Impact of feeding ethanolic extracts of Achyranthes aspera Linn. on reproductive functions in male rats

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Feeding 50% ethanolic extract of A. aspera to male rats resulted in reduced sperm counts, weight of epididymis, serum level of testosterone and testicular activity of 3β-hydroxysteroid dehydrogenase, while motility of the sperm and activity of the HMG CoA reductase were not affected. Cholesterol level in the testis, incorporation of labelled acetate into cholesterol, 17-ketosteroids in urine and hepatic and fecal bile acids were increased. The results suggest that ethanolic extract of A. aspera caused reproductive toxicity in male rats and the action may be by suppressing the synthesis of androgen.

The plant Achyranthes aspera Linn, (Kadaladi), belonging to the family Amaranthaceae, is widely distributed in India. In the Indian system of medicine, the plant is used as a curative in cough, asthma, bronchitis, dyspepsia, flatulence, colic, painful inflammations, dropsy, ophthalmopathy, vomiting, leprosy, skin diseases, pruritus, helminthiasis, strangury, renal and vesical calculi, cardiac disorders, anaemia, vitiated conditions of Kapha and Vata and general debility1.

There is an isolated report that n-butanol fractions of A. aspera possess contraceptive efficacy in female rat and hamster when administered through oral route during the preimplantation period2. However there is no information on its effect on male reproduction. Hence, the effect of oral administration of 50% ethanolic extract of root of A. aspera was studied in male rat.

Preparation of the extract — Root of A. aspera was collected locally and dried at 60°C. Dried material (10%) in 50% ethanol was refluxed at 65°C for 90 min, filtered and evaporated to dryness in a speed vacuum concentrator (Savant sc110).

Animal study — Male albino rats (Sprague Dawley strain, body wt 150±5g), bred in the university animal house and maintained on a normal laboratory diet (Lipton India Ltd, Bangalore, India) were used. Water and food were given ad libitum. The rats were housed in polypropylene cages in a room with temperature maintained at 25±1°C in a 12:12 hr L:D cycle. Rats were randomly divided into two groups (control and experimental) of 6 animals each. The extract was suspended in distilled water and administered through oral route using gastric intubation. Control animals were administered with distilled water. The duration of the experiment was 60 days and the dose was 50mg /100g body weight /day. On day 60, 24 hour urine was collected for estimation of 17- ketosterols. At the end of the experimental period the rats were fasted overnight and sacrificed. The weight of the testis and epididymis were recorded immediately and the testis and epididymis were removed to ice -cold containers for analyses.

Semen was collected from cauda epididymis of healthy male rats and was diluted with normal saline and kept at 37°C. The motility of the sperm was observed under light microscope. Sperm motility and sperm counts were determined by the methods given in WHO manual3. Control animals were administered with distilled water. The duration of the experiment was 60 days and the dose was 50mg /100g body weight /day. On day 60, 24 hour urine was collected for estimation of 17- ketosterols. At the end of the experimental period the rats were fasted overnight and sacrificed. The weight of the testis and epididymis were recorded immediately and the testis and epididymis were removed to ice -cold containers for analyses.

Semen was collected from cauda epididymis of healthy male rats and was diluted with normal saline and kept at 37°C. The motility of the sperm was observed under light microscope. Sperm motility and sperm counts were determined by the methods given in WHO manual3. The lipids were extracted adopting the procedure of Radin et al. Cholesterol was estimated according to the method of Abell et al. Hydroxymethyl glutaryl CoA reductase activity was assayed as described by Rao and Ramakrishnan6 by determining the ratio of hydroxymethyl glutaryl CoA to mevalonic acid. Activities of glucose 6 phosphate dehydrogenase7, malic enzyme8, 17β hydroxysteroid dehydrogenase9 and Δ5-3β hydroxysteroid dehydrogenase10 were determined. Testosterone level was determined by RIA by using the kit for total testosterone purchased from Diagnostic Products Corporation, USA. Bile acids were extracted from liver according to the procedure of Okishio et al11. Fecal sterols and bile acids were extracted by the general procedure of Grundy et al12. Bile acids were estimated by the
enzymatic procedure described by Palmer. In vivo incorporation of 1,2,14C acetate into lipids of testis was carried out as described by Molly Thomas et al. Extraction and estimation of 17 ketosteroids was done. The protein content was estimated after precipitation with TCA by the method of Lowry et al. Statistical analysis of the data was performed using Student’s ‘t’ test.

The results are presented in Table 1.

As a pilot study, a few local plants were screened for spermicidal toxicity. A. aspera was found to have spermicidal action in the in vitro and in vivo studies. Hence, a detailed study on the impact of ethanolic extract of A. aspera in the rat model was undertaken. The treatment did not cause any change in the weight of the testis. Similar results have been obtained on treatment with prototuran, a herbicide which impaired androgen biosynthetic process. The epididymis provides an environment suitable for morphological and biochemical changes in the spermatozoa. Physiological and biochemical integrity of the epididymis is dependent on androgens. The deficiency of androgen causes general regression of epididymal epithelium and a decline in the number of spermatozoa. A decrease in sperm reserve may be a reasonable cause for reduction in the weight of the epididymis. The significant decrease in the serum testosterone level in the treated animals supports this view.

Steroid hormones regulate spermatogenesis and maturation of spermatozoa. Precursor for testicular androgen is cholesterol. The cholesterol levels were increased in the experimental animals. Synthesis of cholesterol was enhanced as evidenced by the increased incorporation of labelled acetate into cholesterol. Catabolism of cholesterol to bile acids was also increased. Drastic reduction in the activity of Δ3-3β-hydroxysteroid dehydrogenase may be the reason for reduced testosterone production and accumulation of cholesterol. NADPH is required for the biosynthesis of testosterone. Decreased activity of the lipogenic enzyme glucose 6 phosphate dehydrogenase may also be a contributory factor for the reduced synthesis of testosterone. Increase in 17 ketosteroids points to increased catabolism of testosterone to produce 17 ketosteroids and the latter are generally inactive or less active than the parent compound. Therefore it can be concluded that the ethanolic extract of A. aspera at a dose of 50mg/100g body weight suppresses the production of testosterone and increases the catabolism of testosterone. The extract may contain a combination of a number of hydrophilic and hydrophobic compounds. Studies are in progress to isolate the active principle.

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References


