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Protective impact of basil (Ocimum basilicum) on adriamycin induced reproductive toxicity in male albino rats

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Abstract: ***The protective effect of basil (Ocimum basilicum) against anticancer drug adriamycin induced testicular toxicity was investigated in albino rats. Animals were divided into four groups. G1 was considered as control. G2 was orally given aqueous O. basilicum extract at a dose level of 20 ml/ kg 5 days / week for 8 weeks. G3 was injected intraperitonealy with ADR at a dose level of 2 mg/kg body weight in saline, once per week for 8 weeks. G4 was i.p.injected with ADR followed by oral administration with aqueous O. basilicum extract 5 days/ week for 8 weeks. Testes were removed and stained with H&E for histological examinations. Bcl-2 and nuclear PCNA were demonstrated immunohistochemicaly. Testosterone and LH were measured in the sera. The results showed that treating animals with ADR caused many histopathological alterations, degeneration of seminiferous tubules and loss of the spermatogenic cells. The interstitial tissue appeared with different vacuoles, blood hemorrhage anddegeneration of Leydig cells were recorded. Immunohistochemical changes were detected as strong expression of Bcl-2 in Leydig cells and decreased expression of PCNA in spermatogonia. Biochemical results showed a decrease in levels of testosterone and LH. Treating animals with ADR and O. basilicum extract caused an improvement in testicular alterations caused by ADR. Moreover, testosterone and LH increased. These findings indicated that O. basilicum extract might be having protective effect against ADR induced testicular toxicity.***

Keywords: Basil, Adriamycin, Testes, Histology, Testosterone, LH.

1. Introduction

Adriamycin (doxorubicin) is an anthracycline antitumor antibiotic and is commonly used in the treatment of a wide range of cancers, including hematological malignancies (1), many types of carcinoma (2) and soft tissue sarcomas (3). It was strongly suggested that the mechanism responsible for testicular toxicity of ADR is due to oxidative stress from lipid peroxidation (4) and cellular apoptosis (5) as being major causes.The use of adriamycin in clinical chemotherapy is limited due to diverse toxicities, including hepatotoxicity, nephrotoxicity and cardiotoxicity (6). The high level of adriamycin could damage membranes, proteins (e.g., enzymes, structural, and receptors), and DNA that may lead to cardiac dysfunction and apoptosis (7). Adriamycin application carries the risk of serious dose-dependent toxicity to other non target tissues. The testis is among the non target tissues that are vulnerable to side effects of adriamycin (8). Adriamycin can noticeably impede spermatogenesis and lead finally to infertility (9).

Recently, plants considered as an important source for the discovery of novel pharmacologically active compounds, with many blockbuster drugs being derived directly or indirectly from plants (10). Basil or sweet basil (Ocimum basilicum L., Lamiaceae family) was employed traditionally as a folk remedy for a wide spectrum of aliments. Among the aromatic herbs, basil has economic importance. The areal part of *Ocimum basilicum* is very good source of minerals and other phytochemicals, which are biologically active substances responsible for various therapeutic potential (11). Basil essential oil is a major aromatic agent with applications in various industries, such as the food, pharmaceutical, cosmetic, and aroma therapy industries (12). The Basil oil has high economic value due to the presence of specific substances such as estragol, lineol, linalool, eugenol, methyl cinamato, limonene and geraniol (13). Recently, basil was shown to rank highest among species and herbal crops for phenolic compounds, essential oils which are associated with decreasing risks of cancer (14,15). Sethi *et al.* (16) found that leaves of ocimum sanctum possess good antioxidant as well as anti-stress potentials in experimental animals. The aim of this study was to assess the potential protective effects of aqueous extract of *O. basilicum* leaves in male rat model against adriamycin - induced testicular toxicity.

2. MATERIALS AND METHODS

Chemicals

Adriamycin (Doxorubicin):

Doxorubicin (Adriablastina produced by Carlo Erba,Italy) was purchased from a local pharmacy in the form of 10 mg/ampoule.

Basil extract:

A fresh leaves of Basil (*Ocimum basilicum*) was collected from the garden in Faculty of Science , Menoufia University, Shebin El- Kom, Egypt. The leaves were rinsed with clean water to remove any foreign matter. Leaves were blended with distilled water. The mixture was strained, the merc pressed and the mixture was filtrated using filter paper. The aqueous extract was used at a dose level of 20 ml/kg *Ocimum basilicum* (17).

Animals and treatments:

Healthy adult male albino rats (*Rattus norvegicus*) weighing 120 ± 5 g were used. They were obtained from the breeding center of experimental animals, Helwan, Egypt. Animals were kept in the laboratory under constant temperature (24±2°C) for at least one week before and throughout the experimental work. They were maintained on a standard diet composed of 55%

corn starch, 20% casein, 15% corn oil, 5% salt mixture and 5% vitaminzed starch (Egyptian Company of Oils and Soap Kafr-El Zayat, Egypt). Water was available *ad libitum.* All the experiments were done in compliance with the guide for the care and use of laboratory animals approved by Faculty of Science, Menoufia University, (Approval No.MNSH167).

Animals were divided into 4 groups:

Group 1: These animals (20 rats) were served as normal controls

Group 2: These animals (20 rats) were treated with oral aqueous *O. basilicum* extract at a dose level of 20 ml/kg for 5 days/wk through 8 weeks.

Group 3: Animals of this group (20 rats) were injected intraperitonealy with ADR at a dose level of 2 mg/kg body weight in sterile saline, once per week for 8 weeks

Group4: Animals of this group (20 rats) treated with ADR at the same dose level as those of group 3 followed by oral administration aqueous *O. basilicum* extract at a dose level of 20 ml/kg for 5 days/wk through 8 weeks.

Histological preparations:

Immediately after decapitation, after 4 and 8 weeks, the animals were dissected, their testes were removed and fixed in 10% formalin. After fixation, the specimens were dehydrated using an ascending series of alcohol, cleared in 2 changes of xylene, and embedded in molten paraffin (melting point: 50-58°C). Sections of 5 mm thickness were cut using rotary microtome and mounted on clean slides. For histopathological examination, the sections were stained with Ehrlich hematoxylin and counter stained with eosin.

Morphometric Study:

The diameter of seminiferous tubules and the germinal epithelial height were measured in animals of control and experimental groups. The diameter of seminiferous tubules was measured by taking the average of the two vertical and horizontal diameter of the tubules. The epithelial height was measured from the spermatogenic cells on the inner surface of the basement memmbrance through the most adanced cell types lining the lumen of the tubules. By magnified power 20X, 10 rounded centrally located seminiferous tubules per sections in 10 sections were measured in each animal. Ocular stage micrometer was used.

Immunohistochemical Study:

For immunohistochemical localization of PCNA, and Bcl-2 fixed wax sections were stained using the avidin-biotin peroxidase method (18). Formalin-fixed paraffin-embedded tissue sections were deparaffinized, endogenous peroxidase activity was blocked with H_2O_2 in methanol and the sections were heated in 0.01 mol/l citrate buffer in a microwave pressure cooker for 20 minutes. The slides were allowed to cool to room temperature, and nonspecific binding was blocked with normal horse serum for 20 minutes at room temperature. The MIB-1 monoclonal antibody was used for detection of nuclear PCNA, a marker of proliferating cells (1:40, code No. M7187, Dako, Cambridge, UK). Anti-Bcl-2 (Dako) monoclonal antibodies were used for detection of bcl-2. Counterstaining was performed using Mayer's hematoxylin (Cat. No. 94585, BioGenex, Menarini Diagnostics, Antony, France).

Image analysis:

Digital images were analyzed by a semi-quantitative scoring system (Image J software, Java based application for analyzing images). The brown-stained immunohistochemical expressions of PCNA and bcl-2 were analyzed by measuring the percentage colored stained area per field area in five randomly high power fields at magnification of 400X

Biochemical studies:

For biochemical study, blood samples were collected in clean dry centrifuge tubes according to the retro-orbital plexus method (19). Blood samples were left to clot at room temperature, and then centrifuged at 3000 round per minute (rpm) for 20 minutes. Luteinizing hormone (LH) concentration in serum was measured by enzyme-linked immunosorbent assay (ELISA), according to Kosasa (20). Testosterone concentration in serum was measured by ELISA assay according to Sizonenko (21).

1) Statistical analysis

The data were expressed as mean ± standard error. Data were analyzed using Student's *t*-test and homogeneity of variances (Levene test) using statistical program of social science (SPSS) software for windows. $P < 0.05$, $P < 0.01$ and $P < 0.001$ values were used.

B. Results

Histopathology

Examination of the testes of control rats showed the typical features of normal seminiferous tubules, spermatogenic cells, intertubular connective tissue and spermatozoa (Fig.1a). Treating animals with ADR revealed different histopathological alterations. After 4weeks, testes of these animals showed congestion of blood vessels (Fig. 1b). Examination of sections of testes of rats treated with ADR for 6 weeks showed intertubular hemorrhage (Fig.1c). After 8 weeks, the alterations were much more pronounced. The intertubular space become congested with blood haemorrhage and possessed degenerative interstitial leydig cells in addition to appearance of vacuoles in between the germ layers (Fig. 1d). The seminiferous tubules were irregular and degenerated and there was decrease in their diameters (Fig .1e). Animals treated with ADR and basil showed that most of the seminiferous tubules appeared with large number of spermatogenic cells with an increase of sperms in comparison to ADR treated animals (Fig.1f).

Morphometry:

Figs (2 & 3) showed that treating animals with adriamycin caused a significant decrease in the diameter of the seminiferous tubules and in their epithelial heights, respectively. On the other hand, animals given adriamycin and basil extract manifested a highly significant increase ($P < 0.001$) in the diameters of the tubules and the epithelial heights than adriamycin treated group. However, No significant change was recorded in the diameter of the seminiferous tubules and their epithelial heights between control and basil treated rats.

Immunohistochemical observations:

Few expression of Bcl-2 was detected in Leydig cells in testes of control animals (Fig. 4a)**.** Testicular tissue obtained from ADR-treated rats for 8 weeks showed strong expression of Bcl-2 in Leydig cells in comparison with the control group (Fig. 4b). Treatment of animals with ADR and basil extract decreased the expression of Bcl-2 (Fig. 4c). A normal expression of PCNA was observed in control rats (Fig. 5a), whereas animals treated with ADR revealed decreased expression of PCNA (Fig. 5b). Animals treated with ADR and basil extract showed an increase in the expression of PCNA compared to ADR-administered rats (Fig.5c). Figures 6&7 showed the area percentage of Bcl-2 and PCNA expression in testes of animals after 8 weeks of treatment.

Biochemical results:

The means of serum testosterone levels in different groups were presented in figure (8). There was no statistical difference in testosterone level between control and basil extract treated groups. Testosterone level significantly ($P < 0.001$) decreased in sera of rats treated with ADR for 4, 6 and 8 weeks, when compared to the control group. When animals treated with both ADR and basil extract, significant elevation in testosterone level was observed as compared to ADR treated rats. Data in figure (9) showed that control group and basil extract treated group were nearly similar in their LH level at the same duration. Rats treated with ADR for 4, 6 and 8 weeks showed significant reduction in serum LH level ($P < 0.001$) when compared to control group. Treating animals with both ADR and basil extract revealed significant elevation in serum LH level when compared to ADR treated group.

3. DISCUSSION:

Adriamycin is a very potent chemotherapeutic drug which has been used against a variety of cancers. Despite its efficiency, ADR has been shown to cause death of healthy cells, especially those undergoing rapid proliferation. It has been shown that ADR causes germ cell death and seminal alterations (22,23). The present study indicated that ADR administration to rats induced histological alterations in the testes. The testes showed degeneration in seminiferous tubules with reduction in germinal epithelial layer, lack of spermatozoa, vascular degeneration of many spermatogonia, sertoli cell damage. The intertubular blood vessels were congested and the number of spermatogenic cells decrease with losting of their normal arrangement and exfoliation of degenerated cell in the lumen of the tubules. The sperm bundles were degenerated in some of the seminiferous tubules and completely absent in others. These results are in agreement with Sakr et al. (24) who found that animals i.p injected with a single dose of 10 mg/kg ADR caused many histopathological alterations in the testes of rats. Patil and Balaraman, (25) reported that ADR at a dose 3mg/kg once a week for 5 weeks caused vacuolization and fibrinoid debris in the seminiferous tubules. Shrunken seminiferous tubules showed loss of germ cell,widening of the interstitial space and severe vacuolization were also observed in interstitial tissues. The morphometric result of the testes in this study revealed that after treatment with ADR there was a significant decrease in the diameter of seminiferous tubules and their epithelial height compared with controls. This was similar to the result of Brilhante et al. (26) who studied the effect of ADR at dose 5 mg/kg for 15 and 22 days and showed a significant decrease in the diameter of seminiferous tubules. Furthermore, Sah et al. (27) demonstrated that there were significant decreases in the diameter of seminiferous tubules after given ADR 10 mg/kg body weight intra-peritoneally to male albino rats. ADR administration at dose (2 mg/kg/once a week) intraperitoneally, for 8 weeks caused significant decrease in reproductive organ weights, epididymal sperm concentration and motility, diameter of seminiferous tubules, germinal cell layer thickness (28).

Immunohistochemical results showed an increase in expression of Bcl-2 in Leydig cells in animals treated with adriamycin. Bcl-2 is specifically considered an important anti-apoptotic protein and has a role in governing the release of cytochrome c from the mitochondria (29). Apoptosis of Leydig cells is involved in the regulation of Leydig cell number and can be induced by cytotoxins (30). Also, adriamycin decreased PCNA positive staining spermatogonia cells. The diminishing in PCNA in testicular germ cells indicates the decrease in proliferative activity and spermatogenesis. These results confirmed those recorded by Uygur et al., (31**)**.

In the present study, a significant decrease in serum testosterone and LH after treatment with adriamycin, these results are in agreement with several authors. Hozayen (32) reported that administered rats with adriamycin (25 mg/ kg; three times interaperitoneally/week for two weeks) caused decrease in testosterone and LH level. High level of testosterone in testis is essential for the normal spermatogenesis as well as for the maintenance of the structural morphology and the normal physiology of seminiferous tubule (33).

There are several hypotheses to explain ADR-induced toxicity. Among them, the free radical hypothesis is the most thoroughly investigated. ADR undergoes one-electron reduction through a metabolic activation caused by NADPH cytochrome P-450 reductase or other flavin-containing enzymes in microsomes (34). This reduction generates ADR semiquinone free radicals. In the presence of molecular oxygen, the semiquinone rapidly reduces oxygen to superoxide, and the intact ADR remains. Superoxide spontaneously converts to hydrogen peroxide or is rapidly converted by superoxide dismutase. The effect of ADR on antioxidant enzymes was studied by many authors. Llesuy and Arnaiz (35) reported that doxorubicin induced decreases in antioxidant enzyme levels and the mechanism of its toxicity would involve a reduction in antioxidant defenses. Kalender et al. (36) studied the effect of ADR on major enzymes participating in free radical metabolism. They found that superoxide dismutase and catalase activity decreased while malondialdehyde levels increased in the ADR-treated group compared to control.

Examination of testes of rats exposed to combined treatment with ADR and basil extract revealed marked improvement in the histopathological, morphometric and immunohistochemical changes compared with those treated with ADR. Restoration of the histological structure and increase in the number of germ cell layers were observed in testes of animals treated with ADR and *O. basilicum* extract . This result runs in parallel with Sakr and Abdel samie. (37) who proved that aqueous extract of basil led to an improvement in histological, morphometric and immunohistochemical alterations in testes induced by diazinon in albino rats. Asuquo et al. (38) found that *O. gratissimum* extract improved the testicular histopathological alterations in diabetic rats. Sakr and Nooh (39) reported that O. *basilicum* extract alleviated cadmium-induced testicular damage and apoptosis in rats. Khaki et al. (40) reported that *O. basilicum* extract protected rats from testicular damage and reduced apoptosis after exposure to an electromagnetic field. In the present work, significant elevation in serum LH and testosterone levels in rats treated with both ADR and basil extract. This was in agreement with Ebong *et al*. (41) who reported that *O.gratissimum* leaf extract administration reversed the low level of testosterone and LH in diabetic animals towards normal.

Enzymatic and non-enzymatic antioxidants serve as an important biological defense against environmental pollutants (42). The antioxidant effects of O. *basilicum* was studied by several investigators. Treatment with *O. basilicum* extract reduced lipid peroxidation and increased the antioxidant enzymes, SOD and CAT in rats intoxicated with diazinon (37) and CCl_4 (43). Khaki, (44) reported that administration of *O. sanctum* extract to animals exposed to 50 Hz EMF resulted in a

significant decrease in LPO levels and significant increase in SOD, CAT, GPx, GSH and ascorbate levels.

Therefore, the results of the present work indicate that O. *basilicum* has protective effect against testicular toxicity induced by adriamycin and this is may be attributed to the antioxidant effects of its components.

REFERENCES

[1] Bagheri, S.; Bayat, Z.; Mahdizadeh, S. J. and Taghizadeh, E. Theoretical study on physicochemical and geometrical properties of the anti-cancer drugs doxorubicin and daunorubicin. J. Chem. Pharm. Res.,2011, 3(4): 370-374.

[2] Prahalathan, C.; Selvakumar, E.; Varalakshmi, P.; Kumarasamy, P. and Saravanan, R. salubrious effects of lipoic acid against adriamycin-induced clastogenesis and apoptosis in wistar rat bone marrow cells. Toxicol.,2006, 222(3): 225-232.

[3] Tacar, O.; Sriamornsak, P. and Dass, C. R. Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. J. Pharm. Pharmacol.,2013, 65(2): 157–170

[4] Yeh, Y.C.; Lai, H.C.; Ting, C.T.; Lee, W.L.; Wang, L.C.; Wang, K.Y. Lai, H.C and Liu, T.J. Protection by doxycycline against doxorubicin-induced oxidative stress and apoptosis in mouse testes. Biochem. Pharmacol, 2007,74(7):969–980.

[5] Shinoda, K.; Mitsumori, K.; Yasuhara, K.; Uneyama, C.; Onodera, H.; Hirose, M. and Uehara, M. Doxorubicin induces male germ cell apoptosis in rats. Arch Toxicol.,1999, 73(4-5): 274-281.

[6] Tallaj, J. A.; Franco, V.; Rayburn, B. K.; Pinderski, L.; Benza, R. L.; Pamboukian, S.; Foley, B. and Bourge, R. C. Response of doxorubicin-induced cardiomyopathy to the current management strategy of heart failure. J. Heart Lung Transplant,2005, 24(12): 2196-2201.

[7] Kumar, D. H. Lou. and P. K. Singal. Oxidative stress and apoptosis in heart dysfunction. Herz.,2002, 27(7): 662-668.

[8] Suter, L.; Bobadilla, M.; Koch, E. and Bechter, R. Flow cytometric evaluation of the effects of doxorubicin on rat spermatogenesis. Reprod. Toxicol.,1997, 11(4): 521–531.

[9] Howell, S. J. and Shalet, S. M. Spermatogenesis after cancer treatment: damage and recovery. J. Natl. Cancer Inst. Monogr.,2005, 34: 12-17

[10].Raskin, I.; Ribnicky, D.M.; Komarnytsky, S.; Ilic, N.; Poulev, A.; Borisjuk, N.; Brinker, A.; Moreno, D.A.; Ripoll, C.; Yakoby, N..; O'Neal, J.M.; Cornwell, T.; Pastor, I. and Fridlender, B. Plants and human health in the twenty-first century. Trends Biotechnol.,2002, 20(12): 522-531.

[11]Tewari, D.; Pandey, H.K.; Sah, A. N.; Meena, H.S.; Manchanda, A. and Patni. P . pharmacognostical, Biochemical and Elemental investigation of Ocimum basilicumplants available in western Himalayas International Journal of Research in Pharmaceutical and Biomedical Sciences, 2012,3(2): 840-845.

[12]Trevisan, M.T.; Vasconcelos Silva, M.G.; Pfundstein, B.; Spiegelhalder, B. and Owen, R.W. Characterization of the volatile pattern and antioxidant capacity of essential oils from different species of the genus Ocimum. J. Agric. Food Chem.2006, 54(12):4378-4382.

[13]Sifola, M.I. and Barbieri, G.G. Yield and essential oil content of three cultivars of basil grown under different levels of nitrogen in the field. Scientia Horticulturae,2006, 108:408–413

[14]Taie, H.A.A.; Salama, Z.A. and Radwan, S. Potential activity of basil plants as a source of antioxidants and anticancer agents as affected by organic and bio-organic fertilization. Not. Bot. Hort. Agrobot. Cluj.,2010, 38(1): 119-127.

[15]Manaharan, T.; Thirugnanasampandan, R.; Jayakumar, R. Kanthimathi, M.S.; Ramya, G. and Ramnath, M.G. Purified Essential Oil from Ocimum sanctum Linn. Triggers the Apoptotic Mechanism in Human Breast Cancer Cells. Pharmacogn Mag.,2016, 12(Suppl 3):S327-31.

[16]Sethi, J.; Sood, S.; Seth, S. and Thakur, A. Protective effect of Tulsi (Ocimum sanctum) on lipid peroxidation in stress induced by anaemic hypoxia in rabbits. Ind. J. Physiol. Pharmacol. 2003; 47(1): 115-119.

[17]Offiah, V.N. and Chikwendu, U.A. Antidiarrhoeal effects of Ocimum gratissimum leaf extract in experimental animals. J Ethnopharmacol.,1999, 68(1-3):327-330.

[18]Ramos-Vara, J.A. Principles and methods of immunehisto-chemistry. Methods Mol Biol.,2011, 691:83-96.

[19]Schermer, S. The Blood Morphology of Laboratory Animals. 3rd Ed., F.A Davis Company, Philadelphia; 1967, p. 42.

[20]Kosasa, T.S. Measurement of Human Luteinizing Hormone. J.Reproductive Medicine,1981, 26: 201-6.

[21]Sizonenko, P.C. Normal sexual maturation. Pediatrician,1987, 14:191-201.

[22].Kato, M.; Makino, S.; Kimura, H.; Ota, T.; Furuhashi, T. and Nagamura, Y. Sperm motion analysis in rats treated with adriamycin and its applicability to male reproductive toxicity studies. J. Toxicol. Sci., 2001,26(1): 51-59.

[23]Kang, Y.J.; Sun, X.; Chen, Y. and Zhou, Z. (2002): Inhibition of doxorubicin chronic toxicity in catalase-overexpressing transgenic mouse hearts. Chem. Res. Toxicol., 15(1):1-6.

[24]Sakr, S. A.; El-shenawy, S.M and Al-Shabka, A.M. Aqueous Fenugreek Seed Extract Ameliorates Adriamycin-Induced Cytotoxicity and Testicular Alterationsin Albino Rats, Reproductive Sciences, 2012,19(1): 70-80.

[25].Patil, L. R. and Balaraman, R. Effect of melatonin on d oxorubicin induced testicular damage in rats. Int. J. Pharm.Tech. Res 2009,1(3):879-84.

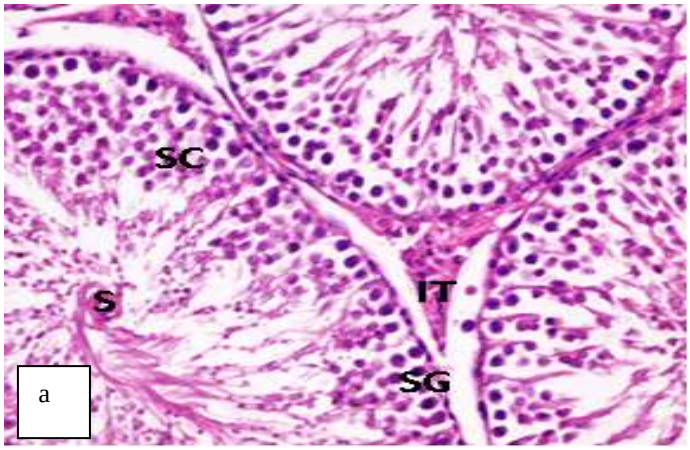
[26]Brilhante, O.; Stumpp, T. and Miraglia, S. Long-term testicular toxicity caused by doxorubicin treatment during pre-pubertal phase. Int J Medicine Med. Sci.,2011, 3(2): 52-60.

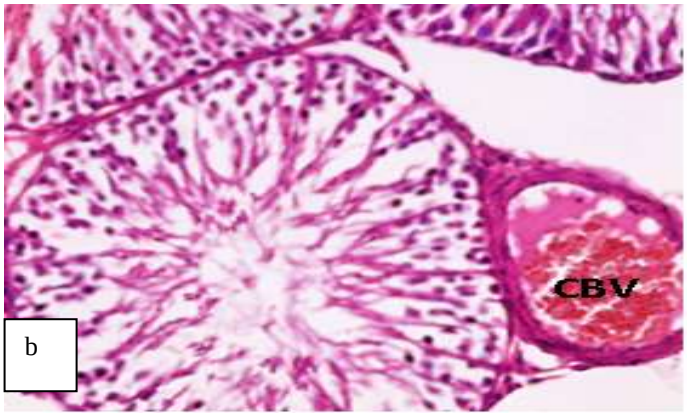
[27]Sah, S. K.; Khatiwada, S.; Chaudhary, D.; Jha, C.B. and Bhattacharya, S. Doxorubicin induced histomorphometric changes in testes of albino rats. Nepal J. Biotechnology,2015, 3(1):10-14.

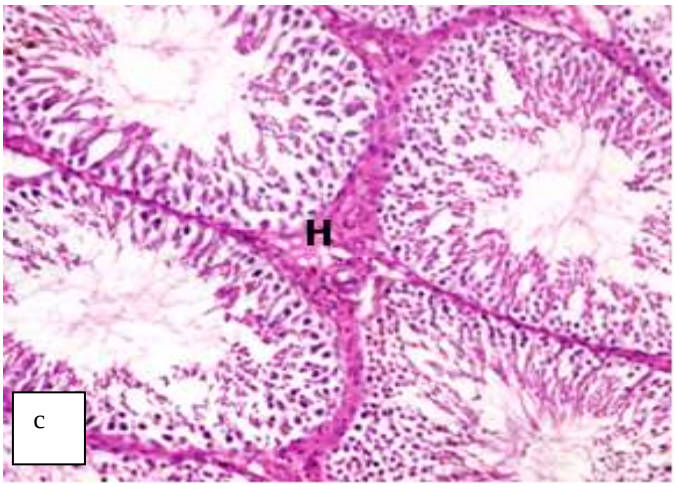
[28]Ceribaşi, A.O.; Sakin, F.; Türk, G.; Sönmez, M. and Ateşşahin, A. Impact of ellagic acid on adriamycin-induced testicular histopathological lesions, apoptosis, lipid peroxidation and sperm damages. Exp. Toxicol. Pathol.,2012, 64(7-8):717-24.

[29]Cory, S. and Adams J. M. The Bcl2 family: regulators of the cellular life-or-death switch. Nat. Rev. Cancer,2002, 2(9): 647-656.

[30]Haider, S. G. Cell biology of Leydig cells in the testis. Int. Rev. Cytol.,2004, 233: 181-241
[31]Uygur, R.; Aktas, C.; Tulubas, F.; Uygur, E.; Kanter, M.; Erboga, M.; Caglar, V.; Topcu, B. and Ozen, O.A. Protective effects of fish omega-3 fatty acids on doxorubicin-induced testicular apoptosis and oxidative damage in rats. Andrologia.2014, 46(8):917-926.
[32]Hozayen. W.G. Effect of Hesperidinand Rutin on Doxorubicin Induced Testicular Toxicity in Male Rats. international journal of food and. nutrition science,2012, 1(1): 31-42.
[33] Sharpe, R.M.; Maddocks, S.; Millar, M.; Saunders, P.T.K.; Kerr, J.B. and Mckinnell, C. Testosterone and spermatogenesis: identification of stage dependent, androgen- regulated proteins secreted by adult rat seminiferous tubules. J Androl.,1992,13:172-184.
[34] Blum, R. H. and Carter, S. K. Adriamycin. A new anticancer drug with significant clinical activity. Ann. Intern. Med.,1974, 80(2): 249-259.
[35] Liesuy, S. F. and Arnaiz, S. L. Hepatotoxicity of mitoxantrone and doxorubicin. Toxicol.,1990, 63(2): 187-198.
[36] Kalender, Y.; Yel, M. and Kalender, S. Doxorubicin hepatotoxicity and hepatic free radical metabolism in rats. The effects of vitamin E and catechin. Toxicol.,2005, 209(1): 39-45.
[37]Sakr, S. A. and Abdel-samie, H.A. Protective effect of *ocmuim basilicum* leaves extract on diazinon-induced reproductive toxicity and oxidative stress in albino rats. EJPMR,2015, 3(1): 375-384
[38]Asuquo, O.R.; Edet, A.G.; Mesembe, O. and Atanghwo, J.I. Ethanolic extracts of Vernonia amygdalina and Ocimum gratissimum enhance testicular improvement in diabetic Wistar rats. Internet J Altern Med.,2010,8(2):1-6.
[39]Sakr, S. A. and Nooh, H.Z. Effect of *Ocimum basilicum* extract on cadmium-induced testicular histomorphometric and immunohistochemical alterations in albino rats. Anatomy & Cell Biol., 2013,46(2): 122–130.
[40]Khaki, A.; Fathiazad, F.; Nouri, M. and Khaki, A.A. Effect of *Ocimum basilicum* on apoptosis in testis of rats after exposure to electromagnetic field. Afr J Pharm Pharmacol,2011, 5(12):1534-1537.
[41]Ebong, P. E.; Efiong, E. E.; Mgbeje, B. I. A.; Igile, G. O. and Itam, E.H. Combined therapy of Moringa oleifera and Ocimum gratissimum reversed testicular damage in diabetic rats. British Journal of Medicine & Medical Research,2014, 4 (11): 2277- 2290.
[42]Tabrez, S. and Ahmad, M. Some enzymatic/ nonenzymatic antioxidants as potential stress biomarkers of trichloroethylene, heavy metal mixture, and ethyl alcohol in rat tissues. Envir. Toxicol.,2011, 26(2): 207-216.
[43]Ogaly H.A., Eltablawy N.A., El-Behairy A.M., El-Hindi H. and Abd-Elsalam R.M. Hepatocyte Growth Factor Mediates the Antifibrogenic Action of *Ocimum bacilicum* Essential Oil against CCl4-Induced Liver Fibrosis in Rats. Molecules, 2015; 20: 13518-13535
[44] Khaki, A. Protective effect of *Ocimum basilicum* on brain cells exposed to oxidative damage by electromagnetic field in rat: ultrastructural study by transmission electron microscopy. Crescent J. Med. Biol. Sci.,2016, 3(1): 1-7.







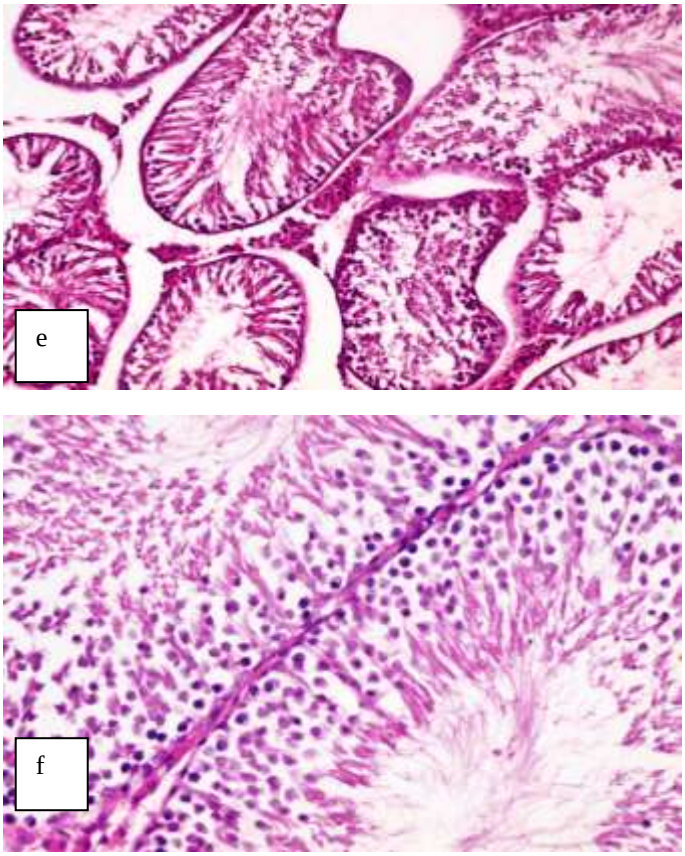


Fig.1. a). Section in testis of a control rat showing normal structure of seminiferous tubules containing different stages of spermatogenesis, SG: spermatogonia, IT: interstitial tissue, SC: spermatocyte, Sp: spermatozo, **b).** adriamycin treated rat after 4 weeks showing congested blood vessel (CBV), **c)**. adriamycin treated rat after 6 weeks showing blood haemorrhage (H) in the intertubular connective tissue, **d).** adriamycin treated rat after 6 weeks showing haemorrhage(H), congested intertubular blood vessels (arrow) and presence of vacuoles(V) (H&E X400). **e)**. adriamycin treated rat after 8 weeks showing irregular shape of seminiferous tubules (H&E X200). **f)**. testis of a rat treated with adriamycin and basil extract showing great improvement in histological appearance of spermatogenic cells, increase of sperms bundle and seminiferous tubule nearly have appearance (H&E X400)

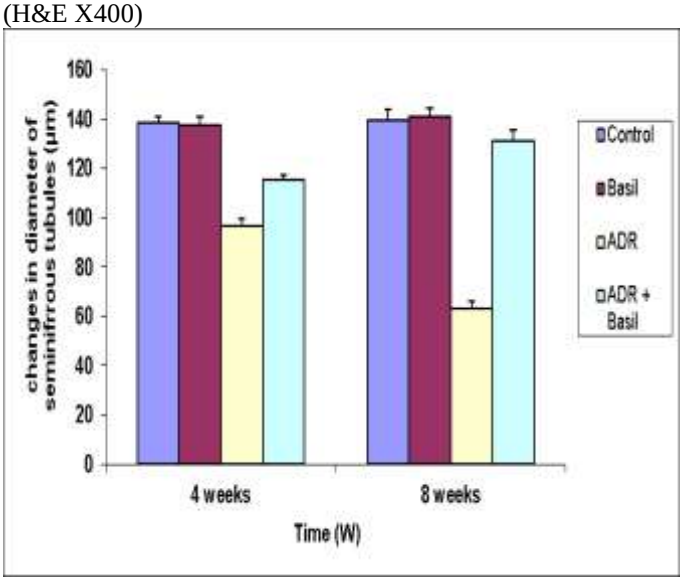


Fig. 2. The effect of different treatments on the diameter of seminiferous tubules

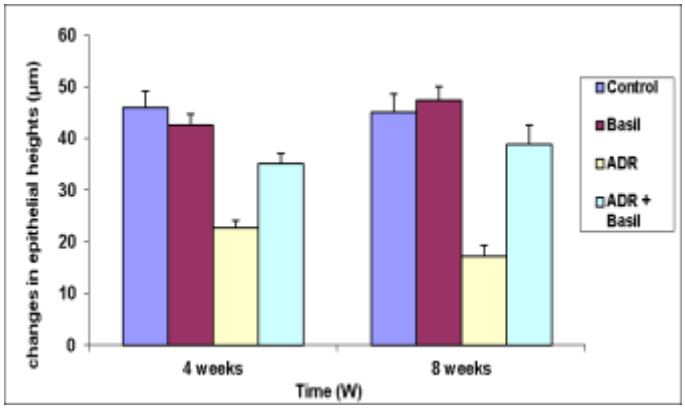


Fig. 3. The effect of different treatments on the height of epithelium of seminiferous tubules.

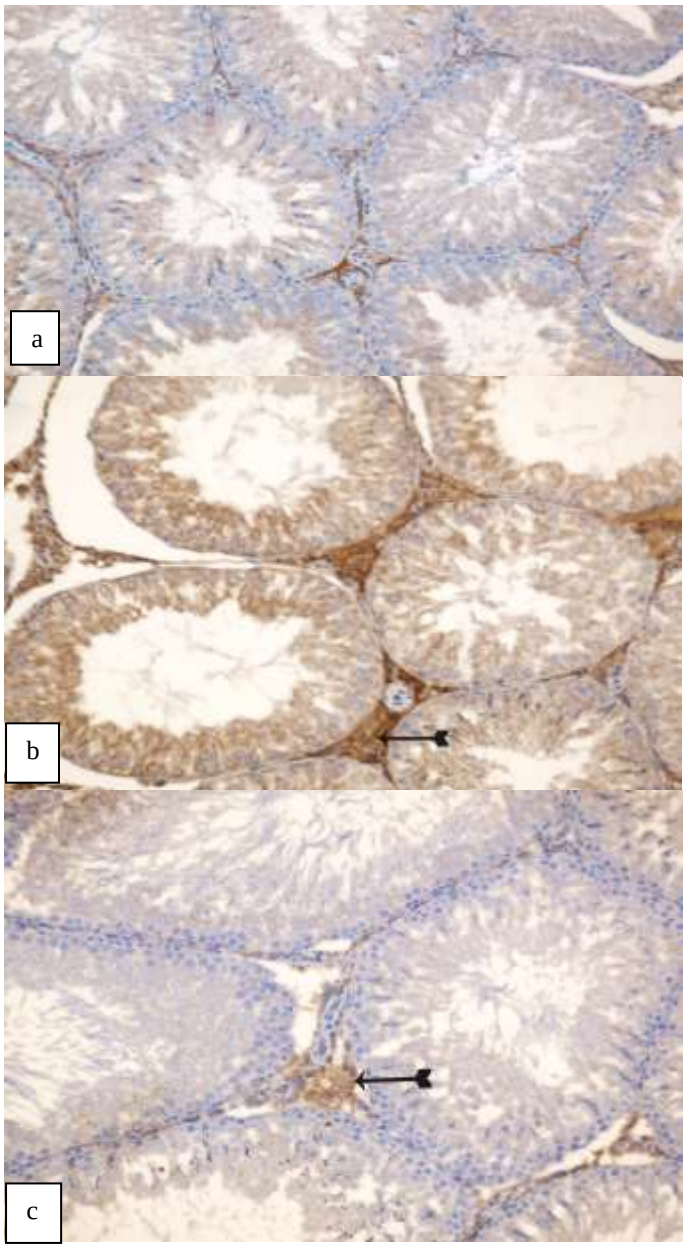


Fig. 4.a). Few expression of Bcl-2 in Leydig cells in testes of control rats, **b).** Increase in expression of Bcl-2 in Leydig cells (Arrow) of a rat after 8 weeks of treatment with adriamycin, **c)**. Decrease in expression of Bcl-2 in Leydig cells (Arrow) of a rat after 8 weeks of treatment with adriamycin and basil extract (immunohistochemical stain, X200).

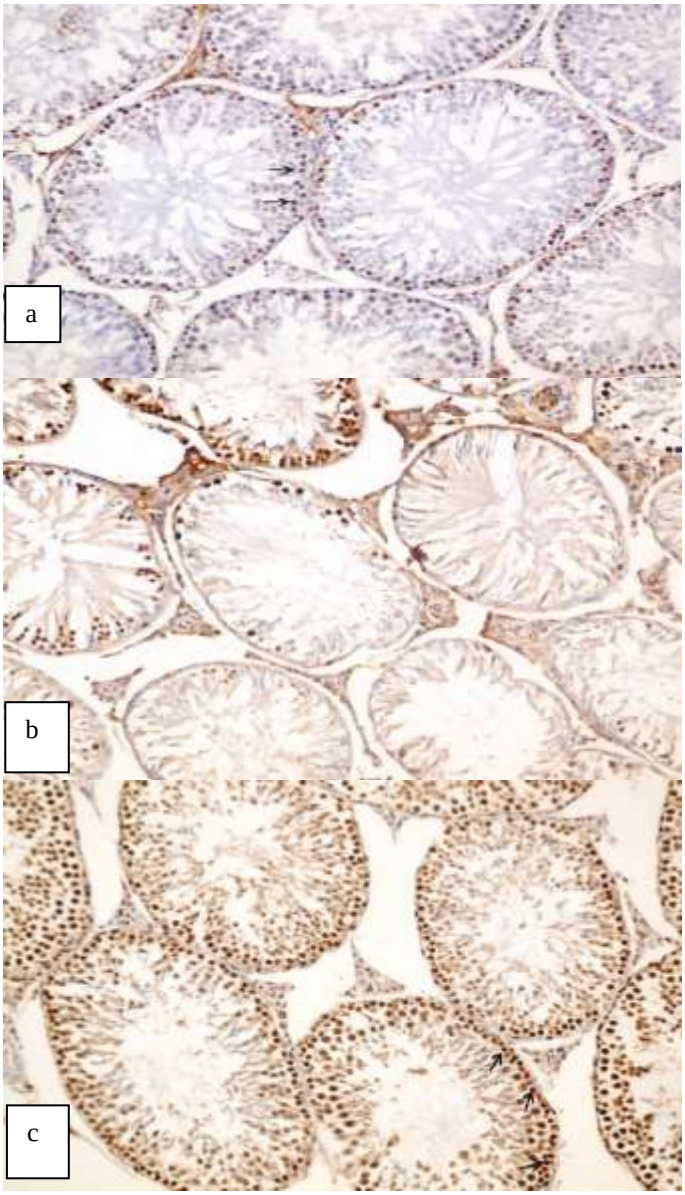


Fig. 5.a). Section in testis of a control rat showing marked expression of PCNA (Arrows), **b).** marked decrease in expression of PCNA in spermatogonia of a rat treated with adriam ycin for 8 weeks, **c)** increase in expression of PCNA in spermatogonia (Arrows) of a rat treated with adriamycin and basil extract for 8 weeks in compared with adriamycin group (immunohistochemical stain, X200).

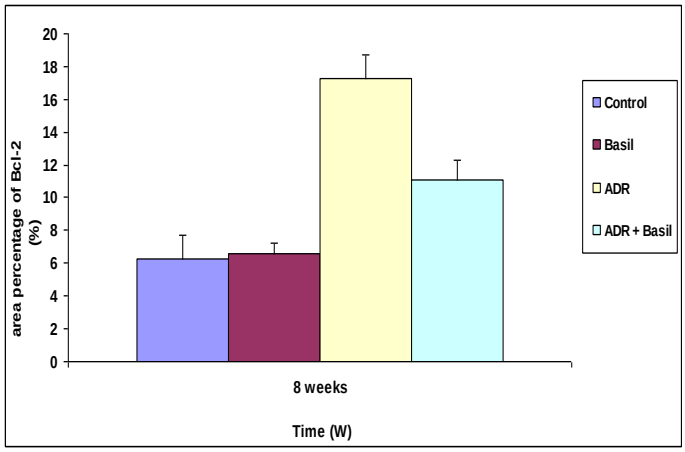


Fig. 6. The area percentage of Bcl-2 expression in testes of animals after 8 weeks of treatment

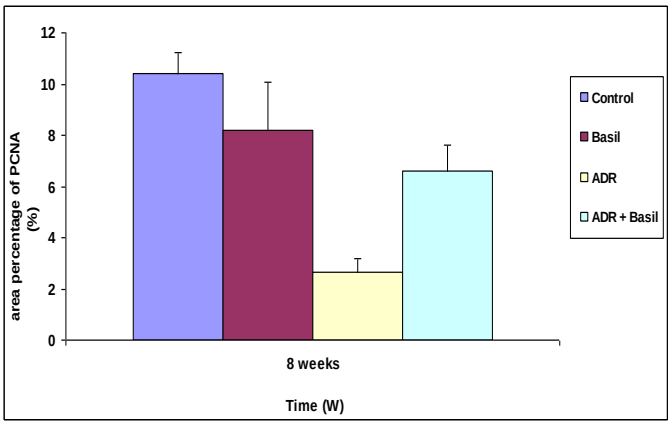


Fig. 7. The area percentage of PCNA expression in testes of animals after 8 weeks of treatment.

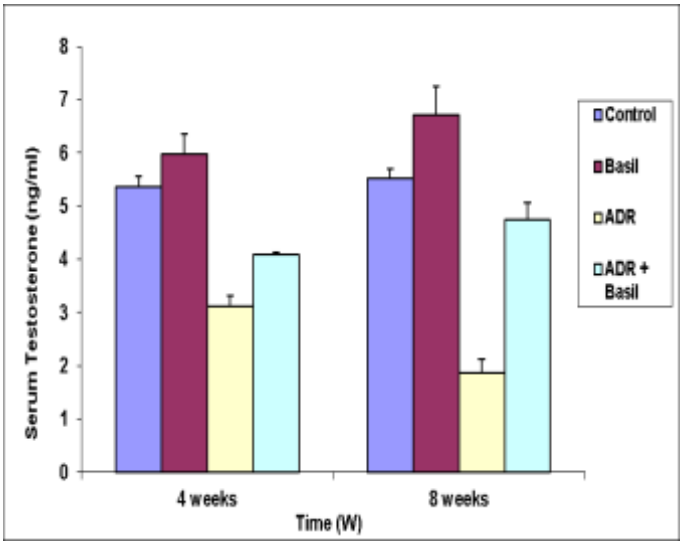


Fig. 8. Effect of Adriamycin and basil on serum testosterone.

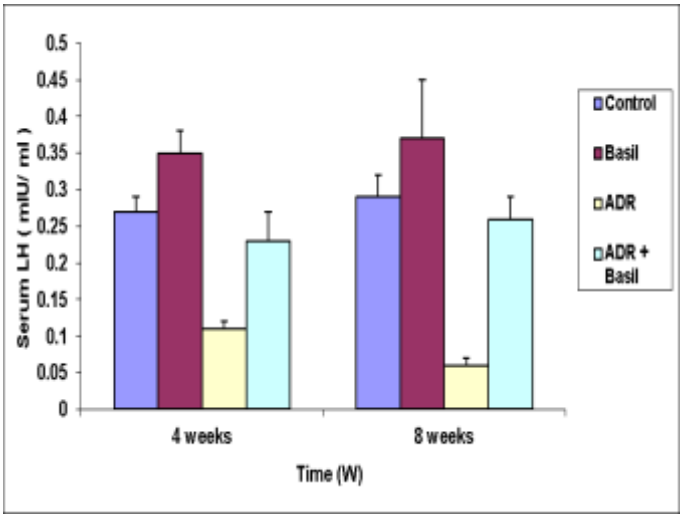


Fig. 9. Effect of Adriamycin and basil on serum LH