Ultrastructural changes in cauda epididymidal epithelial cell types of *Azadirachta indica* leaf treated rats

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To assess if cauda epididymis is a target for the effect of *A. indica* leaves, Wistar strain male albino rats were administered (po) *A. indica* leaves (100 mg/rat/day for 24 days). Transmission electron microscopic analysis revealed that in the cauda epididymal epithelium the nuclei of principal cells were enlarged and the number of coated micropinocytotic vesicles of the apical cytoplasm decreased. Microvilli were missing and mitochondrial cristae and Golgi complex were highly disrupted. The cytoplasm was abounding with lysosomal bodies. The clear cells increased in perimeter and their nuclei increased in size and contained lesser chromatin. The nuclear membrane bulged out. The cytoplasm was vacuolated. Further, there was decrease in size of the lipid droplets, mitochondria, Golgi complex, endoplasmic reticulum and there was accumulation of lysosomal bodies. The changes in the principal and clear cells appear to be due to the effect of the hypoandrogen status caused by treatment with *A. indica* leaves and a direct action on the epididymal epithelium.

**Key words:** Ultrastructure, Cauda epididymis, Epithelial cells, *Azadirachta indica*, Albino rat.

The mammalian epididymis has attracted the attention of investigators because of its important role in sperm maturation. It could be the extragonadal site to interfere within the control of fertility without impairing libido and potency. The anatomical, histological and functional differentiation along the epididymis recorded in several mammalian species formed the basis for further studies on this subject.

India is gifted with abundant natural remedies in the form of herbs, shrubs and mineral elements. In an attempt to prevent conception, tribal people are known to use decoctions prepared from plant materials, both orally and locally. *Azadirachta indica* (syn: *Melia azadirachta*), commonly known as neem, is an important medicinal plant that grows throughout India and Burma. It is known to cause the decrease in the weights of accessory sex glands like epididymis, ventral prostate and seminal vesicles and the effects are reversible after withdrawal of the treatment. The morphological changes in the head of rat spermatozoa and sperm parameters induced by *A. indica* leaves have been reported. Recently, significant reduction in sperm parameters and fructose content of vas deferens and ultra structural changes in prostate gland and vas deferens in albino rats have also been reported.

*In vitro* and *in vivo* studies have shown that the praneeem polyherbal pessary ( formulated from purified ingredients from *A. indica*, *Sapindus mukerossi* fruits and Mentha citrate oil) has potent spermicidal activity on human sperm when applied on the vagina before coitus, it prevents rabbits from becoming pregnant.

The present study has been aimed to elucidate the effect of *A.indica* leaves on the ultrastructural organization of the epithelial cells of rat cauda epididymidis.

**Materials and Methods**

The leaves of *A. indica* were collected and dried in shade. The dried leaves were finely powdered and suspended in distilled water for oral administration to albino rats. Three to four months old male albino rats (Wistar strain) weighing 170-200 g, were obtained from the rat colony maintained in the department. They were housed in cages and were fed on pellet feed ("Gold Mohur", Hindustan Lever Limited, Bombay) and water *ad libitum*.

The animals were divided into two groups each consisting of five animals:

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Group I: Each rat received 1ml of distilled water, po, each day for 24 days and served as controls.

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

The effective dose of 100mg 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 reported elsewhere.[10-12]

After fixation by vascular perfusion, with 3% glutaraldehyde the epididymis was removed rapidly and again fixed in 3% glutaraldehyde for 2-4 hr. The tissue was stored in sodium cacodylate buffer at 4°C (pH 7.2, 0.1 M), washed in buffer and post fixed in 1% osmium tetroxide for 1-2 hr. The tissue was washed again in the buffer, dehydrated in graded series of alcohol, stained en bloc in 2% uranyl acetate for 6 hr, infiltrated with araldite : propylene oxide (1:1) mixture for over-night, again infiltrated with fresh araldite and embedded in araldite beam capsule. The blocks were cut in a Leica LKB Broma ultramicrotome. Ultra thin sections (100-300A) were cut, collected on copper grids and stained in 1% uranyl acetate and lead citrate.[19] The sections were observed in a Joel-TEM 100cx II electron microscope.

Results

In the control rats the principal cells were present along the entire length of the cauda epididymis. These cells have a single round or elliptical nucleus containing granular chromatin. They are characterized by well developed microvilli on the luminal surface. The multivesicular bodies contain amorphous material. Golgi complex is composed of fenestrated cisternae. The endoplasmic reticulum and mitochondria are well developed in the cytoplasm. In higher magnification the cells showed coated vesicles in the vicinity of Golgi complex. Supra nuclear region of the cell showed well developed mitochondrial cristae and endoplasmic reticulum arranged in the form of whorls (Figs 1 and 2).

The most obvious changes in the principal cell of cauda epididymidis of A. indica treated rats were decreased in number of coated microinocytotic vesicles, invagination on the luminal surface, loss of apical microvilli and disruption of mitochondrial cristae and Golgi apparatus. In the nucleus, the chromatin material was pushed to one side, the nuclear membrane was bulged and there was karyokinesis. Apical end of the cells was vacuolated and contained particulate material. The multivesicular bodies were increased and contained a homogenous or heterogenous material. In the apical region the mitochondria atrophied and contributed to vacuolization (Figs 3 and 4).

In the control rats, the clear cells were found in between the principal cells. They contained ovoid nuclei placed slightly above the basal position and contained granular chromatim material. The cytoplasm was abounded with lipid droplets. Microinocytotic vesicles were prominent. The basal cells were elliptical and nuclei were elongated and flattened against the basement membrane (Fig. 5).

In the clear cells of treated rat, microvilli were missing. The size of the cell, nucleus and the cytoplasmic granules were increased. The chromatin material was less, nuclear envelope was bulged, karyolysis and karyohexis were noticed. Mitochondria, Golgi apparatus and endoplasmic reticulum were disrupted. Microinocytotic vesicles were rarely seen. Basal cells appeared decreased (Fig. 6).

Discussion

The epididymis in general and the principal cell in particular, are androgen-dependent and androgen withdrawal is known to cause extensive changes in the principal cell.[21-23] Thus, the changes in the principal cell of A. indica treated rats may reflect a manifestation of hypoaandrogenic status, brought about by A. indica leaf treatment. However, direct action of A. indica leaf on the principal cell cannot be excluded. Microtubules constitute a principal component of the tissue matrix system of the epithelial cells.[24] Vincristine is a microtubule disrupting agent[25-27] and microtubules of the principal cell may be the target for A. indica action. It is possible, therefore, that A. indica may have caused pathological changes in the cauda epididymal epithelial principal cell.

In the present study, principal cell and clear cell underwent ultrastructural changes following A. indica treatment. Among the significant changes observed in the principal cell were decrease in the number of microinocytotic vesicles and reduction in the size of mitochondria and Golgi apparatus. Asha prakash et al.[28] have suggested that absorptive function of principal cell is impaired following administration of
Figs 1-4—(Figs 1, 2) — Electron micrographs of control rat cauda epididymidis. (Fig. 1) — Principal cell (PC) and basal cell (BC) with normal Golgi complex (G). The nucleus (N) and endoplasmic reticulum (ER) are normal. Mitochondria (M) are abundant and intact. At the apical region of principal cell (PC), dense microvilli (Mv) are present. The demarcation between the two principal cells is clear × 4500. (Fig. 2) — The nucleus (N) of Principal cell (PC) is normal. The Golgi complex (G) is evident showing its normal features. Few lysosomal bodies (Ly) are clearly visible. The mitochondria (M) are intact. Supranuclear region of the principal cell shows features typical of the cell. Endoplasmic reticulum (ER) is visible and appears normal × 2000. Figs 3 and 4 — Electron micrographs of cauda epididymidis of A. indica treated rat. (Fig. 3) — The nucleus (N) of principal cell is enlarged. There is bulging of nuclear envelope (small arrow). The chromatin material is reduced. Microvilli (Mv) are disrupted and coated micropinocytotic vesicles (CV) are decreased. The lysosomal bodies (large arrow) show complete disruption. Mitochondrial cristae (M) are disrupted × 9000. (Fig. 4) — The lipid droplets in the principal cells (small arrow) are increased. Multivesicular bodies (*) are also more electron dense. In the nucleus (N) heterochromatization is decreased. The mitochondrial (M) are vacuolated. The endocytic vesicles (open arrow) are few × 5200.
cyproterone acetate an antiandrogen. Thus, the findings in the present study lead us to infer hypoandrogenic status caused due to treatment of A. indica leaves.

The result of the present study further indicated that in response to A. indica treatment, the clear cells undergo hypertrophy, hyperplasia and hyperactivity in an attempt to remove the cell debris reaching the ductus epididymal lumen from the testis in the form of residual bodies, Sertoli cell fragments and dead and deformed sperms. Similar observations have been made in the cauda epididymis of rats treated with vincristine\textsuperscript{27,28}.

In the clear cell of the A. indica treated rats, organelles viz., cytoplasmic vacuoles, electron dense secretory granules and mitochondria, exhibited a reduction in abundance. Also the lipid droplets in the cells of the treated rats were smaller as compared with those in control rats. Further, there was a reduction in the number of micropinocytotic vesicles and multivesicular bodies. These findings indicate that the endocytic functions of the clear cells is affected following A. indica treatment.

The above ultrastructural changes indicate that the principal cell and clear cell are affected, thus altering the composition of epididymal fluid which in turn may affect the sperm maturation. This contention is supported by findings of earlier studies\textsuperscript{2,23,26,28–31} also.

References
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