Effect of *Azadirachta indica* leaves on rat spermatozoa

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Histochemical studies and SEM observations on the morphological changes in the head of the spermatozoa in general, and the acrosome in particular, in *A. indica* treated rats are reported. In the treated rats change in the shape and size of the sperm head, with a dorso-ventral constriction of the middle region of the sperm head i.e., between the anterior and posterior regions was observed. It was rather difficult to differentiate the outer acrosomal and outer plasma membranes. A decrease in the perforatorium or sub-acrosomal material, post nuclear cap and the nuclear material near the basal plate at the base of the sperm head were also observed. The results suggest that the effects are probably due to androgen deficiency and a general disturbance in carbohydrates or polysaccharides located in the sperm head, caused by the antiandrogenic property of the leaves of *A. indica*.

About 320 plants are known to cause disturbance in spermatogenesis. To mention a few, the leaves of * Ocimum sanctum*¹, and *Plumeria alba*² have been reported to cause impairment of spermatogenesis in rats. *Azadirachta indica*, the common neem tree of India, possesses emmenagogue, antiimplantational, spermicidal and antifertility properties³⁴⁵. The leaves of *A. indica* cause impairment of spermatogenesis and exhibit antiandrogenic effect on the testis of the adult albino rat⁶. They are antispermatic⁷ and cause decrease in the weight of accessory glands like seminal vesicles and ventral prostate⁸, and decrease in the serum levels of testosterone in rats⁹, and several such effects appear reversible¹⁰. The present study reports changes in the sperm of rat on treatment with *A. indica*.

The leaves of *A. indica* were collected locally, dried in shade, finely powdered, quantitatively suspended in distilled water and administered through a oral gavage to 3 months old male rats of Wistar strain weighing 190-200 g. They were maintained in a well ventilated animal house with standard pellet diet and water *ad libitum*. The rats were divided into 2 groups of 6 animals each. The rats in group I served as control and were administered with 1 ml of distilled water for 14 days and autopsied 24 hr after the last dose. The rats in group II were given 500 mg/kg body weight of neem leaf powder for 14 days and were autopsied 24 hr after the last dose. A drop of epididymal plasma was subjected to histochemical analysis adopting periodic acid-Schiff (PAS) reaction¹¹, and for SEM observations, one or two drops of epididymal plasma was fixed in 2% glutaraldehyde, centrifuged and washed with 0.1 M sodium cacodylate buffer (pH 7.2), centrifuged again and a thin film was applied on cover slip, dried, coated with Gold and finally observed in SEM (model LEO, 435 VP, Detector SL.I LEO Electron Microscopy Ltd. Cambridge, England).

In the sperm of control rats there was intense PAS-positive reaction (Fig. 1). In the sperm of *A. indica* treated rats the intensity of reaction of the sperm in general and in the acrosome in particular was reduced. Even the acrosomal membrane along the entire length of head of the sperm stained less and the lateral portion of the acrosome and ventral surface of the perforatorium or sub-acrosomal material also stained less (Fig. 2). Sperm abnormalities were also observed which included head agglutination, irregular and detached heads and such sperm stained less with PAS (Figs 3 and 4).

Electron micrograph of the sperm head of control rats (Fig. 5), showed normal parts. In treated rats, changes in the shape and size of sperm head and decrease in size of the acrosomal membrane and outer plasma membrane were noticed. It was rather difficult to differentiate the two membranes. A dorso-ventral

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constriction of the middle region, i.e., between the anterior and posterior regions of the sperm head, decrease in the perforatorium or sub-acrosomal material, post nuclear cap and nuclear material near the basal plate at the base of the sperm head were observed (Figs 6 and 7).

Various plants like Calitris robusta, Cassia javanica, Garcinia indica and Ardisia nerifolia have been reported to possess antifertility activity. Vincristine, an alkaloid, isolated from Vinca rosea causes absence of sperm in seminiferous tubules, depletion of germinal epithelial elements and formation of hypertropic giant cell in rats. Gossypol, a constituent of cotton seed oil suppresses spermatogenesis and damages the germ cells of the hamsters. Plumbagin, isolated from the roots of Plumbago zeylanica causes selective testicular lesions in dogs. A. indica causes varying degrees of arrest of spermatogenesis and degeneration of the nuclei of germinal cells in male mice. Recently, this plant has been reported to possess antispermatic activity. Evidence for reversible androgenic effect of this plant has also been reported.

It is known that sperm reproduction does not proceed optimally to completion without a continuous androgen supply. Studies involving hypophysectomy, castration and androgen replacement therapy reveal that androgen is essential for physiological maturation, and survival of the spermatooza in the epididymis. Sperm possess two principal attributes, viz. motility and the fertilizing ability, which are prerequisites for fertilization. Any negative impact on motility would seriously affect the fertilizing ability. Semen sample/ejaculate containing more than 20% of abnormal sperm is considered poorly fertile. The occurrence of morphologically abnormal sperm is a diagnostic aid for infertility. Acrosome contains several enzymes which are secreted by the Golgi apparatus and endoplasmic reticulum. From histochemical evidence, the presence of carbohydrates or polysaccharides in the acrosome of head of the spermatozoa, which are associated with various enzymatic activities, is indicated. The synthesis of enzymes destined for the acrosome is regulated to some degree by testosterone. An androgen deficiency of Golgi complex due to under-nourishment of Sertoli cells seems more plausible, because androgen development is under the control of hormone dependent cellular events manifested through Sertoli cell-germ cell interactions.

In the present study the morphological changes in the head of the sperm in general, and the acrosome in particular, may have resulted from the alteration in the epididymal milieu and probably due to a general disturbance of carbohydrates or polysaccharides present in the acrosome of the sperm head. It is suggested that morphological changes in the head of sperm as well as its acrosome are probably due to androgen deficiency consequent upon the antiandrogenic property of the A. indica leaves.

References

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