Summary & Conclusion
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The objectives of the present study have been

- to find the male reproductive toxic effect of Plumbagin, a naphthoquinone, obtained from *Plumbago* ssp., which is already in use as therapeutic for several ailments; and
- to find if plumbagin could be recommended for use in male contraception.

Three months old male Wistar strain albino rat of proven fertility were administred with plumbagin dissolved in hot milk at a daily dose of 8mg/kg body wt through intragastric route for 55 days. Rats administered with milk alone served as the control. Cauda epididymidal sperm were subjected to analysis of counts, motility, viability and abnormalities adopting appropriate techniques. Slices of testis and epididymis were subjected to routine histological analysis adopting Bouin’s fixation and H&E staining. Also, slices of testis and segments of epididymis obtained from perfused animals were immersion-fixed in glutaraldehyde, post-fixed in OsO₄ and embedded in low viscosity resin. Semithin sections (1 μm thickness) were stained in TBO and observed in a light microscope. Ultrathin section were stained in uranyl acetate and lead citrate and observed in TEM. Glutaraldehyde-fixed slices of epididymis and spermatozoa were analyzed using scanning electron microscope. Proteins of the various segments of epididymis, namely caput, corpus and cauda were analysed adopting SDS-PAGE and densitometric scanning. Sections of testis and segments of epididymis, fixed in 10% neutral buffered formalin, were analysed adopting immunocytochemical techniques for the localization of cathepsin D and CD68. Blood was also collected and the serum used for the radioimmunoassay of estradiol, testosterone, FSH and LH.

- The plumbagin treatment resulted in severe histopathological changes in the spermatogenic compartment, whereas the androgenic compartment remained unaffected.
- The seminiferous epithelium remained highly disorganized, due to premature exfoliation of cells from the adluminal compartment.
- Several round spermatids reflected death through apoptosis.
- Pachytene spermatocytes were seen to show signs of degeneration.
- The Sertoli cells were affected, causing premature exfoliation of round spermatids either due to disruption of inter-Sertoli cell junctions as well as Sertoli cell-germ cell association due to disruption of Sertoli cell microtubules, actin and vimentine.
- Asynchrony among stages in the cycle of the seminiferous epithelium was observed.
- There was no significant change in the circulating levels of testosterone, FSH, LH and estradiol.
- The cauda epididymidal sperm counts decreased as a consequence of impaired spermatogenesis. Several sperm abnormalities indicated disruption of spermiogenesis. Sperm with two or more axonemes, abnormal axonemal structures and retention of cytoplasmic droplet (CD) by the cauda epididymidal sperm were interesting observations. Retention of CD by the cauda epididymal sperm reflected impairment of specific stimulus from epididymis to shed CD. It also reflects the possibility of synthesis of intra-epididymidal estrogen by CD. A major percentage of the sperm was not viable.
- In the epididymis, principal cells either appeared affected or stimulated in secretion as seen in the widening of the Golgi apparatus and occurrence of secretion granules.
- Heightened secretory activity of principal cell was indicated in the formation of endocytic vesicles in the apical cytoplasm and their digestion in multivesicular bodies.
- Evidence for clear cells, with reticulate microvilli, phagocytosing sperm through the apical pseudopodial processes was obtained
- Evidence for the appearance of microcanaliculi in the corpus epididymal epithelium was also obtained, indicating their role in prevention of extravasation of sperm from the lumen.
- Intraepithelial macrophage activity increased and it is discussed in relation to its role in scavenging cell debris from epididymal epithelium.
- Basal cells increased in counts and it is discussed in relation to its role in detoxification mechanism and also immune surveillance.
Narrow cells with apical blebs protruding and pinching off into the lumen of epididymis, is an indication to prove its role in removal of harmful electrophilic substances.

Apical cells underwent hypertrophy and hyperplasia and were observed to be increased in endocytotic activity.

The presence of lipofuscin inclusion in the principal, basal cells and transformed macrophages were indicative of less efficient lysosomal function.

Evidences for the transformation of basal cells to intra-epithelial macrophages were obtained.

The apical and narrow cells showed higher reactivity of cathepsin D and principal cell responded moderately, indicating the activity of hydrolyses of lysosomes.

Although, histological evidence for transformation of basal cells into macrophages was obtained, immunocytochemical analysis did not prove positive for CD68, and the quantitative expression of it did not show any change between control and treated.

Thus, plumbagin, a phytotherapeutic, is potentially toxic to the male reproductive mechanisms, particularly spermatogenesis and the epididymal physiological maturation of sperm. Therefore, plumbagin being toxic to male reproductive mechanisms, caution be applied while using plumbagin as a therapeutic. In plumbagin, not affecting the Leydig cells, and the circulating levels of hormone and in all the male reproductive toxic manifestation which would potentially impair fertility being reversible, plumbagin would in all prospects be proposed as an agent for testing as a male contraceptive.