Urtica dioica attenuate effect of Doxorobicin-Induced changes on sperm parameters in the mice

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ABSTRACT

Doxorubicin (DXR) is used as an antitumor agent for the treatment of human neoplasm. The use of DXR has adverse effect on reproductive system including testicular toxicity and alteration in semen quality. The aim of this study was to evaluate the protective effects of Urtica dioica *against Doxorobicin-Induced changes on sperm parameters. 24 male mice were randomly divided into 4 groups. Control group received normal saline solution throughout the course of the study.* Urtica dioica (UD) *control group, received UD (100 mg/kg body weight) thrice in a week and DOX (3 mg/kg body weight) once in a week injected intraperitoneally in Doxorubicin (DXR) control group and Urtica dioica- Doxorubicin (UD-DXR) group, received Urtica dioica (100 mg/kg body weight) three times in a week and DOX (3 mg/kg body weight) once in a week through the route for a period of 2 weeks. At the end of experimental period, all animal were sacrificed by cervical dislocation, their epididymes were removed and sperm analysis were done. In mice with DXR administration, epididymal sperm motility, progressive motility, sperm count and viability significantly decrease while sperm cells with abnormal morphology significantly increase when compared with control groups. Co-treatment with UD attenuate toxicity effect of DXR and improve sperm parameters. Results of our study showed that UD diminished DXR-induced testicular toxicity and improve semen parameters, thus suggesting its co-administration as a protective agent during doxorubicin treatment. Further studies should be aimed to determine protective effect of UD against chemotherapeutic agents such as DXR.*

Keywords: Doxorubicin; *Urtica dioica*; antioxidant; semen quality

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INTRODUCTION

Doxorubicin (DXR) (also known as adriamycin), an athracyclin antibiotic, is used as an antitumor agent for the treatment of human neoplasm. Doxorubicin first isolated from cultures of a mutant *Streptomyces peucetius* in the

early 1960s. In clinic the use of DXR as a chemotherapy agent due to its diverse toxicities including cardiotoxicity [1-3], liver toxicity [4], renal toxicity [5] and testicular toxicity [6] is often limited. Adverse effects of DXR on the reproductive system of male may manifest as alteration in semen quality [7, 8], impede spermatogenesis [9] and testicular failure [10] that eventually lead to infertility. The underlying mechanisms of DXR testicular toxicity are not fully understood, though several mechanism including oxidative stress (generation of reactive oxygen species (ROS)) [11], lipid peroxidation [12], cellular apoptosis [9] and breakage of DNA continuity [13] are the most studied candidates.

Furthermore, mammalian sperm cells are rich in polyunsaturated fatty acids (PUFAs) and have low antioxidant capacity that rendering them highly vulnerable to oxidative damage and lipid peroxidation [14-16]. Based on this concept, effect of *Urtica dioica*, as an antioxidant agent to counteract Dox-induced testicular damage have been investigated.

Urtica dioica (UD) (also known as common nettle or stinging nettle), an herbaceous perennial plant, belongs to the family Urticaceae. In folk medicine *Urtica dioica* seeds used for treatment of cancer [17, 18], to treat urinary tract disorder, setting the operating cycle, as well as an anti-inflammatory agent [19]. *Urtica dioica* contains both fat-soluble vitamins (A and D) and also water-soluble vitamins (such as vitamin C and B), minerals (iron, manganese, potassium, and calcium) and proteins [20, 21]. Another assets of the UD are , salicylic acid, lecithin, sterols, thymol, chlorophyll, carotenoids, flavonoids and anti-oxidants [20, 22-25] that promote cleansing, detoxification, anti-inflammatory and antioxidant capacity [26]. Several studies report testicular-protective effect of UD in various condition such as Ischaemia and reperfusion (I/R) resulting from testicular torsion–detorsion [17] and nicotine-induced testicular damage [22], although we have not find any studies investigating effect of UD's in Doxorobicin-Induced changes on sperm parameters. Therefore, the objectives of the present study was to evaluate the protective effects of *Urtica dioica* against Doxorobicin-Induced changes on sperm parameters such as motility, count, viability and morphology in the mice.

MATERIALS AND METHODS

Preparation of extract of U. dioica
U. dioica leaves dried, powdered and percolated by Ethanol (45%).

Animal model and experimental protocol
24 male mice of 6-8 weeks of age were selected from animal house. Animals were housed in polypropylene cages [6 animals/cage]. Cages were maintained in a well ventilated room under standard conditions (25±2°C, 55-60RH, 12:12h light: dark cycle).

The animals were randomly divided into 4 groups with 6 animals per group and were given respective treatment:

Group I served as control (Cont) group received normal saline solution throughout the course of the study.

Group II served as *Urtica dioica* (UD) control group, received *UD* (100 mg/kg body weight) thrice in a week for a period of 2 weeks.

Group III served as Doxorubicin (DXR) control group, received DOX (3 mg/kg body weight) once in a week through the intraperitoneal route for a period of 2 weeks.

Group IV served as *Urtica dioica*- Doxorubicin (UD-DXR) group, received *Urtica dioica* (100 mg/kg body weight) thrice in a week and DOX (3 mg/kg body weight) once in a week through intraperitoneal route for a period of 2 weeks

Sperm analyses
At the end of the experimental period, the animals were sacrificed by cervical dislocation and their epididymes were removed. Epididymal sperm were collected by slicing the caudal epididymis in a petri dish containing 1 ml pre-warmed HTF + 10mg/ml BSA, and incubating for 10 minutes in a CO_2 incubator (5% CO_2 in air at 37°C), to allow sperm swim out into the medium. The diluted sperm suspension were used for the following analysis.

Sperm motility
Total sperm motility and progressive motility were evaluated using a light microscope. For this process, a drop of resulting sperm suspension was placed on a slide and then covered with a cover-slip. The percentage of sperm motility and progressive motility were evaluated under an optical microscope (400× magnification). For each

sample, approximately 200 sperm cells were examined and according to WHO protocol 2010 [27] the sperm cells were classified as progressive, non-progressive and immotile.

Epididymal sperm count
Epididymal sperm count was determined according to WHO protocol 2010 [27]. For this process 10μl of the diluted sperm suspension was transferred to each counting chamber of the hemocytometer and allowed to stand for 5min in a humid chamber to prevent drying. The spermatozoa in both chambers were counted under a light microscope (200× magnification). The data were expressed as the number of spermatozoa per ml.

Sperm viability
According to WHO 2010 protocol [27] viability was assessed by eosin Y (0.5% in normal saline). Briefly, A 5-μl sample of the sperm suspension combined with 5-μl of eosin solution on a glass slide, mixed and covered by a coverslip. The percentage of vital sperm cells were evaluated under a light microscope (400× magnification). In dead cells because of the loss of cellular membrane integrity and functionality, the head of dead sperm cells absorb eosin (become pink), but live sperm cells remain colorless.

Sperm morphology
To determine the percentage of morphologically abnormal sperm cells, a part of sperm suspension was used for smear preparation and each slide was stained using the Diff-Quik (DQ) staining technique. The slides were examined with a light microscope (400× magnification). Total abnormal sperm cells were expressed as percentage.

Statistical analysis
Differences between groups were tested by one-way analysis of variance (ANOVA), using the SPSS software. Post-hoc testing were performed for inter-group comparisons using the least significance difference (LSD) test. The results are expressed as mean ± standard deviation (S.D) and p value <0.05 were considered statistically significant.

RESULTS

Administration of DXR to male mice caused a significant ($P < 0.05$) decrease in the epididymal sperm motility, progressive motility, count and viability when compared with control group and UD group (Table 1). Treatment of male mice with DXR also caused a significant ($P < 0.05$) increase in the abnormal sperm morphology when compared with control group and UD group.

Co-treatment with UD (Group IV (DXR-UD)) caused a significant ($P < 0.05$) increase in the epididymal sperm motility, progressive motility, count and viability and a significant decrease in the abnormal sperm morphology when compared with Group III (DXR). Although in DXR-UD group, sperm motility, progressive motility and sperm vitality significantly ($P < 0.05$) decrease when compared with control and UD group.

In DXR-UD group abnormalities in sperm morphology significantly decrease when compared with DXR group but no significant difference found when compared with control group and UD group.

In male mice group that UD administrated three time in a week for a 2 week-period sperm motility, sperm progressive motility slightly increase when compared with control group and abnormal morphology in sperm cells was decreased, but not statistically significant. Sperm viability and epididymal sperm count in UD group compared with control group was decreased, but not statistically significant.

Table 1. Effect of *Urtica dioica* (UD) and Doxorubicin on epididymal sperm characteristics

Parameters	Group I (Cont)	Group II (UD)	Group III (DXR)	Group IV (DXR-UD)
Sperm motility	72 ± 8.94	76.80 ± 8.10	24.60 ± 4.61 [ab]	45 ± 13.32 [abc]
Progressive motility	61.20 ± 7.01	65.60 ± 10.16	18.80 ± 2.77 [ab]	36 ± 11.22 [abc]
Epididymal sperm count	7.66 ± 2.33	6.83 ± 2.56	2.16 ± 0.75 [ab]	4.83 ± 1.47 [ac]
Sperm viability	75.8 ± 4.32	73.4 ± 8.56	51 ± 3.67 [ab]	59.6 ± 4.39 [ac]
Sperm morphology	29.50 ± 5.89	28.6 ± 4.32	44.33 ± 4.88 [ab]	32.83 ± 4.20 [c]

Values are expressed as mean±S.D. Comparisons are made as follow: 'a' Groups I–IV; 'b' Groups II-IV, 'c' Groups III-IV. Values are statistically significant at p < 0.05.

DISSCUSSION

Doxorubicin as a chemotherapeutic agent is used to treatment a variety of cancers including breast cancer, ovarian cancer, Lung cancer, Neuroblastoma cancer, Leukemia etc [28]. Doxorubicin has many side effect such as testicular toxicity and dysfunction, due to its serious side effect clinical use of this drug was limited.

Doxorubicin affect male fertility potential by decrease quantity and quality of sperm.

Results of our study implie attenuate effect of UD on doxorubicin-induced changs in sperm parameters including epididymal sperm motility, progressive motility, count and viability. Co-treatment with UD in doxorubicine treated male mice were found to significantly increase epididymal sperm motility, progressive motility, count and viability and significantly decrease abnormalities in sperm morphology and minimized the toxic effects of DXR.

A study by kang et al. (2002) suggested DXR testicular toxicity is due to its effect spermatoconic cells [29] also a study conducted by Sjöblom et al. (1998) showed DXR causes an appoptosis at specific stages of seminiferous epithelial cycle [30] that may be explained how DXR decrese epididymal sperm count.

Sperm motility is also a important parameter in fertilty evaluation, low sperm motility affect fetility potential of male and making it more difficult to conceive a child. A process that affects sperm motility and progressive motility is potentially harmful to male fertility. Previous studies suggested that DXR may be has toxic effect on the flagellum of sperm, also DXR decrease citric-acid cycle key enzyme activities [8, 31] in testis and thus impaired sperm motility.

Several studies showed increased oxidative stress and lipid peroxidation following administration of DXR [11, 12, 32-35]. In other studies revealed sperm motility my be decreased due to cascades that occure during lipid peroxidation and result decrease axonemal protein phosphoration and sperm immobilization, also due to increased level of H_2O_2 that diffuse across sperm cell membrane and inhibit enzyme activity and decrease NADPH level [36-39]. Also, elevated level of ROS can cause initiating appoptosis in cell, cell death and ultimately decrese sperm count and increase abnormal sperm morphology.

Co-administration of antioxidants such as UD could contribute to attenuating oxidative stress thus preserving sperm count, motility and viability [40, 41].

According to Xin et al. (2002) DXR has cytotoxic effect on testicular function and sperm parameters, intravenous injection of DXR showed reduction in testicular weight, sperm concentration and sperm motility, and the increase in rate of abnormal sperm morphology. In Xin et al. study revealed that pretreatment with *Lycium barbarum* (a deciduous Shrub) as an herbal antioxidant ameliorated DXR-induced changes [42].

In a study conducted by Rizk et al. (2014), DXR administrated for 3 weeks in an 18 mg kg^{-1} total cumulative dose to male rats. DXR reduced sperm count, increased testicular oxidative stress, inflammatory and apoptotic markers. Rizk et al. used propolis extract to diminish toxic effect of DXR and indicate that treatment with propolis extract prevented DXR-induced changes without reducing its antitumor activity [6]. Kranti et al. (2013) evaluated the protective effect of silymarin, an herbal antioxidant that extracted from seeds of milk thistle, on DXR-induced testicular toxicity. They showed that silymarin maintained sperm count near normal level when co-administrated with DXR [43].

Urtica dioica have been used in folk medicine and has several pharmacological properties including antioxidant, anti-apoptotic and anti-fibrotic activities. In our study *Urtica dioica* showed protective effect against DXR-induced changes on epididymal sperm parameters such as sperm count, sperm motility, sperm viability and rate of abnormal sperm cells.

Our results are in consensus with other studies that showed treatment with DXR cause deleterious effect on sperm parameters and cause testicular toxicity and used of antioxidants reducing toxicity of DXR [29, 32-34, 44, 45].

CONCLUSION

In conclusion, results of the present study indicate that UD protected DXR-induced testicular toxicity and improve semen parameters, thus suggesting its co-administration as a supportive agent during doxorubicin treatment. Further studies could be aimed to determine protective effect of UD against chemotherapeutic agents such as DXR.

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