation period of 1-week and at 50 days of age, animals were divided into nine groups (10 mice/ group) and housed individually in filter-top polycarbonate cages housed in a temperature-controlled (23±1°C) and artificially illuminated (12 hrs 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.

Experimental design

Male albino mice (n=80) at 50 days of age purchased from the Animal House Colony, National Research Centre, Egypt were maintained on standard laboratory diet water *ad libitum* at the Animal House Laboratory, National Research Centre, Egypt. The animals were allocated in different groups as follows: First group: Animals treated with saline solution (control); second group: Animals treated with single dose of DOX (Adriamycin, 20 mg/kg bw) and sacrificed after 24 hrs; third group: Animals treated with single dose of DOX (Adriamycin, 20 mg/ kg bw) and sacrificed after 48 hrs; fourth group: Animals treated with 150 mg/ kg of *Myrtus* sp. for 30 days; fifth group: Animals treated with 150 mg/kg of *Myrtus* sp. for 30 days then treated with 20 mg/kg of DOX on the 31 days and sacrificed after 24 hrs from DOX treatment; sixth group: Animals treated with 150 mg/kg of *Myrtus* sp. for 30 days then treated with 20 mg/kg of DOX on the 31 days and sacrificed after 48 hrs from DOX treatment; seventh group: Animals treated DOX then treated after 24 hrs from DOX treatment with 150 mg/ kg of *Myrtus* sp. for 30 days; and eightieth group: ANIMALS treated DOX then treated after 48 hrs from DOX treatment with 150 mg/kg of *Myrtus* sp. for 30 days. Afterwards, the animals were sacrificed and then the blood and several organs and bone marrow cells were extracted from all treatment groups for the biochemical and genetic analyses.

Sperm abnormalities

After 35 days of the last dose (duration of spermatogenesis) [14], half number of the animals of each treated group (five animals per group) were sacrificed by neck vertebra luxation. The epididymides and testes from each rat were removed and weighed. Sperm was collected as quickly as possible when each rat was dissected. To release sperm, the cauda epididymides were cut in a pre-warmed Petri dish containing 1ml of saline solution at 37°C. After mincing with scalpels, the suspension was stirred and dropped on the grease-free clean slide to determine the motility of sperm using a microscope. Spermatozoa were counted using hemocytometer and a drop of a homogenate smeared on a cleaned slide allowed to air dry and stained with approximately 0.05% aqueous eosin Y. The slides were coded and used for the examination of sperm head and tails abnormalities. For each animal, 500 sperm were examined for morphological abnormalities according to the criteria of Jeong *et al*., [15].

DNA fragmentation analysis

Quantitation of DNA fragmentation was determined via the colorimetric diphenylamine assay as described by Gibb *et al*., [16]. Cells in experimental groups were collected after sacrificing the animals. The cells were suspended in 0.8 ml of 0.01 M PBS and centrifuged at 1500 g for 10 minutes to pellet the cells. The cell pellet was resuspended in 0.7 ml of ice-cold lysis buffer (10 mM Tris, 0.2% Triton X-100, and 1 mM EDTA, pH 8.0) and centrifuged for 15 minutes at 13000 g. Perchloric acid (0.5 M) was added to the pellets containing intact DNA and to the supernatants containing fragmented DNA followed by the addition of 2 volumes of a solution containing 0.088 M DPA, 98% V/V glacial acetic acid, 1.5% V/V sulfuric acid, and 0.5% acetaldehyde solution. The samples were kept at 4°C for 48 hrs. The amount of DNA was determined colorimetrically by the absorbance at 600 nm of the supernatant and the pellet. The absorbance of low molecular weight DNA versus total DNA in each sample was expressed as a relative ratio.

Comet assay

Isolated blood cells of all groups of male mice were subjected to the modified single-cell gel electrophoresis or comet assay [17]. To obtain the cells, the pellet of blood cells were washed with an excess of ice-cold Hank's balanced salt solution (HBSS) and minced quickly into approximately 1 mm^3 pieces while immersed in HBSS, with a pair of stainless steel scissors. After several washings with cold phosphate-buffered saline (to remove red blood cells), the blood cells were dispersed into single cells using a pipette. In brief, the protocol for electrophoresis involved embedding of the isolated cells in agarose gel on microscopic slides and lysing them with detergent at high salt concentrations overnight (in the cold). The cells were treated with alkali for 20 min to denature the DNA and subjected to a 30 minutes electrophoresis under alkaline conditions at 300 mA, 25 V. The slides were stained with ethidium bromide and examined using a fluorescence microscope (Olympus BX60 F-3) with a green filter at ×40 magnification lens (NA=1.3). For each experimental condition, about 100 cells per animal were examined to determine the percentage of cells with DNA damage that appear like comets. The non-overlapping cells were randomly selected and were visually assigned a score on an arbitrary scale of 0-3 (i.e., class 0=no detectable DNA damage and no tail; class 1=tail with a length less than the diameter of the nucleus; class 2=tail with length between ×1 and ×2 the nuclear diameter; and class 3=tail longer than 2×the diameter of the nucleus) based on perceived comet tail length migration and relative proportion of DNA in the nucleus [18]). A total damage score for each slide was derived by multiplying the number of cells assigned to each class of damage by the numeric value of the class and summing up the values. Slides were analyzed by one observer to minimize the scoring variability.

Cytogenetic analysis (CAs)

Mice were subjected to cytogenetic analysis from bone marrow cells using the method of Preston *et al*. [19]. Briefly, mice were injected intraperitoneally (i.p.) with colchicines (0.05 mg/kg) for two and a half hours before sacrifice. Animals were sacrificed and femoral bone marrow cells were flushed with isotonic solution (0.9% NaCl). Hypotonic solution (0.56% KCl) was added to the cell pellet and incubated at 37°C for 30 minutes the solution was fixed, slides were prepared, and stained using 10% Giemsa stain. 50 metaphase spreads for animals were analyzed for scoring the different types of CAs. The results were statistically analyzed using Chi-square test.

Furthermore, spermatocyte cells were prepared for meiotic chromosomal analysis according to Brewen and Preston [20].

Expression of aromatase, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) genes

Isolation of total RNA

Total RNA was isolated from testis tissues of male mice by the standard TRIzol® Reagent extraction method (cat#15596-026, Invitrogen, Germany). Briefly, tissue samples were homogenized in 1 ml of TRIzol® Reagent per 50 mg of the tissue. Afterwards, the homogenized sample was incubated for 15 minutes at room temperature. A volume of 0.2 ml of chloroform per 1 ml of TRIzol® Reagent was added. Then the samples were vortexed vigorously for 15 seconds and incubated at room temperature for 3 minutes. The samples were centrifuged for no more than 12,000 × *g* for 15 minutes at 4°C. Following centrifugation, the mixture was separated into lower red, phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. RNA was remained exclusively in the aqueous phase. Therefore, the upper aqueous phase was carefully transferred without disturbing the interphase into a fresh tube. The RNA was precipitated from the aqueous phase by mixing with isopropyl alcohol. A volume of 0.5 ml of isopropyl alcohol was added per 1 ml of TRIzol® Reagent used for the initial homogenization. Afterwards, the samples were incubated at 15-30°C for 10 minutes and centrifuged at not more than 12,000 × *g* for 10 minutes at 4°C. The RNA was precipitated which was often invisible before centrifugation, formed a gel-like pellet on the side and bottom of the tube. The supernatant was removed completely. The RNA pellet was washed once with 1 ml of 75% ethanol. The samples were mixed by vortexing and centrifuged at no more than 7,500 × *g* for 5 minutes at 4°C. The supernatant was removed and RNA pellet was air-dried for 10 minutes. RNA was dissolved in diethyl pyrocarbonate (DEPC)-treated water by passing solution a few times through a pipette tip.

Total RNA was treated with 1 U of RQ1 RNAse-free DNAse (Invitrogen, Germany) to digest DNA residues, resuspended in DEPC-treated water. Purity of total RNA was assessed by the 260/280 nm ratio (between 1.8 and 2.1). In addition, integrity was assured with ethidium bromide stain analysis of 28S and 18S bands by formaldehyde-containing agarose gel electrophoresis. Aliquots were used immediately for RT.

RT reaction

The complete Poly(A)$^+$ RNA isolated from male mice tissues were reverse transcribed into cDNA in a total volume of 20 µl using RevertAidTM First Strand cDNA Synthesis Kit (MBI Fermentas, Germany). An amount of total RNA (5 µg) was used with a reaction mixture, termed as a master mix (MM). The MM was consisted of 50 mM MgCl2, 5 × RT buffer (50 mMKCl; 10 mMTris-HCl; pH 8.3; 10 mM of each dNTP, 50 µM oligo-dT primer, 20 U ribonuclease inhibitor (50 kDa recombinant enzyme to inhibit RNase activity), and 50 U M- MuLV reverse transcriptase. The RT reaction was carried out at 25°C for 10 minutes, followed by 1 hr at 42°C, and the reaction was stopped by heating for 5 minutes at 99°C. Afterwards, the reaction tubes containing RT preparations were flash cooled in an ice chamber until being used for DNA amplification through quantitative real-time polymerase chain reaction (qRT-PCR).

qRT-PCR

PCR reactions were set up in 25 µL reaction mixtures containing 12.5 µL 1× SYBR® Premix Ex TaqTM (TaKaRa, Biotech. Co. Ltd., Germany), 0.5 µL 0.2 µM sense primers, 0.5 µL 0.2 µM antisense primer, 6.5 µL distilled water, and 5 µL of cDNA template. The reaction program was allocated to 3 steps. First step was at 95.0°C for 3 minutes. The second step consisted of 40 cycles in which each cycle divided into 3 steps: (a) at 95.0°C for 15 seconds; (b) at 55.0°C for 30 seconds; and (c) at 72.0°C for 30 seconds. The third step consisted of 71 cycles which started at 60.0°C and then increased about 0.5°C every 10 seconds up to 95.0°C. At the end of each qRT-PCR, a melting curve analysis was performed at 95.0°C to check the quality of the used primers. Each experiment included a distilled water control.

The quantitative values of RT-PCR (qRT-PCR) of aromatase (Cyp19-F: 5′-ATA CCA GGT CCT GGC TAC TG-3′, Cyp19-R: 5′-TTG TTG TTA AAT ATG ATG CC-3′ [21]), LH (LH -F: 5′-TCT CAC CACCAC CGT CTG TA-3′, LH-R: 5′-TGC AGT CGC TGT AGT CCA TC-3′ [21]), and FSH (FSH-F: 5′-GGG CCA GGA ACT GTG AAA TA-3′, FSH-R: 5′-TCT CAG AAC TGC CGA GGT TT-3′[21]) genes were normalized on the bases of ß-actin (ß-actin-F: 5′-TTG CCG ACA GGA TGC AGA A-3′, ß-actin-R: 5′-GCC GAT CCA CAC GGA GTA CT-3′[21]) expression.

At the end of each qRT-PCR, a melting curve analysis was performed at 95.0°C to check the quality of the used primers.

Calculation of gene expression

First, the amplification efficiency (Ef) was calculated from the slope of the standard curve using the following formulae [22]:

$Ef=10^{-1/slope}$

Efficiency (%) = (Ef−1)×100

The relative quantification of the target to the reference was determined by using the

ΔCT method if E for the target (Cyp19, LH, and FSH) and the reference primers (β-Actin) are the same [22]:

$$Ratio_{(reference/target\ gene)}=Ef^{CT(reference)-CT(target)}$$

Statistical analysis

All data were analyzed using the general liner models procedure of statistical analysis system followed by Scheffé-test to assess significant differences between groups. The values are expressed as mean ± standard error mean. All statements of significance were based on the probability of $p<0.05$.

RESULTS

Sperm abnormalities assessment

The mean values of different types of the abnormalities of sperm morphology were shown in Table 1. The most frequently observed abnormality was a head without hock and amorphous sperm.

The results showed that the frequencies of the morphologically abnormal sperm were significantly increased in male mice treated with DOX at both time intervals (24 and 48 hrs) compared with those in control and *Myrtus* sp. treated mice (Table 1). *Myrtus* sp. treatment did not increase the frequencies of the morphologically abnormal sperm compared with DOX drug groups after 24 or 48 hrs (Table 1). However, the frequencies of the morphologically abnormal sperm were decreased significantly in the groups of mice treated with DOX at 24 and 48 hrs then *Myrtus* sp. for 1-month compared to mice treated with DOX alone (Table 1).

DNA fragmentation

Tables 2 and 3 show the effect the protective action of *Myrtus* sp. against DOX induced toxicity on DNA fragmentation and DNA damage in male mice. The DNA fragmentation and damage levels were significantly higher in mice treated with DOX after both time intervals (24 and 48 hrs) than those in control and *Myrtus* sp. treated mice.

In contrast, the DNA fragmentation and damage levels were decreased significantly in the groups of mice treated with DOX at 24 and 48 hrs then *Myrtus* sp., as well as in groups of *Myrtus* sp. then DOX at 24 and 48 hrs compared to mice treated with DOX alone.

CAs

Cytogenetic analysis in the present study showed that there were structural CAs in bone marrow and spermatocytes (Tables 4 and 5). Structural aberrations consisted of gaps, breaks, deletions, fragments, and centromeric attenuations. The results shown in Tables 4 revealed that there was a significant difference between DOX at 24 and 48 hrs treated mice and the control group. Addition of *Myrtus* sp. after DOX at 24 and 48h resulted in a significant decrease in all types of CAs compared to DOX treated group.

CAs in spermatocytes treated for 24 and 48h of DOX (Table 5) consisted of chain, autosomal, and X-y univalent. The present results showed that DOX treated group had more frequencies of CAs than the control group and *Myrtus* sp. group. Addition of *Myrtus* sp. to DOX treated group decreased the frequency of such aberration in the two periods of treatment.

Expression of LH, FSH, and CYP19 genes

Effect of *Myrtus* sp. and DOX on the expression changes of LH, FSH, and CYP19 genes in testis tissues of male mice is summarized in Figs. 1-3. The present results revealed a significant decrease of gene expression levels of LH, FSH, and CYP19 genes in testis tissues of male mice treated with DOX at both time intervals (24 and 48 hrs) compared with those of the control group and *Myrtus* sp. treated mice. In contrary, the expression levels of LH, FSH, and CYP19 genes in male mice exposed DOX at 24 and 48 hrs then *M. communis* for 1-month were significantly higher than those found in male mice treated with DOX alone (Figs. 1-3).

DISCUSSION

Evaluation of the protective effect of *Myrtus* sp. against DOX inducing reproductive toxicity in male mice was assessed.

The present results demonstrated that DOX increased significantly the sperm abnormalities in male mice. The action could be attributed to the lack of the DNA synthesis. When DNA and RNA syntheses are compared in different experimental conditions, DOX was shown to have varying inhibitory effects. In L 1210 cells, DOX inhibited DNA and RNA syntheses to the same extent [23], while DNA synthesis of the HeLa cells was inhibited more ([24]).

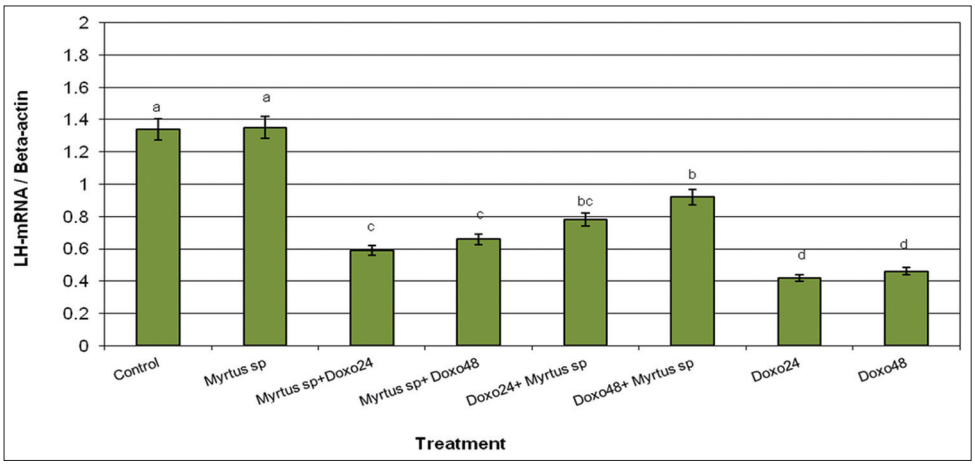

Fig. 1: Expression of luteinizing hormone mRNA in testis tissues of male mice treated with doxorubicin and/or Myrtus sp. analyzed by quantitative real-time polymerase chain reaction. [a,b]Mean with different letters, within the tissue, differ significantly (p≤0.05)

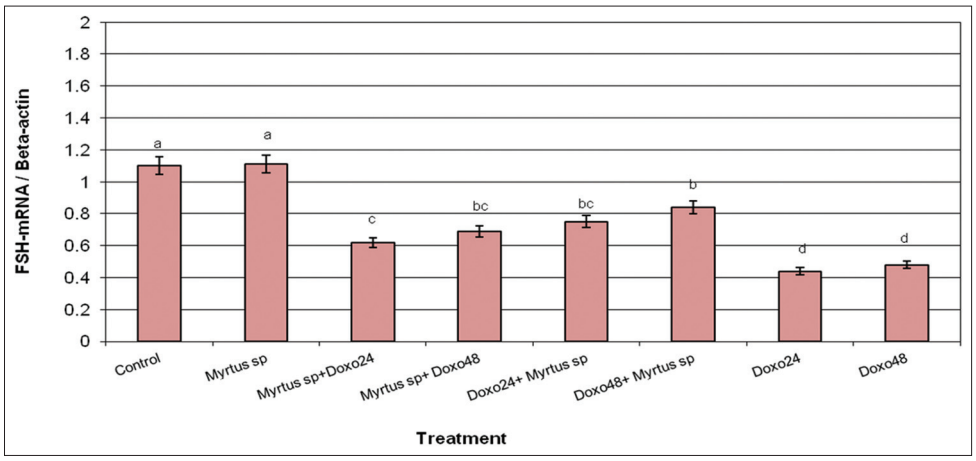

Fig. 2: Expression of follicle-stimulating hormone mRNA in testis tissues of male mice treated with doxorubicin and/or Myrtus sp. analyzed by quantitative real-time polymerase chain reaction. [a,b]Mean with different letters, within tissue, differ significantly (p≤0.05)

Table 1: Sperm abnormalities in male mice after treatment with DOX and/or *Myrtus* sp.

Group	Types of sperm head abnormalities					Total abnormal heads	Total abnormal tails	Total abnormal sperms (head+tail)
	Amorphous	Without hook	Sperm with two heads	Big shape	Small shape			
Control	4.5±0.6[a]	0.5±0.3[a]	0.0	0.0	0.0	5.0±0.8[a]	3.0±0.8[a]	8.0±0.7[a]
Myrtus sp.	5.0±0.7[a]	0.75±0.3[ab]	0.0	0.0	0.0	5.75±0.9[a]	3. 5±0.6[a]	9.25±0.5[a]
Myrtus sp.+Doxo24	12.0±0.9[c]	2. 5±0.6[cd]	0.0	0.0	0.75±0.5[a]	15.25±1.0[d]	4.5±0.6[a]	19.75±0.8[d]
Myrtus sp.+Doxo48	10.5±0.9[bc]	2.25±0.3[cd]	0.0	0.0	0.25±0.3[a]	13.0±0.9[cd]	4.25±0.6[a]	17.25±0.5[c]
Doxo24+*Myrtus* sp.	9.5±1.0[b]	2. 0±0.4[bc]	0.0	0.0	0.25±0.3[a]	11.75±1.0[c]	4.0±0.7[a]	15.75±0.9[c]
Doxo48+*Myrtus* sp.	6.5±0.6[a]	1.75±0.3[abc]	0.25±0.3[ab]	0.25±0.3[ab]	0.0	8.75±0.8[b]	3.75±0.5[a]	12.5±0.9[b]
Doxo24	21.5±0.6[e]	3.5±0.6[d]	0.5±0.3[b]	0.0	0.0	25.5±0.5[f]	6.5±0.4[b]	32.0±0.7[f]
Doxo48	16.5±0.3[d]	3.0±0.4[cd]	0.0	0.5±0.3[b]	0.75±0.5[a]	20.75±0.3[e]	4.5±0 0.5[a]	25.25±0.5[e]

Data were expressed as mean±SE. Means with different superscript letters (a, b, c, d, e) are significantly different (p<0.05), DOXO: Doxorubicin

Moreover, the current study revealed that DOX increased DNA damage, CAs, and alterations in the gene expression of reproductive genes (LH, FSH, and CYP19) in tissues of male mice.

In agreement with our observations, Burke *et al.* [25] reported that the gene expression alterations may attribute to Ca2+ ATPase pathway. The mice over-expressing sarcoplasmic reticulum Ca2+ ATPase gene, which is one of the targets of DOX exposure have provided important insights into the mechanistic basis of DOX cardiotoxicity.

In the last two decades, much emphasis has been placed on natural antioxidants. Therefore, both the naturally occurring nutritive and non/nutritive antioxidants have become a major area of scientific research [26]. The protection provided by fruits and vegetables against mutagenicity and cytotoxicity has been attributed to the various natural antioxidants they contain [27]. Among them, phenolic substances such as tocopherols (vitamin E and related compounds), various classes of flavonoids, phenolic acid, tannins, lignans, *etc.*, are of special significance [28].

We have evaluated the *Myrtus* sp. extract as protective agent against toxicity of the DOX drug. The present study found that *Myrtus* sp. extract recovered the levels the abnormalities in sperm, DNA damage and alterations in gene expression.

In the agreement**,** Sumbul *et al*. [29] reported that the antioxidant activities of the fruit extracts of *Myrtus* sp. were determined by using 2,2-diphenyl-1-picrylhydrazyl and b-carotene-linoleic acid assays. The methanol extracts of *M. communis* fruits exhibited a high level of free radical scavenging activity. Flavonoids and anthocyanins in *Myrtus* sp. extract were checked for antioxidant activity by TEAC assay and the free radical activity. The myrtle extract showed interesting free radical scavenging activity [30].

Moreover, several studies indicated anti-mutagenic effects of several extracts of *M. communis*. Antimutagenic activity of the essential oil of myrtle was studied by collecting the plant samples from the two distant localities and they were assayed against spontaneous and t-BOOH-induced mutagenesis in *Escherichia coli* oxyR mutant IC202, a bacterial strain deficient in removing radical oxygen species. When the oxidative mutagen was used, essential oil expressed higher reduction of mutagenesis in a concentration-dependent manner [31].

Furthermore, the current results revealed that *Myrtus* sp. extract increased the expression of reproductive genes. Only one study investigated the effect of *Myrtus* sp. extract on the gene expression. Hayder *et al*. [32] investigated the *in vitro* antioxidant and antigenotoxic potentials of myricetin-3-O-galactoside and myricetin-3-O-rhamnoside from *M. communis* regarding the modulation of expression of genes involved in the cell defense system using cDNA microarray. They found that both the compounds induced

Table 2: DNA fragmentation in mice after treatment with DOX and/or *Myrtus* sp.

Groups	DNA fragmentation	
	Rang	M+SE
Control	7.44-8.64	8.04±0.23^{a}
Myrtus sp.	10.2-11.4	10.78±0.34^{ab}
Myrtus sp.+Doxo24	22.34-26.46	24.38±1.18^{e}
Myrtus sp.+Doxo48	18.0-20.93	20. 09±1.09^{d}
Doxo24+*Myrtus* sp.	14.7-16.0	15.46+0.39^{c}
Doxo48+*Myrtus* sp.	11.0-13.25	12. 05±0.65^{b}
Doxo24	36.28-41.2	38.92+1.3^{g}
Doxo48	32.86-36.34	34.16+1.1^{f}

Means with different superscript letters (a, b, c, d, e, f, g) are significantly different (p<0.05), DOXO: Doxorubicin

Table 3: DNA damage using comet assay in male mice after treatment with DOX and/or *Myrtus* sp.

Treatments	Number of animal	Number of cells		Class				DNA damaged cells (%)
		Analyzed	Comet	0	1	2	3	
Control	5	100	4	96	4	0	0	4
Myrtus sp.	5	100	5	95	4	1	0	5
Myrtus sp.+Doxo24	5	100	43	57	16	14	13	43
Myrtus sp.+Doxo48	5	100	32	68	12	10	10	32
Doxo24+*Myrtus* sp.	5	100	24	76	9	8	7	24
Doxo48+*Myrtus* sp.	5	100	17	83	7	6	4	17
Doxo24	5	100	89	11	30	29	30	89
Doxo48	5	100	74	26	26	25	23	74

DOXO: Doxorubicin

Table 4: CAs in bone marrow cells of male mice after treatment with DOX and/or *Myrtus* sp.

Treatments	Number of animals	Structural CAs					Total CAs
		Gap	Break	Deletions	Fragment	Centric attenuation	
Control	10	0.20±0.20	0.0±0.0	0.0±0.0	0.0±0.0	0.40±0.24	0.60±0.40^{a}
Myrtus sp.	10	0.40±0.24	0.40±0.24	0.20±0.20	0.40±0.24	0.60±0.24	2.0±0.63^{b}
Myrtus sp.+Doxo24	10	1.40±0.40	1.60±0.40	2.60±0.24	2.60±0.24	4.20±0.58	12.40±1.16^{e}
Myrtus sp.+Doxo48	10	1.60±0.45	1.60±0.40	1.60±0.40	1.60±0.24	2.80±0.20	9.20±1.01^{d}
Doxo24+*Myrtus* sp.	10	0.80±0.20	0.60±0.24	1.20±0.37	1.40±0.24	2.60±0.24	5.80±1.01^{c}
Doxo48+*Myrtus* sp.	10	0.40±0.24	0.40±0.24	0.40±0.54	0.60±0.24	1.40±0.24	3.20±0.73^{b}
Doxo24	10	6.0±0.44	7.20±0.37	8.20±0.80	8.20±0.37	9.20±0.37	39.20±2.13^{g}
Doxo48	10	3.80±0.20	4.60±0.24	4.80±0.37	4.00±0.54	4.60±0.50	21.60±1.28^{f}

CAs: Chromosomal aberrations, DOXO: Doxorubicin. Means with different superscript letters (a, b, c, d, e, f, g) are significantly different (p<0.05)

Table 5: CAs in spermatocyte cells of male mice after treatment with DOX and/or *Myrtus* sp.

Treatments	Number of animals	Structural CAs			Total CAs
		Chain	Autosomal	x-y univalent	
Control	10	0.20±0.20	0.40±0.24	0.40±0.24	1.0±1.0^{e}
Myrtus sp.	10	0.40±0.24	0.60±0.24	1.20±0.37	2.20±0.80^{ef}
Myrtus sp.+Doxo24	10	2.60±0.24	3. 40±0.24	6.00±0.70	12.00±1.1^{bc}
Myrtus sp.+Doxo48	10	1.60±0.24	2.00±0.31	4.20±0.58	8.00±1.0^{c}
Doxo24+*Myrtus* sp.	10	1.60±0.24	2.20±0.37	3.40±0.50	7.20±1.06^{d}
Doxo48+*Myrtus* sp.	10	1. 0±0.0	1.40±0.24	2.00±0.44	4.40±0.67^{e}
Doxo24	10	6.20±0.58	6.40±0.50	9.60±0.50	22.20±1.28^{a}
Doxo48	10	4.80±0.37	4.60±0. 60	6.00±0.54	15. 20±1.5^{b}

CAs: Chromosomal aberrations, DOXO: Doxorubicin. Means with different superscript letters (a, b, c, d, e, f, g) are significantly different (p<0.05)

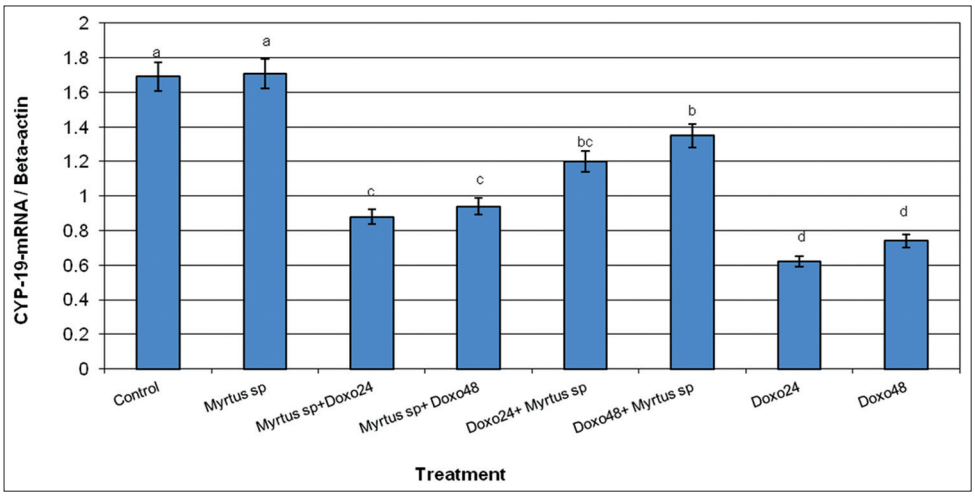

Fig. 3: Expression of CYP19 mRNA in testis tissues of male mice treated with doxorubicin and/or Myrtus sp. analyzed by quantitative real-time polymerase chain reaction. [a,b]Mean with different letters, within the tissue, differ significantly (p≤0.05)

an inhibitory activity against nifuroxazide, aflatoxin-B1, and H_2O_2 induced mutagenicity, which modulated the expression patterns of cellular genes involved in oxidative stress, in DNA damaging repair and in apoptosis.

CONCLUSION

The protective effect of *Myrtus* sp. extract against DOX induced reproductive toxicity was examined. In light of the results obtained, we can conclude that myrtle extract showed considerable anti-genotoxic potential, which was probably due to the antioxidant activity of its components.

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