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Ultrastructural changes in prostate gland and vas deferens induced by *Azadirachta indica* leaves in albino rats

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Abstract

Objective: The purpose of the present study was to investigate the effect of *Azadirachta indica* leaves on the ultrastructural changes in the prostate gland and vas deferens of albino rates. Materials and methods: Male adult albino rats (Wistar strain) were administered *A. indica* leaf powder (orally) at a daily dose of 100 mg/rat for 24 days. At autopsy, the tissues after fixation by vascular perfusion were processed for the ultrastructural studies. The section were scanned on the electron microscope. Results: The ultrastructural observations revealed structural changes in the cytoplasmic organelles. The microvilli were lacking and hte lysosomal bodies were scattered and disturbed. Chromatin material in the nuclei was decreased and the cytoplasm was highly vacuolated. The nuclei were indented and the mitochondria were atrophic. The microfilaments in the lumen were lacking, coated pits and the pear shaped vesicles were disturbed. Lipid droplets and lipofusion material was seen in the electron dense bodies of the cell and the Golgi complex was highly disturbed. These changes indirectly indicate androgen deficiency. Conclusion: It is suggested that the effects may be possibly, due to direct or indirect action of anti-androgenic properties of *A. indica* leaves leading to androgen deficiency.

Key words: *Azadirachta indica*, albino rats, prostate, vas deferens ultrastudy.

1. Introduction

The structural and functional integrity of the accessory sex glands are controlled by androgens [1-3]. The prostate gland as a male accessory sex organ depends for its growth and maintenance on the supply of testicular hormones, notably testosterone. It is known that prostatic tissue is androgen dependent [4,5]. Numerous plant products and natural medicines are reported to inhibit male fertility and thereby have potential as chemical contraceptives [6,7].

The Indian neem tree, *Azadirachta indica* (Syn: *Melia azadirachta*) is recognised since long, for its unique properties. The bark, leaves and seeds of *A. indica* contain margosine, a bitter alkaloid,

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margosic acid, an acid fraction and margo-scopicrin, a cristalline bitter principle isolated from the oil [8].

It also contains nimbin, nimbinnin, nimbidin and azadirachtanin which are believed to be the active constituents of the whole plant [9-11]. Three compounds have been isolated from the seeds namely, *Meliantrial [12], Salannin* [13] and *Azadirachtin* [14]. *Azadirachtin* occurs in several forms, having the basic triterpenoid structure as common to all of them [15,16]. From the seed cake, four major components of Azadirachtin have been isolated namely, Azadirachtin-A, Azadirachtin-B, Azadirachtin-C and Azadirachtin-D [17].

A.indica leaves possess antiandrogenic and antifertility properties [18-21]. *A. indica* also causes a decrease in the weight of accessory sex glands like ventral prostate and seminal vesicle and the effects appear reversible [22, 23].

Recently, the morphological changes in the head of rat spermatozoa and effects on sperm parameters induced by *A. indica* leaves have been reported [24, 25]. The Praneem polyherbal pessary (purified ingredients from *A. indica* leaves, sapindus mukerossi fruits and mentha citrate oil) has potent spermicidal action on human sperm *in vitro* and *in vivo.* When applied in the vagina before mating, it prevented rabbits from becoming pregnant [26].

The purpose of the present study was to investigate the effect of *A. indica* leaves on the ultrastructural changes in the prostate gland and the vas deferens of rats.

2. Materials and methods

Male rats of the Wistar strain, 3-4 months old and 190-200/gm body weight were acclimatized to laboratory conditions and received a standard rat pellet diet and water *ad libitum*. *A. indica* leaves were collected from Karnatak University Campus in the month of October and November at 8 am. Fresh leaves of *A. indica* were collected and dried in shade, powdered and suspended in distilled water for oral administration (by gavaging) to albino rats. A voucher specimen has been deposited at the Herbarium of the Botany laboratory, Karnataka University Dharwad and it was cross verified with the collected material.

The animals were divided into two groups comprising of five animals each.

Group I : Each rat received 1ml of distilled water through oral route each day for 24 days and served as control.

Group II : Each rat was administered 100 mg of leaf powder in 1 ml of distilled water by oral route each day for 24 days.

The effective dose of 100/mg and the period of treatment *viz.* 24 days have been arrived at after preliminary studies on dose and duration response studies in our laboratory and have been reported elsewhere [21-23].

After fixation by vascular perfusion with 3% glutaradehyde, the prostate gland and vas deferens were removed rapidly and again fixed in 3% glutaradehyde for 2-4 h. The tissues were stored in sodium cacodylate buffer at 4°C [pH 7.2, 0.l M], washed in the buffer and post fixed in 1% osmium tetra oxide for 1-2 h. The fixed tissues were washed again in the buffer, dehydrated in graded series of alcohol, stained in 2% uranyl acetate for 6 h, infiltrated with araldite: propylene oxide (1:1) mixture overnight and again infiltrated with fresh araldite and embedded in araldite beam capsules.

The blocks were cut with the ultramicrotome (Leica LKB Broma). Ultrathin sections of 100-300 A° were cut, collected on copper grids and stained in 1 % uranyl acetate and lead citrate (Reynolds, 1963) [27]. The sections were scanned on an electron microscope (Joel-TEM 100 x II).

3. Results and discussion

3.1 Prostate Gland

In control animals, prominent nuclei were clearly seen with other normal cell organelles. The nuclear pores and the Golgi regions were normal (Fig.1,3). Well developed ergastoplasmic sacs and mitochondria were closely spaced at the apical region. At the apical surface small projections of the microvilli and electron dense bodies (lysosomes) were distinctly visible (Fig. 2, 3). The finger print like whorls of endoplasmic reticulum were prominent (Fig. 3).

In the treated animals, structural changes in cytoplasmic organelles were observed. The nuclei were distrurbed and indented. The organelles were atrophied and there is commencing of vacuolization. The endoplasmic reticulum were completely disturbed and dilated (Fig. 4, arrow).

Ergastoplasmic sacs were completely disturbed and dilated. In the apical region, the mitochondria were atrophic, contributing to vacuolization (Figs. 5,6, arrow). Lipid droplets and electron dense material were scattered. Pinocytotic vesicles on periphery of the cell were clearly visible. In the cytoplasm, there is an increased amount of lysosomal bodies containing homogenous or heterogeneous material (Fig. 6).

3.2 Vas Deferens

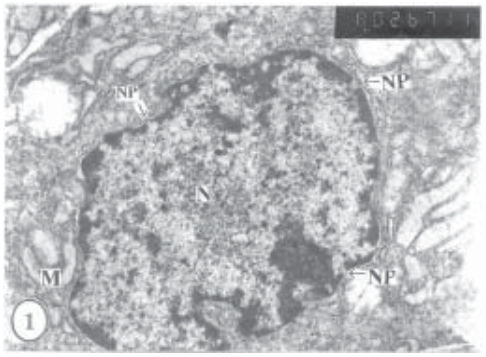
In control animals, the epithelial cells of the vas deferens were tall and columnar, with long microvilli at the apex. The nuclei were regular and contained abundant heterochromatin mass. Columnar cells are with abundant cytoplasmic organelles including cisternae of endoplasmic reticulum, Golgi complex, mitochondria and other cell organelles were clearly visible (Fig. 7,10).

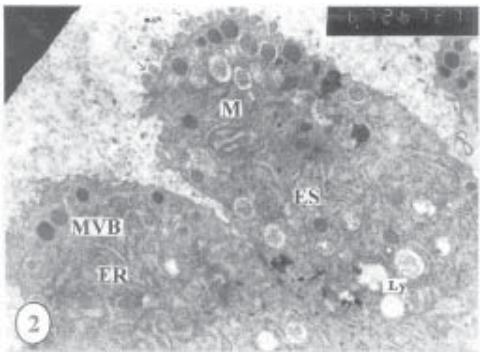
In apical region of the cell, coated pits, small coated vesicles and pear shaped vesicles were visible. Basal cells show cytoplasmic organelles and numerous microfilaments in the lumen (Fig. 8, 9). In the treated animals the nuclei of the principal cells, were irregularly outlined and displayed deep indentations. The Golgi complex was highly disturbed and dilated. In the cytoplasm, there was atrophy of the mitochondria and small pale irregular multivesicular bodies were present.

The supranuclear region of the principal cell showing membrane bound granules were identified as lysosomes. Lipid droplets and lipofusion materials were seen in the electron dense body of the cell (Fig. 12,14). Apical region of the principal cell showed disturbed coated pits, small coated vesicles, and pear shaped vesicles. The micro filaments in the lumen were lacking (Fig. 13) In the cytoplasm, inter digitating lateral plasma membrane along the side of basal region of the principal cell is observed (Fig. 14).

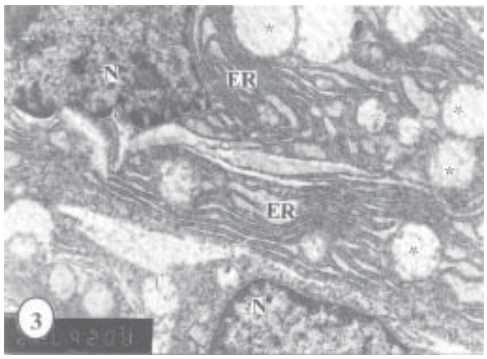
The antifertility action of non-steroidal chemicals in male rats do not appear to involve directly on the endocrine system [28]. The direct action of androgens on the prostate gland changed the morphology and metabolism of testosterone [29]. Androgenic steroids regulate the development and size of the mammalian prostate gland [30]. Hence, the prostate is indeed an androgen dependent organ [22,23,31,32].

In the present study, due to *A. indica* leaves treatment, the prostate microvilli were lost, the number of electron dense bodies were scattered and lysosomal bodies were disturbed. The epithelial cytoplasm showed a system of membrane bound ergastoplasmic channels studded with the discrete Golgi body, associated with secretory granules. Similar observations have been made by Gittinger (1972) [33], in explants grown in non-supplemented medium whereas the epithelium showed severe regression.





Electron micrographs of control rat prostate gland (Fig 1-3): Fig. 1. Normal structure of Nucleus (N). There is a nuclear pore (NP) (arrow). Mitochondria (M) is normal in appearance X 4000. **Fig. 2.** The lamellated ergastoplasmic sacs (ES) appear normal, few multivesicular bodies (MVB) are present. Endoplasmic reticulum (ER) and lysosome (Ly) like bodies are normal. At the apical surface of the tubule, a small projection of the microvilli is seen (arrow) X 3000.



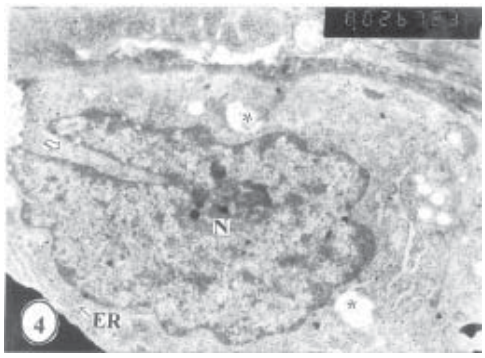
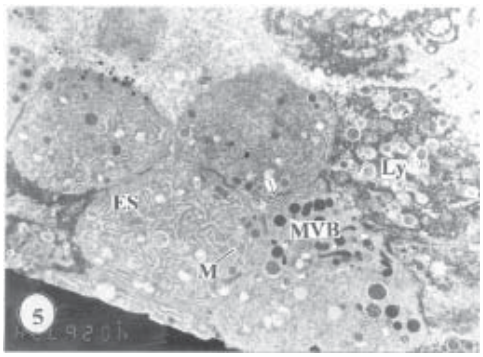


Fig. 3. The finger print like whorls of the endoplasmic reticulum (ER) Secretory vacuoles (*) contain granular Components X 6400. **Electron micrographs of rat prostate gland treated with *A indica.* (Fig 4-6) : Fig. 4.** Nucleus (N) irregular and deeply indented in shape (arrow) . Secretory granules are vacuolated (*). Dilated and disrupted endoplasmic reticulum (ER) are also seen. X 4560.



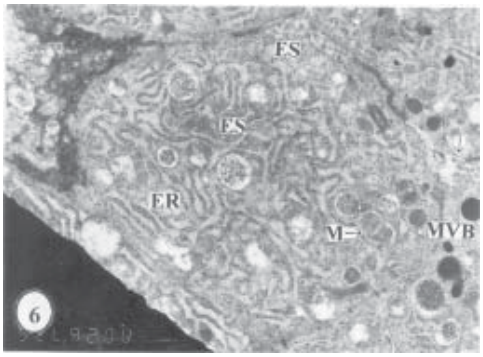
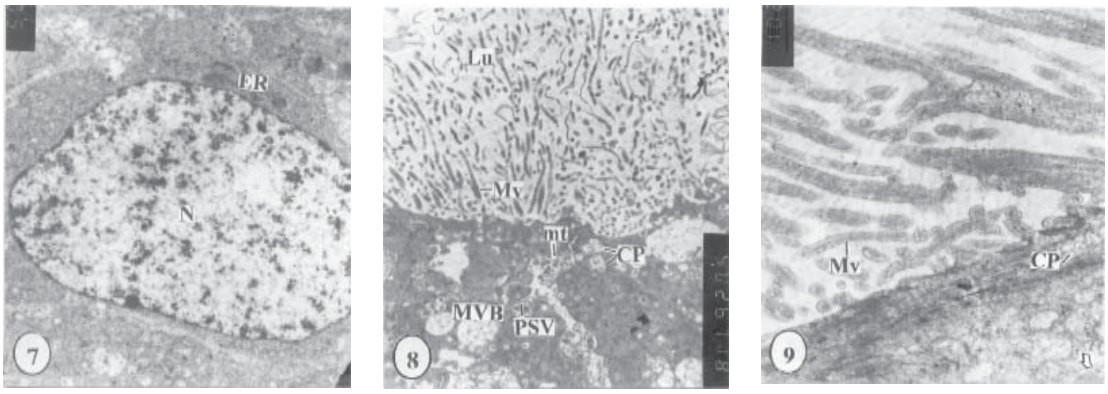


Fig. 5. Lysosome (Ly) like bodies are seen to be increased. Mitochondria (M) are atrophied and have started vacuolization. The number of multivesicular bodies (MVB) have increased in number. Ergastoplasmic sacs (ES) are dilated and disrupted X 1600. **Fig. 6.** With higher magnification, the mitochondiral cristae (M) are disrupted and have started vacuolization. Ergastoplasmic sacs (ES) and Endoplasmic reticulum (ER) are dilated and disrupted. Secretory granules are degenerated and fully disturbed (arrow) X 5600.



Electron micrographs of control rat vas deferens (Fig 7-10) : Fig. 7. The structure of nucleus (N) is normal, surrounded by endoplasmic reticulum X 1600. **Fig. 8.** The apical region of a principal cell showing pear shaped vesicles (PSV) coated pits (CP), Lumen (Lu), Microvillus (MV), microtubules (mt) and multivesicular bodies (MVB). X 2500. **Fig. 9.** Apical region of a principal cell showing coated pits (CP), microvilli (MV) and secretory granules (hollow arrow) X 600.

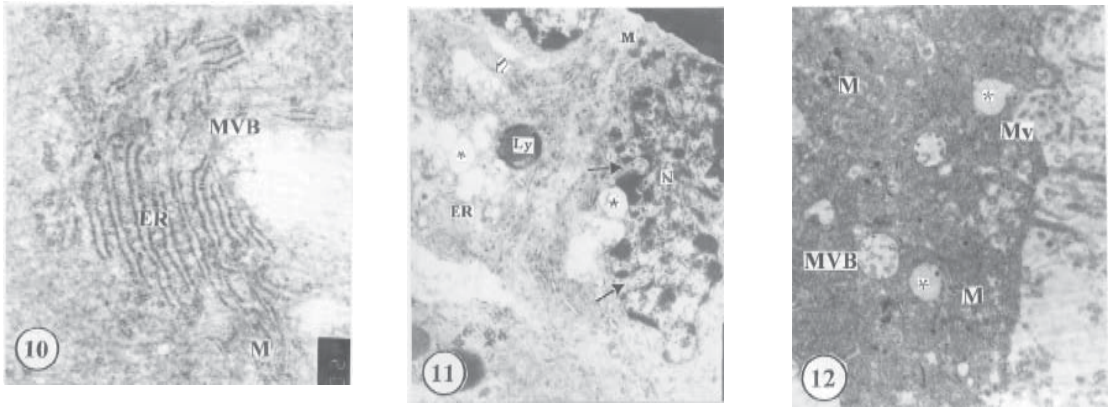


Fig. 10. The whorls of the Endoplasmic reticulum (ER) and mitochondria (M) appear normal. Multivesicular bodies (MVB) are also visible X 6800.

Electron micrographs of rat vas deferens treated with *A. indica* leaves (Fig 11-14): Fig. 11. The Nucleus (N) is irregularly outlined and displays deep indentation (arrows), mitochondria (M) are packed and disturbed. Supranuclear region of a principal cell shows several membrane bound granules identified as lysosomes (Ly). Endoplasmic reticulum (ER) is dilated and disrupted. There is commencing of vacuolisation and degeneration (hollow arrow) in the secretory granules. Uncoated vesicles (*) are more in number. X 7200. **Fig. 12.** Multvesicular bodies (MVB) contain a small round mass of granulated electron dense material. Mitochondria (M) are disturbed. Microvilli (MV) are almost lacking. The large uncoated vesicles (*) contain wispy material. X 5600.

There were several dilated Golgi bodies, microvesicles and mitochondrial cristae were rather closely packed and disturbed. The endoplasmic reticulum was disturbed and dilated, similar to those of castrated rats [34].

The cytoplasm appeared highly vacuolated, nuclei contained less chromatin material, particularly the mitochondria were few and atrophied leading to vacuolization. The observations made are similar to the studies of Song. *et al.* (1991) [35] in ultrastructural observation of prostate gland in rats with chronic fluorosis, and in denervated Sprague Dawley rats [36] .

Hence in the present study, the changes in the prostate indicate androgen deficiency, charactersied by vacuolization of cytoplasm,

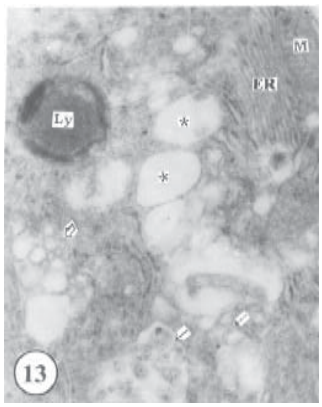


Fig. 13. In the higher magnification of apical region of principal cell, the mitochondria (M) are packed and disrupted. Endoplasmic reticulum (ER) are dilated and disturbed. Membrane bound granules show homogeneous electron dense material and a paler, purely granulated region, indicating that these elements are lysosomal (Ly) in nature. There is commencement of vacuolization and degeneration in secretory granules (*). There is increase in number of uncoated vesicles (hollow arrow) and coated pits (arrow) are disturbed. X 1200.

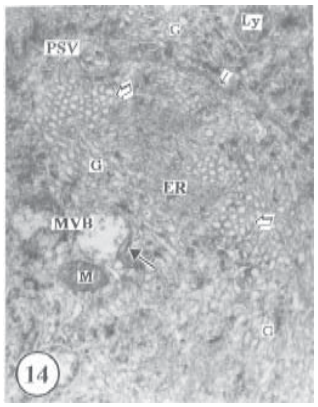


Fig. 14. A pale multivesicular body (MVB) showing a plaque of fuzzy material on the cytoplasmic face of its delimiting membrane (arrow head). Endoplasmic reticulum (ER) are dilated and disturbed. The Gogli Complex (G) are dilated and disrupted. Lipid droplets (hollow arrow) appear more in number. Mitochondria (M) are closely packed and disturbed. In the cytoplasm, inter-digitating lateral plasma membrane, along the side of basal region of the principal cell is observed (arrow). X 2600.

Abbreviations: N - Nucleus; mt - Micro tubules; ER - Endoplasmic reticulum; CP - Coated pits; Ly - Lysosome; PSV - Pear shaped vesicles; M - Mitochondria; MV - Microvilli; MVB - Multivesicular bodies; Lu - Lumen; G - Golgi complex.

decrease or impairment of mitochondria to varying extent and deposition of lipid droplets. The organelles were atrophied and there is commencement of vacuolization. The nuclei became indented with less chromatin material. Ergastoplasmic sacs and Golgi regions were dilated and disturbed. Pinocytotic vesicles appeared on the periphery of the cells.

The structural and functional integrity of the vas deferens is controlled by androgens [37-39]. The vas deferens plays an important role in the transport of sperms [40]. In addition to this it is absorptive in nature as manifested by signs of pinocytosis in the lumen and presence of multivescicular bodies and abundant lysosomes [40-43].

In the present study, microfilaments in the lumen were lacking, the nuclei of the cells were irregularly outlined and displayed deep indentation. Golgi complex was highly disturbed and dilated, endoplasmic reticulum was also disturbed throughout the cytoplasm. Similar observations have been made in human vas deferens caused by carcinoma [44].

In apical region of the principal cells, coated pits, small coated vesicles and pear shaped vesicles were disturbed. These observations are similar to vas deferens of rats, treated with native ferritin and cationic ferritin[45].

It is suggested that the effects may be possibly due to the direct or indirect action of the *A indica* leaves on ventral prostate and vas deferens.

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