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MALE REPRODUCTIVE TOXICITY OF DMPA ON SEMINAL VESICLE OF INDIAN PALM SQUIRREL, *Funambulus pennanti*

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ABSTRACT
The effect of Depot Medroxy Progesteron Acetate (DMPA) on the seminal vesicle of male squirrel was investigated and an attempt was made to assess whether testosterone could be used to protect seminal vesicle from DMPA toxicity. The changes in the seminal vesicle consisted of regression of the gland, degeneration of the epithelium and invasion of macrophages into epithelium as well as lumen, restriction in the ramification of secretory epithelium with less secretory zone, increased peripheral connective tissue suggesting DMPA's role of an alteration in function of accessory glands,which are essentially indirect manifestations of hypo-androgenism, however, administration of exogenous testosterone appeared to protect seminal vesicle from DMPA.

KEYWORDS: *Funambulus pennanti*, seminal vesicle, hypo-androgenism, DMPA.

INTRODUCTION

The seminal vesicles are among the most important male accessory glands contributing to about 60% of the seminal plasma (Mann and Lutwak-Mann,1981). Seminal vesicular secretion is rich in fructose, proteins, prostaglandins, complex carbohydrates and enzymes involved in the clotting of the ejaculate (Gonzales et al., 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 (Agrawal and Vanha-Pertualla, 1987; Zubkova and Robaire, 2004; Troedsson et al., 2005). The potent immune-suppressive activities of the water-soluble fraction of the seminal vesicle fluid and its role in reproductivity immunity have also been described (Rozeboom et al., 1999; Alghamdi et al., 2004). The seminal vesicle is an androgen dependent organ in terms of both structure and function (Gonzales et al., 1994; Almenara et al.,2000;Nishino et al.,2004). The seminal vesicle possesses 5α-reductase activity, which converts testosterone to dihydrotestosterone, the active hormone. More recently it has been demonstrated that seminal vesicles contain LH/hCG receptors, thus making this accessory reproductive organ a potentional target of direct regulation by LH (Tao et al., 1998; Nishino et al., 2004). Depo provera or Medroxy progesterone acetate (DMPA) is a synthetic hormone and has a close structural similarity to natural progesterone. When used alone it causes impotency (Frick et al., 1982), therefore, an androgen replacement like esters of testosterone is necessary to maintain sexual function to avoid the consequences of androgen deprivation (Nieschlage and Behre, 1990).

MATERIAL AND METHODS

Drugs

Depot Medroxy Progesteron Acetate (DMPA) injection (Upjohn USA).

Sustanon: (250ml of Sustanon contain testosterone Propionate-30mg, Testosterone Phenyl Proprionate-60mg, Testosterone Isocaproate-60mg, Testosterone Decanoate-100mg (Maxpro).

Animals

A total 18 adult male squirrels weighing between 100 to 150gms were trapped alive in and around Nagpur City during the breeding period from January to July, 2007 (Reddi and Prasad, 1968). They were fed in the morning and in the evening daily with the soaked grams, chappatis, breads and cooked rice, dal, fruits, vegetables, ground nuts and water. The animals were housed at constant temperature (28±2ºC) and relative humidity (60±10%) with a 12h light: 12h dark cycle.

Treatment

One week after arrival, male squirrels were assigned to one of the two schedules of DMPA and combination treatment (Sustanon). The control animal received same amount of saline (Table-1).

TABLE 1. Experimental Design

Number of animals and sex	Treatment	Dose mg/Kg BW	Route	Duration
6 males (control)	Olive oil	E.V.	I.P.	30 days
6 males (Experimental)	DMPA	1mg daily	I.P.	30 days
6 males (Experimental)	DMPA+Sustanon	0.1mg DMPA+1mg Sustanon daily	I.P.	30 days

Abbreviations : E.V. = Equal volume, I.P. = Intraperitoneal, B.W. = Body weight

Histological assessment

The animals were sacrificed using chloroform 24 hours after the last day of each experiment. Immediately the seminal vesicles were excised, weighed on an analytical balance and fixed in Bouin's fluid for 24hrs and preserved in 70% alcohol, dehydrated in ethanol and embedded-in paraffin wax. The sections cut in 5 µm were stained with haematoxylin and counter-stained with eosin. Measurements were taken with an occular micrometer wherever essentional.

Testosterone evaluation

For the determination of testosterone level in blood, Palm squirrels were anesthetized by ether and 2ml of blood was drawn by cardiac puncture with 2ml sterile syringe. The blood was allowed to clot at room temperature for half an hour. The clotted blood was sent to NRPL Pathology laboratory, Nagpur for further processing by enzyme linked-florescent assay (Delahunt and Hirsutism, 1993).

Statistical analysis

To indicate individual variations in each corresponding region, the mean values and standard deviation (mean ± SD) for measurements from three animals were calculated. The statistical significance of differences for these values in different regions was assessed using t-test (Delgaard et al., 2008). A significant level of $P<0.05$ was accepted.

RESULTS

DMPA treatment led to abdominal bloating, excess of fat deposits and therefore resulting into significant increase in body mass, whereas combination treatment resulted into an insignificant increase in body mass (fig.1 and fig. 2).

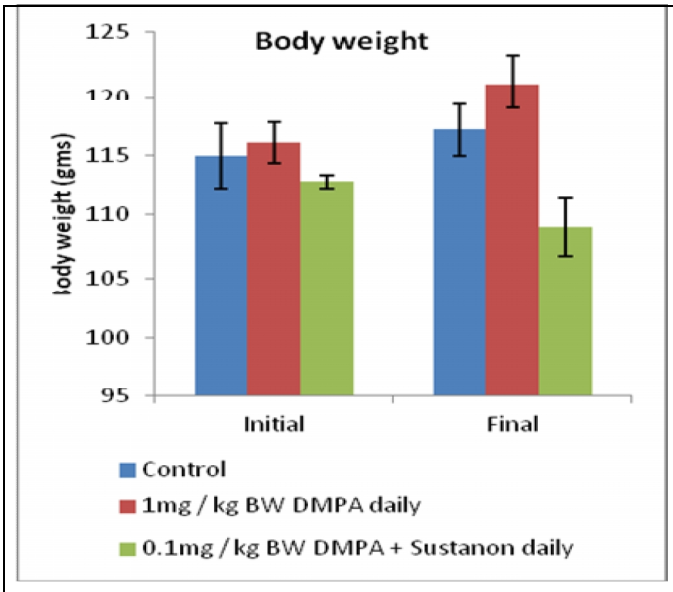

Fig. 1. : Body weight changes over the course of 30 days treatment with DMPA and DMPA+Sustanon, expressed as weight on the last day of treatment divided by weight on the first day of treatment (Control n=6, DMPA n=6,DMPA+Sustanon n=6). DMPA+Sustanon treated squirrels did not gain as much weight as the control squirrels. *P<0.05

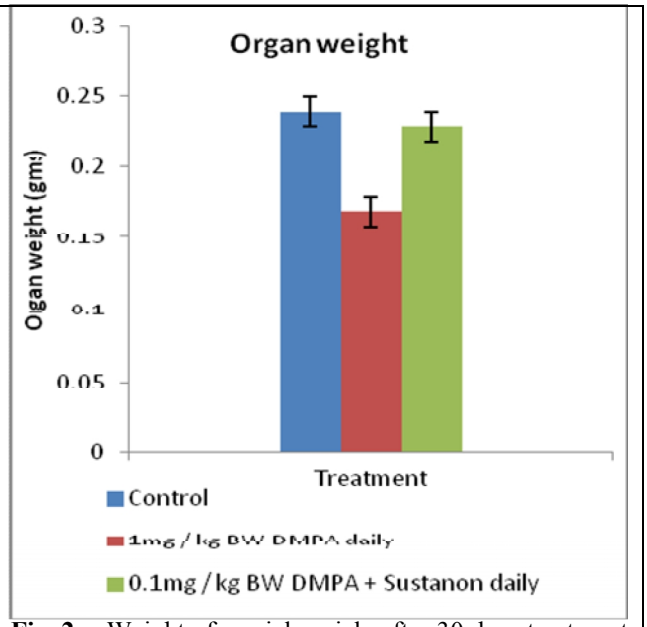

Fig. 2. : Weight of semial vesicle after 30 days treatment with vehicle or DMPA and DMPA+Sustanon (Control n = 6, DMPA n = 6, DMPA+Sustanon n=6). There was a significant decrease in the weight of seminal vesicle of the DMPA treated animals.* $P < 0.001$.

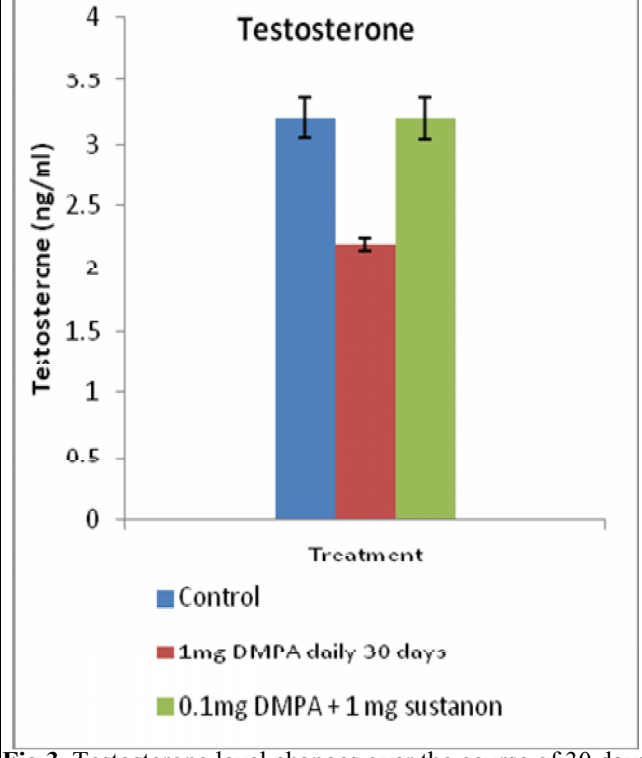

Fig.3. Testosterone level changes over the course of 30 days treatment with DMPA and DMPA+Sustanon squirrel (Control n = 6, DMPA n = 6, DMPA + Sustanon, n = 6) There was a significant decrease in DMPA treated group when compared to vehicle, level was maintained to control values in combination group. * P < 0.001.

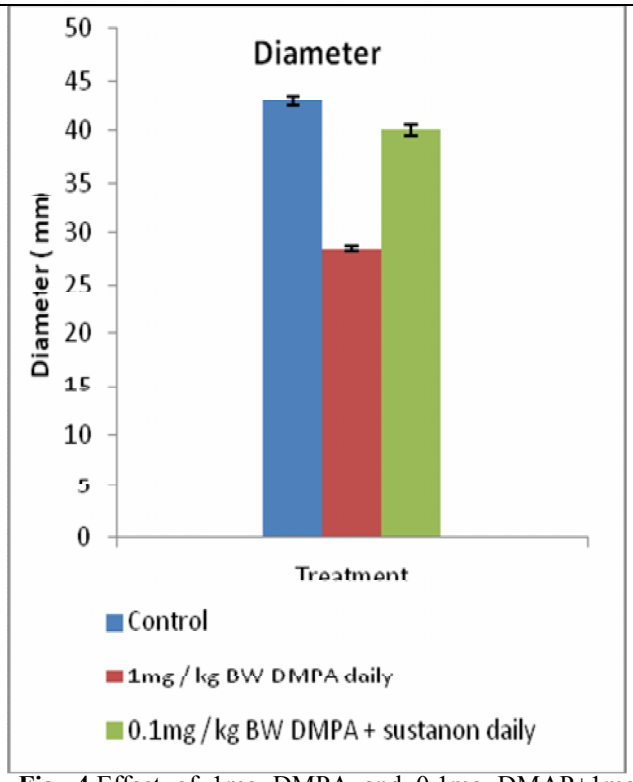

Fig. 4. Effect of 1mg DMPA and 0.1mg DMAP+1mg Sustanon for 30 days on diameter of seminal tubule of squirrel (Control n = 6, DMPA n = 6, DMPA+Sustanon n=6). There was a significant decrease in the diameter of DMPA treated group when compared with the vehicle, combination treatment maintained it near to control values. * P < 0.05.

Evaluation of testosterone

The DMPA treatment (1mg/KgBW/day) showed significant decrease (P < 0.1) but the DMPA+Sustanon treatment (0.1mg DMPA+1mg Sustanon daily) resulted into insignificant increase (P < 0.001) in serum testosterone concentration when compared to control values (fig.3).

HISTOPATHOLOGICAL CHANGES

Vehicle-treated controls

The seminal vesicles were composed of tubular alveoli and the mucosa was not thrown into folds. Epithelium folded, few basal cells also occupied the basal region, lumen contained densely stained secretory material, lamina propria surrounding the epithelial cell was comprised of cellular connective tissue containing some smooth muscles rich in elastic fibres, however, some of the acini were lined by tall columnar epithelial cells with a prominent basal nucleus thrown into crypt (figs. 5 and 8).

Chronic DMPA treatment for 30 days (1mg/KgBW/day)

Reduction in the size of the tubules was observed when compared to the control (fig.4). Each tubule was bound by a rim of muscular coat, which was also made of loose strands of smooth muscle fibers. The intertubular connective tissue was moderately thick. Of interest was an extensive vacuolation in infra and supranuclear region of each cell giving a foamy appearance to all the tubules. The secretory epithelium was thrown into long crypts manifested towards lumen thus obliterating the central lumen into a number of tubules. Most of the tubules were devoid of secretion (figs. 6 and 9).

Chronic DMPA+Sustanon treatment for 30 days (0.1mg DMPA+1mg Sustanon daily)

0.1mg DMPA+Sustanon 30 days could maintain the secretory activity and tubule size (fig. 3). Each tubule was lined by pseudostratified secretory epithelium sometimes running straight or thrown into club-like folds or thrown into finger-like crypts, and there was insignificant increase in connective tissue (figs. 7 and 10).

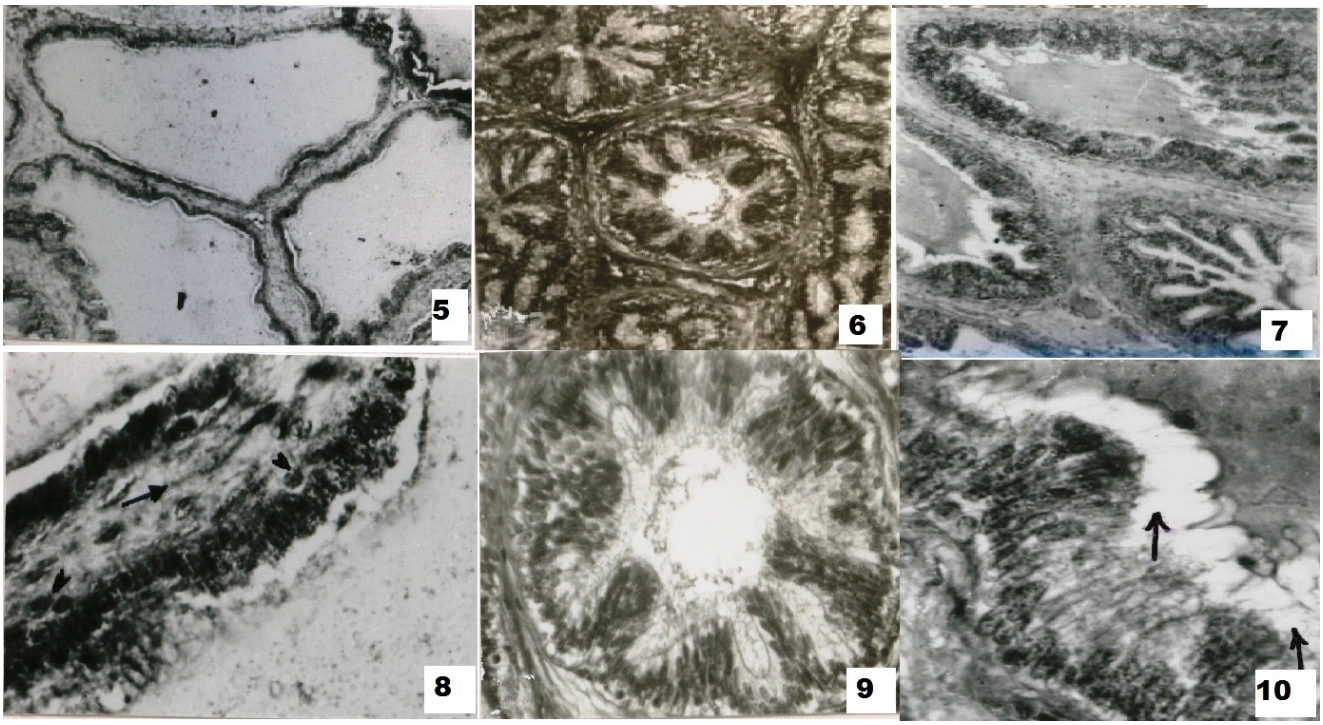

Fig. 5 : Seminal vesicle vehicle-treated control squirrel. Note distended acini/tubules within the fibromuscular area. The tall columnar epithelium is folded, lumen contains copious densely-stained secretory material X100.

Fig. 6 : Seminal vesicle 1mg DMPA injected daily for 30 days. Tubules with crypt-like long mucosal foldings obliterating the lumen isolated by moderate connective tissue. Lumen lacks the secretion X 100.

Fig. 7 : Seminal vesicle 0.1mg DMPA +1mg Sustanon for 30 days could maintain the secretory activity and tubule size X 100.

Fig. 8 : Vehicle-treated secretory epithelium. Lamina propria surrounding the epithelial cells is comprised of cellular connective tissue containing some smooth muscles rich in elastic fibres (arrow),please note few basal cells, basal in position (arrow head) X 1000.

Fig. 9 : Single tubule further magnified from fig.6. Secretory epithelium is long columnar with elongated nuclei, which appear many in numbers. The cells show secretory zone and condensation, pyknosis and displacement of nuclei. Lumen shows no secretion X 1000.

Fig. 10 : 0.1mg DMPA +1mg Sustanon 30 days treated seminal vesicle. Some secretory epithelial cells show light supra nuclear zone (arrow) X 400.

DISCUSSION

Seminal vesicular secretion is important for semen coagulation, sperm motility, stability of sperm chromatin and suppression of immune activity in female reproductive tract (Gonzales, 2001). The accessory glands are morphologically and physiologically dependent on the production of the androgen and the circulating androgen which are in turn LH dependent (Lee et al.,1994; Nemeth et al.,1998;Nishino et al.,2004; Sastry and Kashmiri,2011). DMPA being anti-androgenic causes depression of plasma testosterone with decrease in weight, size of accessory glands and other regressive changes (Bhasin et al.,1997; Sastry and Gupta.,2008).DMPA is also a potent inhibitor of testicular 5α-hydroxysteroid oxidoreductase activity, itself binding to catalytic binding sites of the substrate like DHT (5α-dihydroxytestosterone) thus reducing androgen binding protein (ABP) production which would have helped in the maintenance of the accessories, since it is carrier of testosterone (Wong et al., 1978) or through hormone target cell interaction or the expression of seminal vesicle protein or by direct action on the metabolism of testosterone compartment of testis which are in the control of adenohypophysis.

The reduction or the shrinkage of the acini were in equivalence with the increase of fibro-muscular tissue defining stromal-epithelial interaction (Chang and Wang, 1992; Blanchere et al., 2001; Dunker and Kreiglstein, 2002). The above mentioned changes also points to androgen dependency of the gland but administration of exogeneous testosterone (Sustanon) restores the original condition. It was interesting to note that in the combination treated group seminal vesicles achieved their secretory capacities, weight and general architecture near to control values as described by Kragt et al., 1974;Flickinger et al.,1977 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 (Almenara et al., 2000; Nishino et al.,2004).

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