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Toxic Effects of anAnticancer Drugs Doxorubicin and Cyclophosphamide on Seminal Vesicle

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Abstract

Anticancer drugs Doxorubicin and Cyclophosphamide are widely used to treat different types of cancer. The goal of present study is to elucidate toxic effects of Doxorubicin and Cyclophosphamide on accessory male sex gland, seminal vesicle and testosterone level.For this male Wistar rats were divided into experimental (5mg and 10mg/KgBW drugs for 10days) and control group,received drugs intraperitoneally.Treated rates revealed remarkable alteration in the histopathology ofseminal vesicle and significant decrease in testosterone levels. Thus Doxorubicin and Cyclophosphamide treatment resulted into dose and duration dependent histopathological changes in the seminal vesicle as well as on testosterone concentration.

Keywords: Doxorubicin, Cyclophosphamide, Seminal vesicle, Testosterone.

Introduction

The seminal vesicles (SVs) are among the most important male accessory sex glands which produce and act as reservoir for seminal fluid (Mckay and Sharma, 2018). They are located in the pelvis superior to the rectum, inferior to the fundus of the bladder and posterior to the prostate. Each seminal vesicle consists of a single, coiled, blind-ending tube giving off several irregular pouches. Histologically, the seminal vesicles are composed of 3 layers. These include an inner mucosal layer, consisting of pseudostratified columnar epithelium with goblet cells and a lamina propria; a muscular

layer, with an inner circular and outer longitudinal smooth muscle arrangement; and finally, an outer adventitial layer composed of loose areolar tissue.Seminal vesicles are an androgen dependent organ that secretes a significant fraction of the fluid that eventually becomes seminal fluid(Bromfield, *et.al.*, 2018; Mckay and Sharma, 2018). In most species the major contribution to semen volume is provided by the seminal vesicle glands, in human seminal vesicle glands contribute between 70% and 85% of ejaculate volume, bull seminal vesicle glands produce approximately 50% of ejaculate volume, and in the mouse seminal vesicle glands produce approximately 90% of the ejaculate volume (Kierszenbaum and Tres, 2011). Seminal vesicular secretion is rich in fructose, proteins, prostaglandins, complex carbohydrates and enzymes involved in the clotting of the ejaculate (Gonzales and Villena, 2001). It also provides nutrients for the spermatozoa and optimizes the conditions for transport, sperm motility, viability, elimination of non-viable spermatozoa from the uterus in both the male and female reproductive tracts (Zubkova and Robaire, 2004; Troedsson *et al*., 2005).

Doxorubicin and Cyclophosphamide are anticancer drugs used for the treatment of different types of the cancer. Doxorubicin, an anthracycline antibiotic, is a widely used chemotherapeutic agent. It is well-known to cause innocent organ/tissue damage due to its cumulative toxicity. It alters sperm development, production, structural integrity and motility rates in association with increased cellular apoptosis (Bromfield, 2018).Cyclophosphamide belonging to the class of Oxazaphosphorines is a bioactivated metabolite and alkylating agent that show cytostatic effects by forming covalent DNA adducts. The cytotoxicity of Cyclophosphamide is mediated by alkylation of DNA at the N7 position of guanine and the formation of DNA–DNA cross-links, DNA–protein cross-links, and single and breaks (Crook *et al*., 1986).The present piece of work is carried out to study the toxic effect of Doxorubicin and Cyclophosphamide on seminal vesicle and on testosterone level.

Materials and Methods

Drugs

Doxorubicin hydrochloride injection by Oplax (50 mg/25 ml) Markans Pharma Ltd., Mumbai, India.

Cyclophosphamide, an anticancer drug, with the chemical formula C7H15Cl2N2O2P and molecular weight, 261.086 g/mol., manufactured by Candila Healthcare Limited, Goa was used.

Experimental Animals

Adult, healthy Wistar rat, *Rattus norvegicus*, with average body weight of 200-250g obtained from Shree Farma, Bhandara (MS) were used for the study. Animals were maintained in the laboratory under an absolute hygienic condition as per the recommended ethical standards. They were housed in polypropylene box cages, bedded with rice husk and kept at constant temperature 28±2°C and relative humidity with 12h light: 12h dark cycle. They were fed with pelleted diet and water ad libitum.

Treatments

Various sets of experiments were performed for each drug and were compared with the vehicle treated control. The drugs were administered intraperitoneally. The doses used are summarized in Table 1.

Table 1: Experimental Design for control and experimental animals

No. of animals and sex	Treatment	Dose (mg/KgBW)	Route	Duration
6 Male (Control)	Saline	E.V.	I.P.	10 days
6 Male (Experimental)	Doxorubicin	5mg	I.P.	10 days
6 Male (Experimental)	Doxorubicin	10mg	I.P.	10days
6 Male (Experimental)	Cyclophosphamide	5mg	I.P.	10 days
6 Male (Experimental)	Cyclophosphamide	10mg	I.P.	10 days

Abbreviations: - E. V. = Equal Volume, I.P. = Intraperitoneal, B.W. = Body Weight

Histological assessment

Animals were sacrificed using chloroform 24 hours after the last day of each experiment. Immediately the seminal vesicles were excised, fixed in Bouin's fluid for 24hrs and post preserved in 70% alcohol. The tissues were dehydrated by passing through graded series of alcohol, cleared in xylol and after embedding in paraffin blocks were prepared and cut in numerous parallel 5µm sections. For routine histological study the sections were stained with Ehrlich's haematoxylin and counter-stained with eosin.

Enzyme-Linked Immunosorbent Assay for Measurement of Testosterone

For the determination of testosterone level in blood, rats were anesthetized by ether and 2 ml of blood was drawn by cardiac puncture with sterile syringe. The blood was allowed to clot at room temperature for half an hour. The clotted blood was then used for measurement of serum testosterone by ELISA (Delahunt and Hirsutism, 1993).

Statistical Analysis

To indicate individual variations in each corresponding region, the mean values and standard deviation (mean ± SD) were calculated from six animals. The statistical significance of differences for these values in different regions were assessed using 't-test' (Dalgaard, 2008). A significant level of $P < 0.05$ was accepted.

Results

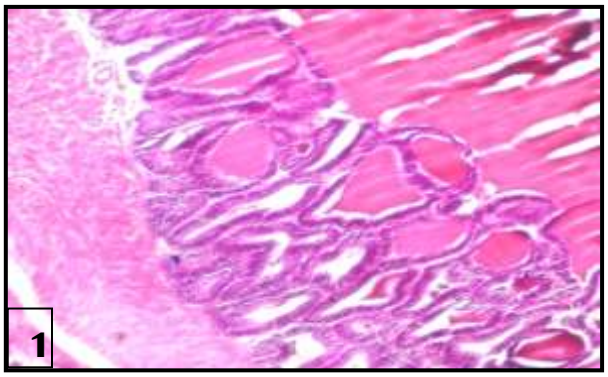
Doxorubicin and Cyclophosphamide treatments reveal the behavioral changes such as sluggishness, low appetite, withdrawn mood, hair fall of body and oral mucositis etc., however, mortality rate were zero percent. A decrease in weight of body and seminal vesicle was notified in both the treated group of animals in comparison to controls being significant for higher dose

Histopathological study of Seminal vesicle

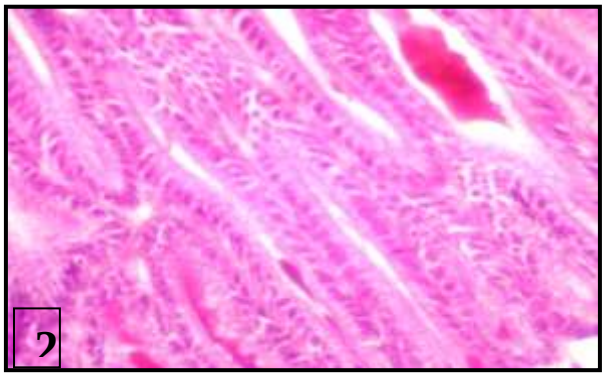
Vehicle treated control group

The seminal vesicle in rat is a paired curved structure, about 2-3cms in height, one end draining into urethra, the gland is ensheathed into thick fibro-muscular connective tissue. The seminal vesicle of the control rat is composed of a large number of acini embedded within the fibromuscular connective tissue. The acini were lined by tall columnar epithelial cells containing a prominent basal nucleus. Few basal cells, almost rounded in shape and basal in position were also observed between the columnar epithelial cells. Large number of dense secretory granules were visible in the apical cytoplasm. The lumen of acini was filled with the darkly stained secretory material. Lamina propria surrounding the epithelial cells was comprised of cellular connective tissue containing some smooth muscles rich in elastic fibres (figs.1 and 2).

Vehicle treated control



Seminal vesicle is a saccular gland composed of central cavity and peripheral pouches of anastomosing glandular structures filled with copious amount of secretion. The gland is encapsulated in thick connective tissue capsule X 100.



Mucosal folds lined by tall columnar epithelium. Few basal cells are present in between the columnar epithelial cells X 400.

Doxorubicin treated group

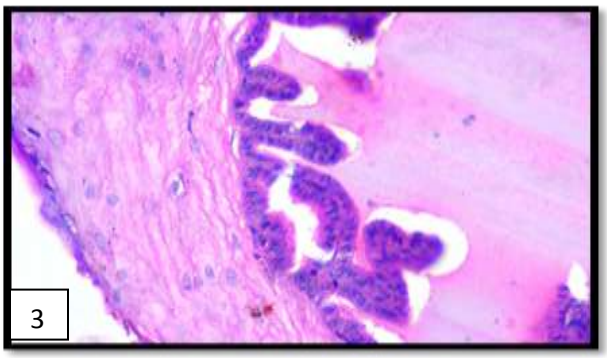
5mg/KgBW treated group

There was a remarkably thickened fibrous peripheral connective tissue encapsulating the acini with infiltration of lipid globules which further progressed to the epithelium appearing as groups of cells having flame-like appearance with tapering cytoplasm, syncytium and with a look of "orphan annie eye" simulating papillary carcinoma (figs.3 to 4).

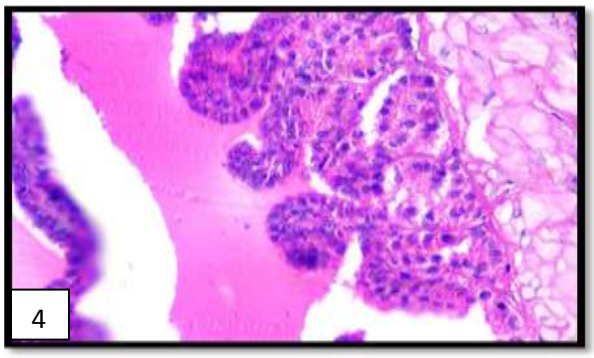
10mg/KgBW treated group

The treatment has caused heavy thickening of fibro-muscular tissue encapsulating the gland. Restriction in the ramification of secretory epithelium resulted into scanty secretion. Blood vessels showed vasodilation as well as fibrosis of the wall and apoptosis of the endothelial cells in other arteries (figs.5 and 6).

5mg//KgBW treatment with Doxorubicin

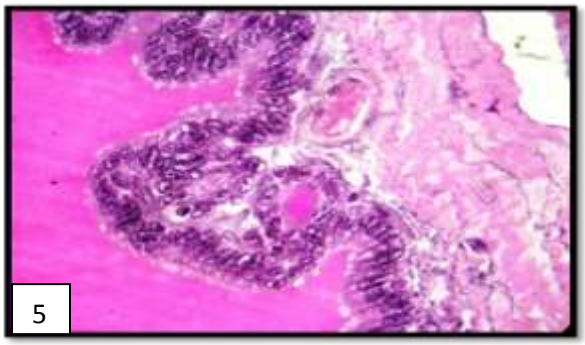


Remarkably thickened fibrous peripheral connective tissue encapsulating the acini, nuclei regressed and disorganized X100

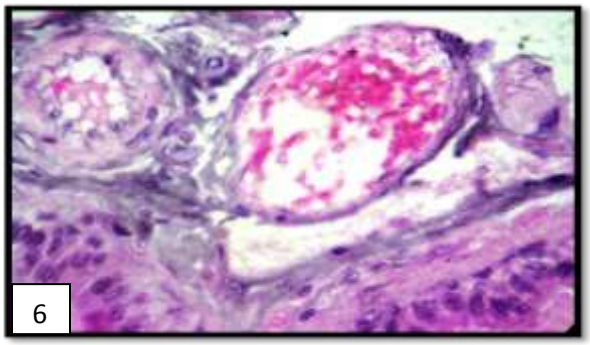


Another foci from the regimen. Lipid infiltration of the fibro-muscular tissue. Secretory epithelium shows "Flame cells" with tapering cytoplasm leading to carcinoma X400.

10mg//Kg BW treatment with Doxorubicin



Peripheral fibrous muscular tissue demonstrates lipid infiltration.



Arteriosclerotic alterations of hyperthermic blood capillaries, one vein and other artery showing vasodilation typically with thickened perivascular wall.

Cyclophosphamide treated group

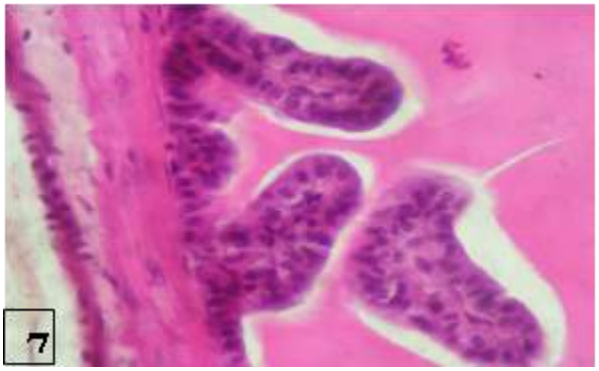
5mg/KgBW treated group

5mg/KgBW Cyclophosphamide dose resulted in the restriction of the secretory epithelial cells of the seminal vesicle with regression in its height, depletion in the quality of the colloidal secretion, its condensation. The nuclei underwent pyknosis and displacement (figs. 7 and 8).

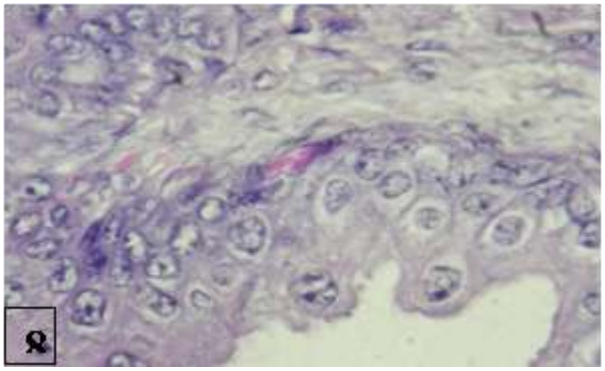
10mg/KgBW treated group

Increase in the dose level resulted in the formation of autophagic vesicle in the mucosal folds of seminal vesicle. These sequestrated autophagic vesicles at advance stage of development underwent intra-lysosomal degradation bathing in the lumen containing recognizable fragments of cytoplasm (figs. 9 and 10).

5mg//KgBW treatment with Cyclophosphamide

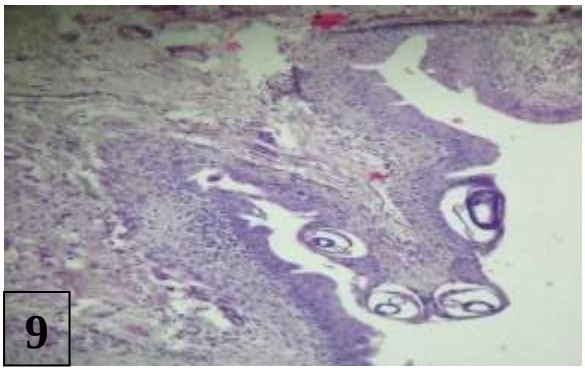


5mg/KgBW treatment resulted into restriction in the ramification of the secretory epithelium, regression in the epithelium height, less secretory zone, therefore depletion in the quality of colloid, condensation, pyknosis and displacement of nuclei.

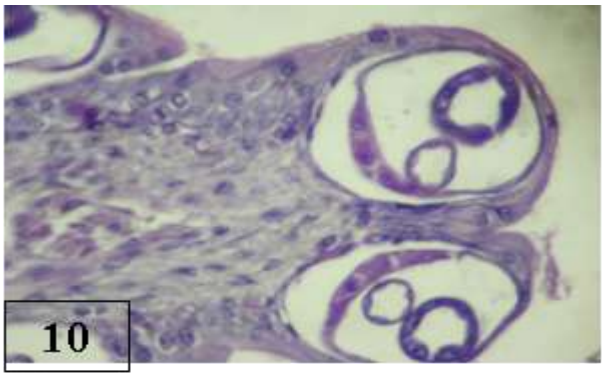


Protruding monstrous (monster) epithelial cells (MEC) of the seminal vesicle in a clustered pattern with acidophilic cytoplasm, hyperchromatic nuclei, lipochrome pigment in the cytoplasm, very large bizarre nuclei simulating carcinoma cells X 1000.

10mg//Kg BW treatment with Cyclophosphamide



Mucosal fold demonstrating autophagic vacuoles at advanced stage of development X 400.



Sequestrated autophagic vacuoles at late stage of intra lysosomal degradation bathing in the lumen containing recognizable fragments of cytoplasm X 400

Testosterone

Level of the testosterone was significantly decreased after the high doses of Doxorubicin and Cyclophosphamide treatment when compare with control.

Discussion

The primary function of the seminal vesicles (SVs) gland is to produce a supportive fluid to maintain sperm viability and functionality during transport through the male reproductive tract at ejaculation and female reproductive tract to facilitate fertilization of the ovum and nutrition of sperm as well as (in rodents) the formation of a copulatory plug after ejaculation (da Silva *et al*., 2016).Seminal vesicle has a highly convoluted pseudostratified columnar epithelium with active protein secretory machinery. Also secretes fructose, prostaglandins, metallothionein-1 (Mt-1), and transglutiminase-4 (TGM4) (Jonsson *et al*., 2006; Kawano and Yoshida, 2007; Balaji *et al*., 2008). SVs secretory functions are androgen dependent (Cunha and Donjacour, 1987), however, castration or any chemical insult results in involution due to, cytological degeneration, apoptosis, gross reduction in secretion. Alteration in androgen-estrogen balance can also affect adult male accessory sex organs resulting in aberrant histological changes and even prostatic squamous metaplasia (Rivas *et al*,. 2003; Bianco *et al*., 2006) as noticed in the present work.

The seminal vesicle in the control rats appeared consisting of a fan-like structure embedded within the fibro-muscular connective tissues. The alveoli were filled with copious amount of colloid and were intricately ramified. The secretory epithelium was pseudostratified and trabeculate. The secretory crypts were lined by tall columnar epithelium, basal nuclei but large supra-nuclear secretory zone. After the administration 5mg and 10mg/KgBW Doxorubicin, there were an increase in the peripheral connective tissue, lipid infiltration, restriction in the ramification of the secretory epithelium and similarly after the administration of 5mg and 10mg/KgBW Cyclophosphamide, there were an increase in the peripheral connective tissue, restriction in ramification of the secretory epithelium, condensation and pyknosis and displacement of nuclei, monstrous epithelial cells and autophagic vacuoles.

There were reduction in the amount of colloid, the regressed epithelium in both drug treatments showed less secretory zone directly suggesting the depletion of secretory activity, condensation, pyknosis, and displacement of nuclei.After the Doxorubicin and Cyclophosphamide treatment for 5 days, there was an increase in the peripheral connective tissue, too far restriction in the ramification of the secretory epithelium and similarly there was reduction in the amount of colloid. The regressed epithelium showed less secretory zone directly suggesting the depletion of secretory activity, condensation, pyknosis, and displacement of nuclei. The remarkable thickening of the fibromuscular coat around each acinus along with the stroma indicates that it plays an important role in hormonal

functioning of the accessory glands, suggestive of an alteration in the function of accessory glands. The reduction or the shrinkage of the acini were in equivalence with the increase of fibro-muscular tissue defining stromal-epithelial interaction (Dünker and Kreiglstein, 2000; Sastry and Kashmiri, 2011).According to Sridevi *et al*., 2012 doxorubicin caused histopathological changes and impaired seminal vesicle function, the changes were transitory and could be revert back to normal if sufficient withdrawn time is given.

The abundant development of the fibro-muscular tissue around the gland as well as in between the acini suggests as observed in the present study, a disturbance of epithelial-stromal interactions. Stromal-epithelial interactions play a critical role in the development of accessory glands such as prostate and Cowper's similar to seminal vesicle, and a factor transforming growth factor-beta-2 (TGF-β) has been suggested to be a potential mediator of these interactions (Blanchere *et al*., 2001).

Androgens and stromal-epithelial interactions are pivotal in the development and function of the male reproductive system (Mooradian *et al*., 1987); disruption of these can result in pathologies later in life. Epithelial cells are normally pseudostratified and columnar in adult seminal vesicle, with epithelial cell height reported to be androgen dependent (Nishino *et al*., 2004), possibly acting as a more sensitive marker of androgen action than gross changes in gland weight (Nishino *et al*., 2004). Androgen receptor (AR) from smooth muscle cells increase epithelial cell proliferation, implying that androgen signalling via the smooth muscle normally suppresses epithelial cell proliferation, this may be a direct effect on the epithelial cells themselves or an indirect effect due to changes in the proportion of smooth muscle in the seminal vesicle, i.e. as reported in the prostate (Lee, 1996). Interestingly, epithelial cell-specific ablation of AR in the prostate also results in increased proliferation. These results could imply that epithelial cell proliferation is differentially regulated in the prostate and seminal vesicle or that it is a balance of androgen action via either epithelial and/or smooth muscle cells that is important and impairing either is detrimental to normal tissue homeostasis.

Serum testosterone level decreased by the after Doxorubicin and Cyclophosphamide administration may affect directly, perhaps through hormone target cell interaction or the expression of seminal vesicle proteins. Alternatively Doxorubicin may affect the metabolism of an androgen which is important for the functional normality of accessory glands (Welsh *et al*., 2010) and also proved by castration effect or the sex organs are androgen dependent and thus reflect the availability of androgens. The Doxorubicin and Cyclophosphamide cause reduction in weights of accessory sex organs which

indicates the atrophy of glandular tissue and also reduction in secretory ability, thus reflecting the decreased level of testosterone as reported earlier as well as in the present study (Abdella and Ahmad, 2009; Hozayen, 2012; Das *et al*., 2012; Sastry and Kashmir, 2011; Kashmiri and Sastry, 2015).

Theabove mentioned changes also points to androgen dependency of the gland since the male accessory glands are highly dependent on androgenic hormones to maintain their normal structure and function and is also very sensitive to the level of circulatory androgen. HenceDoxorubicin and Cyclophosphamide treatment resulted into dose and duration dependent histopathological changes in the seminal vesicle as well as on testosterone concentration.

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