Effects of Purslane Shoot and Seed Ethanolic Extracts on Doxorubicin-Induced Testicular Toxicity in Albino Rats

Walaa G. Hozayen[1]; Osama M. Ahmed[2,3] and Haidy T. Abo Sree[3]

[1]Biochemistry Division, Chemistry Department, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt
[2]Physiology Division, Zoology Department, Faculty of Science, Beni-Suef University, Beni- Suef, Egypt
[3]Faculty of Oral and Dental Medicine Nahda University, Beni-suef, Egypt
walaahozayen@hotmail.com[1]; osamamoha@yahoo.com[2]; haidyalshafeey@yahoo.com[3]

Abstract: The clinical usefulness of anthracycline antineoplastic antibiotic, doxorubicin (DOX) is restricted since it has several acute and chronic side effects. The effect of doxorubicin (4 mg/kg b.w/week) without or with oral administration of purslane *(Portulaca oleracea)* shoot ethanolic extract (50mg/kg b.w./day) and purslane seed ethanolic extract (50mg/kg b.w./day) co-treatments for 6 weeks was evaluated in adult male rats. Serum testosterone luteinizing hormone (LH), follicle stimulating hormone (FSH) level were assayed. Testis lipid peroxidation (indexed by MDA) and antioxidants like glutathione (GSH), glutathione-S-transferase (GST), peroxidase (POX), superoxide dismutase (SOD), catalase (CAT) levels in testis were assessed. The data revealed a significant decrease in serum levels concentration of testosterone, LH and FSH levels in doxorubicin-injected rats. In addition, testis glutathione, glutathione transferase, peroxidase, SOD and CAT levels were decreased while lipid peroxidation concentration in the testis was increased as a result of doxorubicin injection. Co-administration of ethanolic purslane and seed extracts potentially improved the adverse changes in serum testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH) levels with an increase in testis antioxidants levels and reduction in lipid peroxidation. In conclusion, it can be suggested that dietary purslane extract supplementation may provide a cushion for a prolonged therapeutic option against DOX testicular toxicity without harmful side effects.

1. Introduction

Doxorubicin (DOX) obtained from soil actinomycetes *Streptococcus peucetius* is used for the treatment of solid tumors such as those arising in the breast, bile ducts, endometrial tissue, esophagus and liver, osteosarcomas, soft-tissue sarcomas and non-Hodgkin's lymphoma (**Tikoo *et al*., 2011**). DOX is known as a powerful anthracycline antibiotic widely used to treat many human cancers, but significant cardiotoxicity (**Kuznetsova *et al*., 2011**), hepatptoxicity (**Patela *et al*., 2010**), nephrotoxicity (**Mohana *et al*., 2010)** and testicular toxicity (**Trivedi *et al*., 2011)** limits its clinical application**.** Mitochondria are considered to be one of the primary targets of DOX through mitochondria-mediated apoptosis, remarkable modification of mitochondrial membranes (e.g. *via* binding with cardiolipin), which is also associated with changes in various mitochondrial functional parameters and activities of respiratory chain complexes (**Trivedi *et al*., 2011)**. Moreover, doxorubicin significantly damages energy-transferring and -signalling systems like creatine kinase and AMP-activated protein kinase (**Kuznetsova *et al*., 2011)**. DOX has been found to induce deterioration of sperm motion and sperm content resulting into the adverse effects on male fertility (**Kato *et al*., 2001**). Impaired spermatogenesis by acute

exposure of rats to DOX has been reported (**Suter *et al*., 1997)**. It has been reported that oxidative stress from lipid peroxidation is mainly responsible for the toxicity produced by DOX (**Lebrecht *et al*., 2007 and Simunek *et al*., 2009**). Reactive oxygen species have been known to cause an increase in the concentration of lipid hydroperoxides and loss of membrane polyunsaturated fatty acids in the spermatozoa (**Elangovan *et al*., 2006**). Doxorubicin causes disturbances in the balance between oxidative stress and antioxidant defence system leading to tissue injuries (**Karaman *et al*., 2006)**.

A number of studies were conducted for antioxidants screening from the natural medicine aiming to minimize oxidative injury by DOX. Several natural antioxidants have been shown to alleviate the DOX-induced cell damage without compromising its anti-tumor efficacy in the animal studies (**Xin *et al*., 2011).** *Portulaca oleracea L*, commonly known as purslane, is a warm climate, annual, green shoot (**Al-Quraishy *et al*., 2012**). Recent research indicates that purslane offers better nourishment than the major cultivated vegetables due to its shoot that is a rich source of X9-3-fatty acids, α-tocopherols, ascorbic acid, β-carotene and glutathione. Its seeds also contain a high percentage of α-linolenic acid (LNA) (**Al-Quraishy *et al*., 2012**). These features contribute to the anti-

oxidative properties of purslane which derive from the following pharmacologically active substances, including: 28% flavonoids, that are nearly exclusively flavonol-O-glycosides; 8% terpenoids (principally ginkgolides A, B, C and bilobalide); 6–12% organic acids; and >0.5% proanthocyanidins defined as flavonoid-based polymers. Purslane is effective as an antioxidant agent (**Dkhil et al., 2011**) as well as providing nourishment for testis and other organs. Experimental evidence has also shown that purslane has an anti-oxidative effect in heart tissues in mice by increasing superoxide dismutase activity (**Al-Quraishy et al., 2012**). Other outhors reported that the purslane contains many compounds, including alkaloids, omega-3 fatty acids, coumarins, flavonoids, polysaccharide, cardiac glycosides, anthraquinone glycosides. and containing β-sitosterol (**Mohamed et al., 2011**).

In continuation with the previous literature, the present study was designed to investigate the preventive effect of ethanolic extract of purslane shoot parts and seeds on testis function and oxidative stress in doxorubicin-treated rats.

2. Material and methods
2.1. Experimental animals:

Male Wister albino rats weighing about 140-180g were used as experimental animals in the present investigation. They were obtained from the animal house of Research Institute of Opthalmology, El-Giza, Egypt. They were kept under observation for about 15 days before the onset of the experiment to exclude any intercurrent infection. The chosen animals were housed in plastic cages with good aerated covers at normal atmospheric temperature ($25\pm5^{o}C$) as well as 12 hours daily normal light periods. Moreover, they were given access of water and supplied daily with standard pellet diet *ad libitum*. All animal procedure are in accordance with the recommendations of the Canadian committee for care and use of animals (**Canadian council on Animal care [CCAC], 1993**).

2.2. Chemicals and drugs

Doxorubicin was purchased from EBEWE pharma Ges.m.b.H.Nfg.KG A-4866 Unterach, AUSTRIA. Purslane shoot parts and seeds were purchased from Harraz Medicinal shoot company, Cairo, Egypt (WWW.harrazegypt.com). Testosterone kits obtained from Biosource Company, (Belgium). FSH kits obtained from Monobind, INC. (USA). LH kits obtained from Monobind, INC. (USA). Chemicals used in measurement of antioxidants from Sigma Chemical Company, USA.

2.3. Shoot and seed extract

The shoot parts of the plant and seeds were dried in the shade. They were powdered by an electric grinder, then they were exhaustively extracted with 80% ethanol. The solvent was removed by evaporation under reduced pressure using Bucchi Rotary Evaporator (**Wang et al., 2012**).

2.4. Experimental Animal grouping and experimental design:

The animals of the present experiment were allocated into 4 groups:

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

2-Doxorubicin–administered control: The rats of this group were intraperitoneally administered doxorubicin at a dose of 4 mg/Kg b.w./week for 6 weeks (**Trivedi et al., 2011**).

3-Doxorubicin-administered group treated with purslane shoot extract: This group was treated similarly to group 2 and was orally treated (by oral gavage) with purslane shoot extract at dose level of 50 mg/kg b.w./day for 6 weeks (**Ali and Bashir, 1994; Fayong Gong et al., 2009**).

4-Doxorubicin-administered group treated with purslane seeds extract: This group was treated similarly to group 2 and was orally treated (by oral gavage) with purslane seed extract at dose level of 50 mg/kg b.w./day for 6 weeks (**Ali and Bashir, 1994; Fayong Gong et al., 2009**).

2.5. Preparation of blood and tissue homogenates

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

2.6. Assay of Testis function parameters:

Testosterone concentration in serum was determined according to the method of **Andreyko et al. (1986)** using reagent kits purchased from Biosource Company, (Belgium). LH concentration in serum was determined according to the method of **Braunstein et al. (1976)** using reagent kits purchased from Monobind, INC. (USA). FSH concentration in serum was determined according to the method of **Odell et al. (1968)** using reagent kits purchased from Monobind, INC. (USA).

2.7. Assay of Lipid peroxidation and antioxidant parameters

Testis oxidative stress and antioxidant defense parameters were estimated using chemicals purchased from Sigma Chemical Company (USA) and using

Jenway Spectrophotometer (Germany), glutathione (GSH) content in homogenates was determined according to the chemical method of **Beutler et al. (1963)** with little modification**.** Lipid peroxidation concentration in homogenates was determined according to the chemical method of **Preuss et al. (1998).** Peroxidase (POX. EC 1.11.1.7) activity in homogenates was estimated according to the modified chemical method of **Kar and Mishra (1976)**. Superoxide dismutase (SOD EC 1.15.1.1) activity in homogenates was determined according to the chemical method of **Marklund and Marklin (1974)**. Glutathione-S-transferase (GST. EC 2.5.1.18) concentration in homogenates was determined according to the chemical method of **Mannervik and Guthenberg (1981)**. Catalase (CAT, EC 1. 11. 1. 6) activity in homogenates was assayed according to the chemical method of **Cohen et al. (1970)**.

2.8. Histological examination:

After sacrifice and dissection at specific time intervals, pieces of testis from all groups were immediately removed from each animal, fixed in 10% neutral buffered formalin and transferred to Department of Histopathology, Faculty of Veterinary medicine, Beni-Suef University, Egypt for preparation, sectioning and staining with haemotoxylin and eosin (H&E) **(Bancroft and Stevens, 1982).**

2.9. Statistical analysis

The data in the present study were analyzed using the one-way analysis of variance **(ANOVA) (PC-STAT, University of Georgia, 1985)** followed by LSD test to compare various groups with each other. Results were expressed as mean ± standard error (SE) and values of $P>0.05$ were considered non-significantly different, while those of $P<0.05$ and $P<0.01$ were considered significantly and highly significantly different, respectively.

3. Results

3.1. Biochemical changes

The doxorubicin-administered rats showed a highly significant decrease ($P<0.01$) in serum level of testosterone, FSH and LH recording percentage decreases of -32.81, -38.32 and -48.91 % respectively as compared to normal control group. The treatment of doxorubicin-administered rats with purslane shoot ethanolic extract induced a significant increase of the serum testosterone ($P<0.01$), FSH ($P<0.05$) and LH ($P<0.01$) levels; the recorded percentage increases were 26.67, 23.91 and 64.14% respectively as compared to doxorubicin-administered rats. The treatment of doxorubicin-administered rats with purslane seed ethanolic extracts induced a highly significant increase ($P<0.01$) in serum testosterone, FSH and LH levels recording percentage increases of 37.78, 32.61 and

62.81% respectively as compared to doxorubicin-administered rats (Table 1).

Testis lipid peroxidation exhibited a highly significant increase ($P<0.01$) recording percentage increases of 144.31% as compared to normal control group. The antioxidants levels of glutathione, catalase, SOD, peroxidase and glutatione-S-transferase in testis of doxorubicin-intoxicated rats showed a highly significant decrease ($P<0.01$) recording percentages of -72.7, -85.51, -46.91, -37.62 and -36.42% respectively as compared to normal control group. The treatment of doxorubicin-administered rats treated with purslane shoot ethanolic extract of induced a highly significant increase of the serum glutathione, catalase, SOD, peroxidase and glutatione-S-transferase levels ($P<0.01$); the recorded percentage increases were 163.37, 311.20, 65.81, 31.22 and 32.34% respectively. Testis lipid peroxidation of doxorubicin-administered group treated with purslane shoot ethanolic extract exhibited a highly significant decrease ($P<0.01$) recording percentage decreases of -19.3%. The treatment of doxorubicin-administered rats with purslane seed ethanolic extract induced a highly significant increase ($P<0.01$) in serum glutathione, catalase, SOD, peroxidase and glutatione-S-transferase levels ($P<0.01$) recording percentage increases of 241.98, 500.00, 81.25, 53.72 and 50.32% respectively as compared to doxorubicin-administered rats while lipid peroxidation was highly significant decreased ($P<0.01$) recording percentage decrease of -38.6% as compared to doxorubicin-administered rats. Thus, the seed ethanolic extract seemed to be more potent in improving the antioxidant defense system than shoot ethanolic extract (Table 2).

3.2. Histological changes:

Microscopical examination of all testis sections of a control group revealed a normal tissue architecture. The testis is formed of a number of seminiferous tubules separated by intertubular spaces. Within these spaces lie the interstitial tissues. These tissues are formed of a number of cells known as Leydig cells and blood vessels. Each seminiferous tubule has a normal spermatogenic cell lineages formed of a number of spermatogonia, spermatocytes, spermatids and spermatozoa arranged around central lumen. Alternating with spermatogonia, there are nutritive cells known as sertoil cells (Figures 1a and 1b).

Administration of doxorubicin led to vacuolation of spermatogenium cells, loss of spermatocytes, spermatids and spermatozoa, odema in the intertubular tissue and necrosis of the most lining epithelium of the seminiferous tubules (Figures 2a, 2b, 2c and 2d).

The treatment of doxorubicin-administered rats with purslane shoot part and seed ethanolic extracts led to a marked improvement of seminiferous tubules architecture (Figure 3a, 3b, 4a and 4b). However, mild vacuolation, necrosis and loss of tubularepithilial cells are found. In addition, few hydropic cells are observed.

Table 1: Effect of purslane shoot and seed ethanolic extracts on serum testosterone, FSH and LH levels in doxorubicin-administered rats.

Parameters Groups	Serum Testosterone (ng/ml)	%	Serum FSH (mIU/ml)	%	Serum LH (mIU/ml)	%
Normal	0.67 ± 0.03^a	-	4.62 ± 0.07^a		7.48 ± 0.22^a	--
Doxorubicin	0.45 ± 0.02^c	-32.81	2.85 ± 0.20^c	-38.32	3.82 ± 0.17^c	-48.91
Doxorubicin + Shoot extract	0.57 ± 0.03^b	26.67	3.53 ± 0.12^b	23.91	6.2 ± 0.14^b	64.14
Doxorubicin + Seed extract	0.62 ± 0.03^{ab}	37.78	3.78 ± 0.06^b	32.61	6.22 ± 0.12^b	62.81
LSD at the 5% level	7. 01		0.37		0.53	
LSD at the 1% level	0.11		0.51		0.69	

-Data are expressed as Mean ± SE. - The number of animals in each group is six

-Percentage changes (%) were calculated by comparing doxorubicin-administered rats with normal and treated groups with doxorubicin-administered rats

Table 2: Effect of purslane shoot and seed ethanolic extracts on testis lipid peroxidation and various antioxidants in doxorubicin - administrated rats.

| Parameters Groups	MDA (nmol/ 100mg tissue/hr)	%	GSH (nmol/ 100mg)	%	CAT $(k.10^3)$	%	SOD $(U/g . 10^3)$	%	PXO (U/g)	%	GST $(mU/ 100g.10^2)$	%
Normal	32.02 ± 2.39^d	-	35.62 ± 1.21^a	-	108.75 ± 7.27^a	-	15.58 ± 0.16^a	-	55.66 ± 0.39^a	-	89.11 ± 2.27^a	-
Doxorubicin	78.23 ± 1.78^a	144.31	9.72 ± 0.56^c	-72.71	15.75 ± 2.71^c	-85.51	8.28 ± 0.10^d	-46.91	34.73 ± 0.34^d	-37.62	56.62 ± 1.33^c	-36.42
Doxorubicin + Shoot Extract	63.13 ± 1.90^b	-19.33	25.59 ± 0.60^b	163.27	64.77 ± 7.31^b	311.2	13.73 ± 0.26^c	65.81	45.48 ± 0.52^c	31.22	74.93 ± 2.45^b	32.34
Doxorubicin + Seed Extract	48.02 ± 2.67^c	-38.62	33.24 ± 0.84^a	241.98	94.47 ± 7.71^a	500.0	15.04 ± 0.14^b	81.25	53.39 ± 0.49^b	53.72	85.12 ± 1.99^a	50.32
LSD at the 5% level	6.54		2.48		19.37		0.56		1.30		6.06	
LSD at the 1% level	8.91		3.38		26.41		0.77		1.77		8.26	
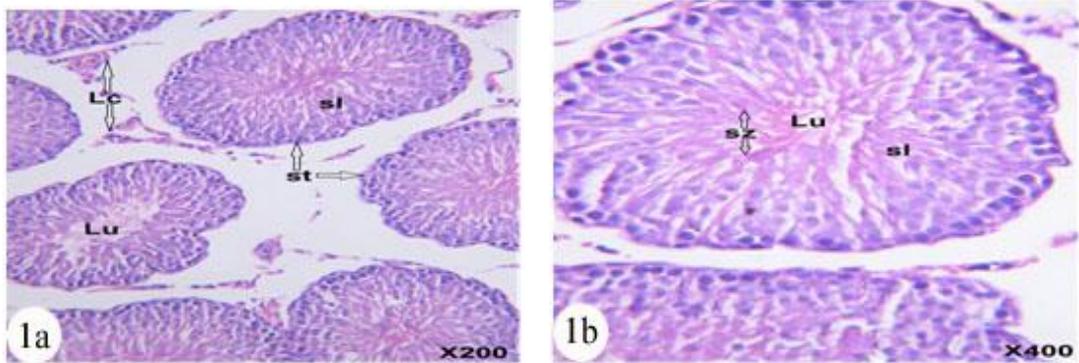
Figure 1: Photomicrographs of testis section showing normal histological Structure of testis. St: seminiferous tubules; Lu: Lumen; sz: spermatozoa or sperms; sl: spermatic lineages and Lc: Leydig cells (Figures 10a and 10b).
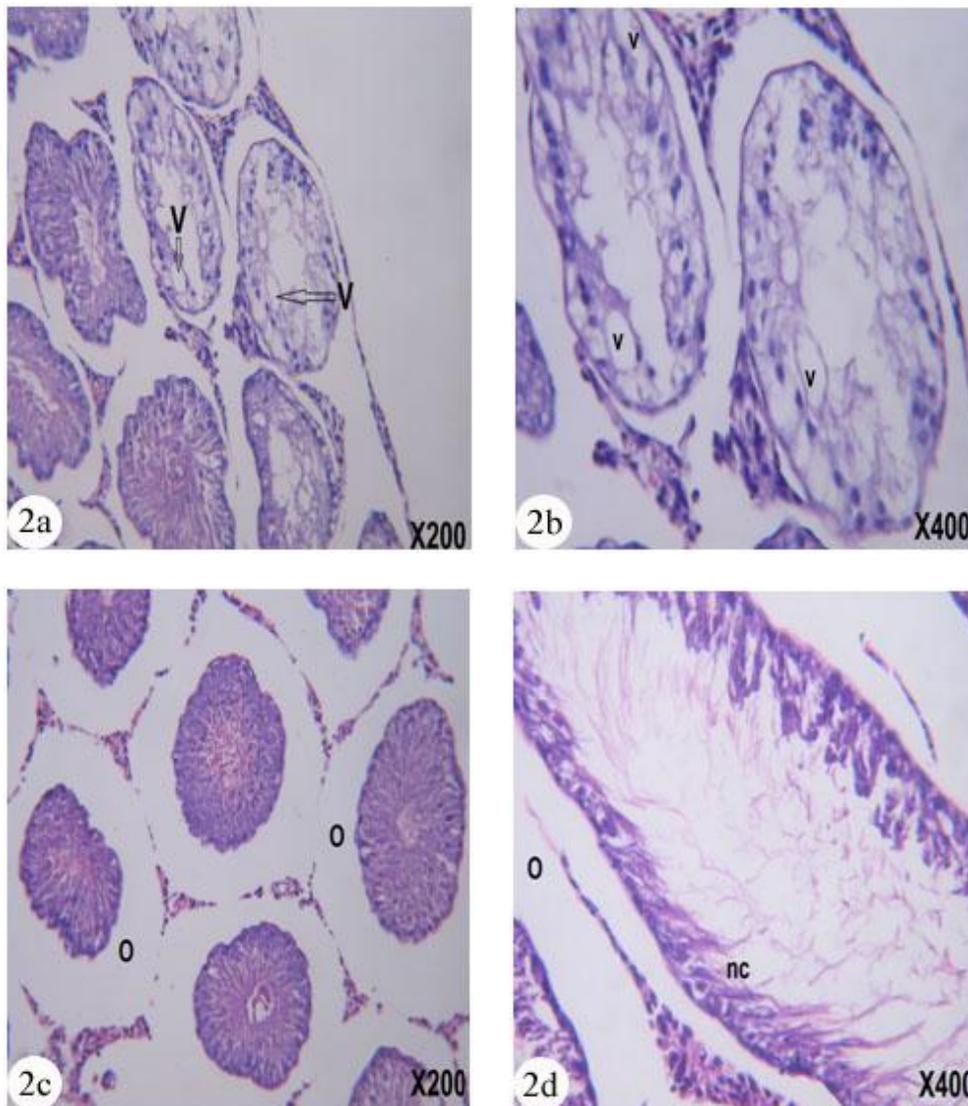

Figure 2: Photomicrographs of testis section of doxorubicin-administered rats showing vacuolation of spermatogenium cells and loss of spermatocytes, spermatids and spermatozoa, vacuolation (V) of spermatogenium cells and loss of spermatocytes, spermatic and spermatozoa are also noticed (Figure 2b). Odema (O) of the intertubular tissue (Figure 2c) as well as vacoultion and necrosis (nc) of the most lining epithelium of the seminiferous tubules (Figure 2d) can be observed.
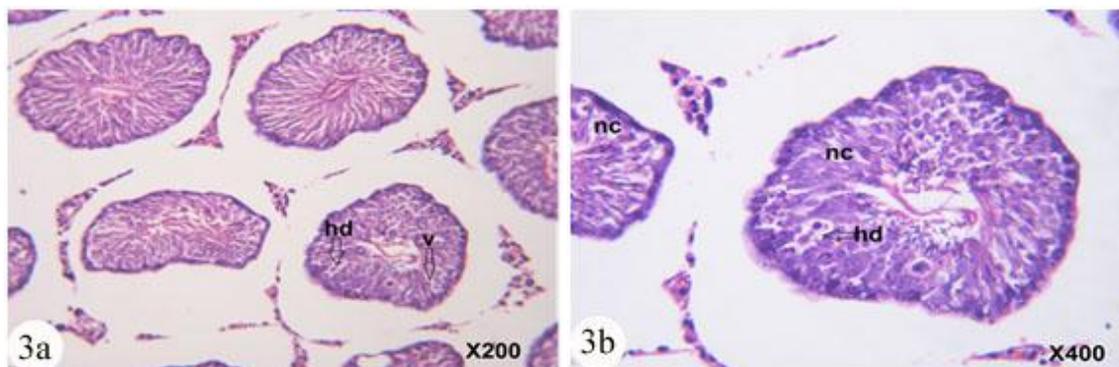
Figure 3: Photomicrographs of testis section of doxorubicin-administered treated rats with purslane shoot ethanolic extract showing moderate voculation (v) and hydropic degenerations (hd) of the lining epithelium of the semniferous tubules. Few number of the seminiferous tubules showed moderate loss and decrease in necrosis of the lining epithelium of the seminiferous tubules (Figures 3a and 3b).
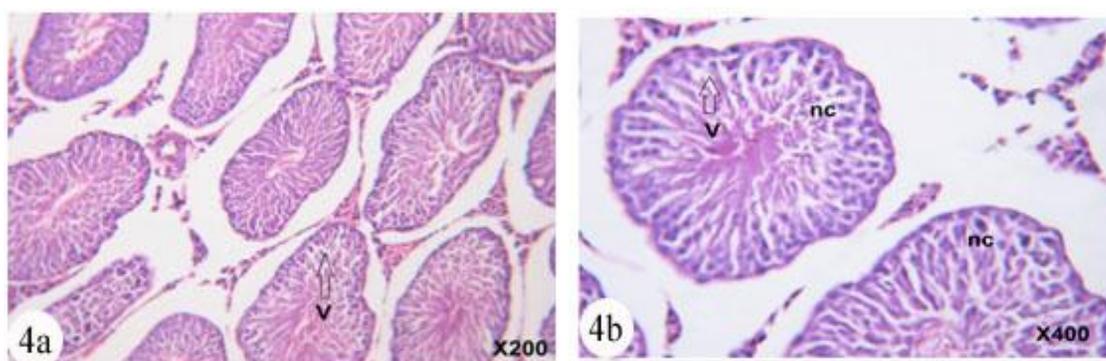

Figure 4: Photomicrographs of testis section of doxorubicin-administered treated rats with purslane seed ethanolic extract showing mild voculation (v) of the lining epithelium of the seminiferous tubules. Few numbers of the seminiferous tubules showed moderate necrosis (nc) and vacoulation (v) of the lining epithelium of the seminiferous tubules (Figure 4a and 4b).

4. Discussion

DOX has been widely used for the treatment of various hematological and solid tumors. DOX shows its chemotherapeutic activity by stabilizing a reaction intermediate in which DNA strands are cut and covalently linked to tyrosine residues of topoisomerase II. This results into inhibition of the negative super coiling of DNA and thereby blocking further DNA transcription and replication. DOX treatment possesses the risk of serious dose-dependent toxicity to other non-target tissues, among which testis is one of such tissues vulnerable to the side effects of this potent chemotherapeutic agent **(Trivedi *et al*., 2011)**.

The present study revealed that intraperitoneal injection of 4 mg DOX/kg b.w. for 6 weeks induced male sexual and testicular dysfunction manifested biochemically by a significant decrease of serum testeosterone, FSH and LH levels. DOX treatment also resulted in a decrease in sperm number and vacoulation and necrosis in spermatogonium cells within the seminiferous tubules as well as odema in the interstitial spaces. These results are in agreement with **Yeh *et al*. (2009) and Trivedi *et al*. (2011).**

The previous deleterious biochemical alterations of the present study were associated with a marked elevation of testis lipid peroxidation and a significant decrease in the level non-enzymatic antioxidant, glutathione and activities of enzymatic antioxidants, catalase, superoxide dismutase, peroxidase and glutathione-S-transferase. These results are in concurrence with those obtained by many other authors **(Kato *et al*., 2001**; **Lebrecht *et al*., 2007; Simunek *et al*., 2009)** who stated that one of the most prevailing hypothesis of testis damage from DOX administration is the ability of the drug to produce reactive oxygen species (ROS) and reduce antioxidant defense mechanism. They also revealed that the increased lipid peroxidation play a critical role in testicular toxicity. Reactive oxygen species have been known to cause an increase in the concentration of lipid hydroperoxides and loss of membrane polyunsaturated fatty acids in the spermatozoa **(Elangovan *et al*., 2006).** In the present

study, DOX treatment led to significant increase in testis lipid peroxidation and decrease in the GSH levels in the testis as compared to the control group. The increase in lipid peroxidation was associated with necrosis and vacoulation in spermatogonium cells. These results are in accordance with those obtained by **Trivedi et al. (2011)**.

Histopathological examination of testis sections of DOX-administration rats supported the previous biochemical results. The testis showed that DOX administration led to vacuolation of spermatogenium cells and loss of spermatocytes, spermatids and spermatozoa. Some seminiferous tubules showed odema of the intertubular tissue, vacuoltion and necrosis of the most lining epithelium of the semniferous tubules which indicated severe germ cell toxicity induced by DOX. These results are in agreement with **Yeh et al. (2009 and Trivedi et al. (2011)** who showed that DOX administration led to significant reduction in the sperm count and increase in the percent abnormality in sperm head morphology. In testis section, increased disorganization, vacuolization, decreased spermatogonia, spermatocytes and spermatids counts were observed. Some of the seminiferous tubules were found to be completely deprived of the cellular structure.

The treatment of DOX-administered animals with purslane shoot and seed ethanolic extracts successfully improved the elevated serum levels of testosterone, FSH and LH. These results are in agreement with **Jalali et al. (2012)** who reported that the purslane extract can be protective against reproductive toxicity due to its contents such as omega-3 fatty acids and β-carotene (**Liu et al., 2000**), vitamins and essential amino acids, α-tocopherols, ascorbic acid, and glutathione as well as phenolics and coumarins (**Spina et al., 2008**). Organic acids are also present (**Fontana et al., 2006)** and alkaloids had been reported to be important chemical constituents of this species (**Xiang et al., 2005).** Concomitant with the present study**, Tawfeq (2008)** reported that the purslane extract caused a significant reduction in the doxorubicin-induced toxicity in mice.

These ameliorations in biochemical serum parameters of testis function are associated with the improvement in testis histology changes. The seed extract seemed to be more potent than shoot extract. The testis showed moderate voculation of the lining epithelium of the seminiferous tubules. Few number of the seminiferous tubules showed moderate loss and decrease in necrosis and vacoulation of the lining epithelium of the seminiferous tubules as a results of purslane shoot and seed extracts

The improvement of tesis function and integrity may be mediated *via* the antioxidant activity of purslane shoot and seed extracts. This is confirmed by the current study who revealed a significant decrease of lipid peroxidation and increase in CAT, SOD, peroxidase and glutathaione-S-transferase activities and glutathione level.

Conclusion

The purslane shoot and seed ethanolic extracts successfully ameliorated the deleterious effects of doxorubicin on serum testosterone, FSH and LH levels and improved the testis function and integrity. These alleviative effects may be mediated *via* the enhancement of the antioxidant defense system and suppression of oxidative stress. However, further clinical studies are required to assess the efficacy and safety of purslane shoot and seed extracts on doxorubicin-induced male sexual and testicular dysfunctions in human beings.

References

1. Ahmed O. M (2001): Histopathological and biochemical evaluation of liver and kidney lesions in streptozotocin diabetic rats treated with glimepiride and various plant extracts. J. Union Arab Biol., 16A:585-625.
2. Ali, B.H.; Bashir, A.A. (1994): Effect of fish oil treatment on gentamicin nephrotoxicity in rats. Ann. Nutr. Metab. , 38(6): 336-9.
3. Al-Quraishy, S.A.; Mohamed, A.; Dkhil. A.B.; Ahmed, E.; Abdel Moneim, B.C. (2012): Protective effects of *Portulaca oleracea* against rotenone mediated depletion of glutathione in the striatum of rats as an animal model of Parkinson's disease. Pesticide Biochemistry and Physiology, 103(2):108–114.
4. Andreyko, J.L.; Bhavnani, B.R.; Nisker, J.A. (1986): Role of serum androgens and sex hormones binding globulin capacity in the evaluation of hirsutism in women, Clin. Biochem., 19(1): 58-61.
5. Bancroft, J.D.; and Stevens, A. (1982): Theory and practice of histological techniques, 2nd edition. Churchill Livingestone, 374-375.
6. Beutler, E.; Duren, O. and Kelly, B. M. (1963): Improved method for the determination of blood glutathione. J. Lab Clin. Med., 61(5): 882-888.
7. Braunstein, G.D.; Rasor, J.; Alder, D.; Danzer, H. and Wade, M.E. (1976): Serum human luteinizing hormone levels through normal pregnancy, American Journal of Obstetrics and Gynecology, 126:678-81.
8. Canadian council on Animal care (CCAC) (1993): Guide to the care and use of Experimental Animals., CCAC, Ottawa, Ontario, Canada, 1-298.
9. Cohen, G.; Dembiec, D. and Marcus, J. (1970): Measurement of catalase activity in tissue. Analytical Biochemistry, (34): 30-38.

10. -Dkhil, M.A.; Abd ElMoneim, A.E.; Al-Quraishy, S.; Saleh, R.A. (2011): Antioxidant effect of purslane (*Portulaca oleracea*) and its mechanism of action. J. Med. Plants Res., 5: 1589–1593.

11. Elangovan, N.; Chiou, T.J.; Tzeng, W.F. and Chu, S.T. (2006): Cyclophosphamide treatment causes impairment of sperm and its fertilizing ability in mice. Toxicology, 222: 60–70.

12. Fayong, G.; Fenglin, L.; Lili, Z.; Jing, L.; Zhong, Z. and Guangyao, W. (2009): Hypoglycemic Effects of Crude Polysaccharide from Purslane. Int. J. Mol. Sci., 10: 880-888.

13. Fontana, E.; Hoeberechts, J.; Nicola, S.; Cros, V.; Palmegiano, G.B. and Peiretti, P.G. (2006): Nitrogen concentration and nitrate/ammonium ratio affect yield and change the oxalic acid concentration and fatty acid profile of purslane (*Portulaca oleracea* L.) grown in a soilless culture system. J. Sc. Food Agric. 86: 2417–2424.

14. Jalali, A.S.; Shapour, H.; Hassan, M. (2012): *Achillea millefolium* inflorescence aqueous extract ameliorates cyclophosphamide-induced toxicity in rat testis: stereological evidences. Chinese Journal of Natural Medicines, 10(4): 0247-0254.

15. Kar, M. and Mishra, D. (1976): Catalase, Peroxidase, and Polyphenoloxidase Activities during Rice Leaf Senescence. Plant Physiol., 57(2):315-319.

16. Karaman, A.; Fadillioglu, E.; Turkmen, E.; Tas, E. and Yilmaz, Z. (2006): Protective effects of leflunomide against ischemia reperfusion injury of the rat liver. Pediatr. Surg. Int., 22: 428–434.

17. Kato, M.; Makino, S.; Kimura, H.; Ota, T.; Furuhashi, T. and Nagamura, Y. (2001): Sperm motion analysis in rats treated with adriamycin and its applicability to male reproductive toxicity studies. J. Toxicol. Sci., 26: 51–59.

18. Kuznetsova, A.V.; Raimund, M.; Albert, A.; Valdur, S. and Michael, G. (2011): Changes in mitochondrial redox state, membrane potential and calcium precede mitochondrial dysfunction in doxorubicin-induced cell death, Biochimica et Biophysica Acta (BBA) – Molecular Cell Research, 1813(6): 1144-1152.

19. Lebrecht, D.; Geist, A.; Ketelsen, U.P.; Haberstroh, J.; Setzer, B. and Walker, U.A. (2007): Dexrazoxane prevents doxorubicin-induced long-term cardiotoxicity and protects myocardial mitochondria from genetic and functional lesions in rats. Pharmacol. Br. J., 151: 771–778.

20. Liu, L.; Howe, P.; Zhou, Y.F.; Xu, Z.Q.; Hocart, C. and Zhang, R., (2000): Fatty acids and β-carotene in Australian purslane (*Portulaca oleracea*) varieties, J. Chromatogr, A 893: 207–213.

21. Mannervik, B. and Guthenberg, C. (1981): Glutathione transferase (human placenta). Methods Enzymol, 77: 231-235.

22. Marchand, D.J. and Renton, K.W. (1981): Depression of cytochrome P-450-dependent drug biotrnsfomation by adriamycin. Toxicol. Appl. Phamacol., 58: 83–88.

23. Marklund, S. and Marklin, G. (1974): Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem., 47:469-474.

24. Mohamed, I. K.E. (2011): Effects of Portulaca oleracea L. seeds in treatment of type-2 diabetes mellitus patients as adjunctive and alternative therapy, Journal of Ethnopharmacology, 137(1): 643-651.

25. Mohana, M.; Sarika, K.; Prakash, G. and Sanjay, K. (2010): Protective effect of *Solanum torvum* on doxorubicin-induced nephrotoxicity in rats. Food and Chemical Toxicology, 48(1): 436-440.

26. Odell, W.D.; Parlow, A.F.; Cargille, C.M. and Ross, G.I. (1968): Radioimmunoassay for human FSH: physiological studies. J. Clin. Invest., 47: 2551-62.

27. Patela, N.; Ceci, J.; George, B.; Corcoranb, and Sidhartha, D.R. (2010): Silymarin modulates doxorubicin-induced oxidative stress, Bcl-xL and p53 expression while preventing apoptotic and necrotic cell death in the liver. Toxicology and Applied Pharmacology, 245(2): 143-152.

28. PC-STAT (1985): One way analysis of variance. Version IA (c) copyright. Programes coded by Roa, M.; Blane, K. and Zonneberg, M. University of Georgia, USA.

29. Preuss, H. G.; Jarrel, S. T.; Scheckenobac, R.; Lieberman, S. and Anderson. R. A. (1998): comparative effects of chromium vanadium of Gymnema Sylvester on sugar-induced blood pressure elevations in SHR. J. American College of Nutrition, 17 (2): 116-123.

30. Simunek, T.; Sterba, M.; Popelova, O.; Adamcova, M.; Hrdina, R and Gers, V. (2009): Anthracycline-induced cardiotoxicity: overview of studies examining the roles of oxidative stress and free cellular iron. Pharmacol. Rep., 61:154–171.

31. Spina, M.; Cuccioloni, M.; Sparapani, L.; Acciarri, S.; Eleuteri, A.M.; Fioretti, E. and Angeletti, M. (2008): Comparative evaluation of flavonoid content in assessing quality of wild and cultivated vegetables for human consumption. J. Sci. Food Agric., 88: 294–304.

32. Tawfeq, A.A. (2008): Protective effect of purslane on rat liver injury induced by carbon tetrachloride. Saudi Pharmaceutical Journal, 16, Nos: 3-4.

33. Tikoo, K.; Mukta, S.S. and Chanchal, G. (2011): Tannic acid ameliorates doxorubicin-induced ▶ cardiotoxicity and potentiates its anti-cancer activity: Potential role of tannins in cancer chemotherapy. Toxicology and Applied Pharmacology, 251(3): 191-200.

34. Trivedi, P.P.; Kushwaha, S.; Tripathi, D.N. and jena. G.B. (2011): cardioprotective effects of Hesperetin against Doxorubicin-induced oxidative stress and DNA damage in rat. Food and Chemical Toxicology, 11(3):215-25.

35. Trivedi, P.P.; Kushwaha, S.; Tripathi, D.N. and Jena, GB. (2011): Hesperetin protects testicular toxicity of doxorubicin▶ in rat: Role of NFκB, p38 and caspase-3. Food and Chemical Toxicology, 49(4): 838-847.

36. Wang, W.; Dong, L.; Jia, L.; Xin, H.; Ling, C.; Li, M. (2012): Ethanol extract of *Portulaca oleracea* L. protects against hypoxia-induced neuro damage through modulating endogenous erythropoietin expression. The Journal of Nutritional Biochemistry, 23(4) : 385-391.

37. Xiang, L.D.; Wang, W.; Wang, R.; Ding, Y. and Du. L. (2005): Alkaloids from *Portulaca oleracea L.* Phyto Chem., 66: 2595–2601.

38. Xin, Y.; Li-Li, Wan, J.; Peng and Cheng, Guoʾ. (2011): Alleviation of the acute doxorubicin-induced ▶ cardiotoxicity by *Lycium barbarum* polysaccharides through the suppression of oxidative stress. Food and Chemical Toxicology, 49 (1): 259-264.

39. Xueqin, X.; Lishuang, Y. and Guonan, C. (2006): Determination of flavonoids in *Portulaca oleracea L.* by capillary electrophoresis with electrochemical detection. Journal of Pharmaceutical and Biomedical Analysis, 41(2): 493-499.

40. Yeh, Y.C.; Liu, T.J.; Wang, L.C.; Lee, H.W.; Ting, C.T.; Lee, W.L.; Hung, C.J.; Wang, K.Y.; Lai, H.C. and Lai, H.C., (2009): A standardized extract of *Ginkgo biloba* suppresses doxorubicin-induced oxidative stress and p53-mediated mitochondrial apoptosis in rat testes. Br. J. Pharmacol., 156: 48–61.

8/22/2013