Effects of short term treatment of solasodine on cauda epididymis in dogs

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Oral administration (80 mg/kg body wt/day for 30 days) of solasodine (extracted and isolated from the berries of the Solanum xanthocarpum) to intact dogs significantly decreased the epithelial cell height of cauda epididymides. The cells became atrophic and the lumen was devoid of spermatozoa. Castration followed by the administration of solasodine further reduced the epithelial cell height in comparison to castrated controls. Concurrent treatment of solasodine along with testosterone propionate was unable to restore the normal epithelial lumen parameters. Total protein, sialic acid, glycogen and acid phosphatase activities were significantly reduced in solasodine treated cauda epididymides. These results suggest antiandrogenic potency of solasodine.

Materials and Methods

Solasodine used was extracted and isolated from the Solanum xanthocarpum commonly known as kant kari; the parts of which are known to have some medicinal uses in dysurea, mepisteralisis, sore throat and pain relieving. Extraction procedure—Shade dried and powdered berries of S. xanthocarpum were exhaustively extracted with CH₃OH. The crude methanolic extract obtained after concentration (under reduced pressure) was treated with 10% HCl for 2-3 hr., filtered and the filtrate was basified with NH₃ solution and was finally extracted with CHCl₃. The CHCl₃ extract following concentration under reduced pressure was resuspended in 80% CH₃OH and a pure sample of solasodine was obtained after recrystallizing 80% CH₃OH at 197-199°C, m.p. (yield 0.42%). It gave single spot on TLC (Si gel plate) using CHCl₃: CH₃OH (19:1) as developing system (Rf=0.24) and spot on plate was visualized by spraying Dragendorff’s reagent. Finally the identification of the isolated sample was confirmed by m.p. (197-199°C), mmp (undepressed) and superimposable IR spectra of the authentic sample which was obtained from Malti. Chem. Research Centre Baroda (Gujrat), India.

Animals and Treatment- Adult healthy male dogs (30) weighing 12-17 kg were used and divided into following 6 groups of 5 each. Castration of animals was carried out under nembutal (25-30 mg/kg body weight) anaesthesia. Seven days after castration animals were included in the study.

Group 1 (G1) - Control : fed with placebo mutton capsules.
Group 2 (G2) - Intact + solasodine: solasodine powder (80 mg/kg body weight wrapped in mutton capsules fed orally/day)
Group 3 (G3) - Castration: castrated bilaterally through the scrotal route under aseptic conditions.
Group 4 (G4) - Castration + solasodine: solasodine powder (80 mg/kg body weight wrapped in mutton capsules fed orally/day)
Group 5 (G5) - Castration + testosterone propionate (TP): intramuscular TP (8 mg) injection every alternate day.
Group 6 (G6) - Castration + solasodine + TP: solasodine powder (80 mg/kg body
weight) wrapped in mutton capsules fed orally/day and TP (8 mg) im injection every alternate day.

After 30 days of treatment the final body weight of all the animals was recorded and they were biopsied and epididymides were dissected out and weighed on a torsion balance after clearing fat and connective tissue.

Half of each cauda epididymides were traced with camera lucida at demonstration of solasodine and epididymis weights, like with epididymides were frozen for biochemical estimations of total protein, sialic acid, glycogen, and acid phosphatase. The remaining halves of cauda epididymides were fixed in Bouin's fluid. Paraffin sections (6 μm thick) were cut and stained with Harris Haematoxylin and Eosin for histological changes. The remaining halves of cauda epididymides were frozen for biochemical estimations of total protein, sialic acid, acid phosphatase, and glycogen. Quantitative estimation of total cholesterol was also carried out.

Cell height of luminal epithelial cells (100) of cauda epididymides were traced with camera lucida at 360 X and length of each tracing was measured, averaged and expressed in terms of luminal epithelial cell height. Data were analysed using Student's t test.

### Results

**Body weight**—Solasodine administration did not cause any significant change in the body weights of treated dogs (groups 1-6). Bilateral castration significantly (P<0.05) lowered epididymal weight as compared to controls (group 1). Testosterone administration to castrated animals resulted in a significant increase in epididymal weight. Simultaneous administration of solasodine and TP failed to restore the epididymis weights, like with TP (Table 1).

**Histology**—Solasodine caused a reduction in tubular diameter and also significantly (P<0.001) reduced the epithelial cell height (Figs 1 and 2; Table 1). Castration too presented a comparable picture with a reduced cauda epididymal lumen and epithelial cell height, stereocilia were absent and inter-tubular stroma was greatly increased (Fig. 3). Castration with solasodine treatment further affected the epithelial cellular integrity. Epithelial cell height dropped significantly (P<0.001) as compared to castration alone (Fig. 4). Testosterone replacement resulted in 42.74% restoration in tubular diameter and cell height (Table 1). The stereocilia also reappeared (Fig. 5). The beneficial effects of TP on epididymal parameters were counteracted when solasodine was supplemented with TP. The percentage of restoration in epididymal weight and epithelial cell height were 12.1 and 44.05 respectively (Fig. 6).

**Biochemical estimation**—Solasodine administration caused significant (P<0.001) reduction in protein and sialic acid contents in cauda epididymides. While glycoprotein and acid phosphatase activities followed a similar trend, total cholesterol was estimated higher in all the solasodine treated groups (Table 2).

### Discussion

Oral administration of solasodine for 30 days caused no loss in body weights in dogs but a significant (P<0.05) decrease in epididymal weight was observed. The epithelial cell height in the lumen was also considerably affected as a result of such a treatment. Biochemical parameters such as total protein, sialic acid, glycoprotein and acid phosphatase activity were drastically reduced as compared to controls. Total cholesterol however, was estimated higher in the treated groups.

Following solasodine feeding, the most outstanding feature in the cauda epididymides was the complete

| Table 1 — Changes in body weight, epididymis weight and lumen epithelial cell height following castration and solasodine treatment |
|-----------------|-----------------|-----------------|
| Groups          | Body wt (kg)    | Epididymides (mg/kg body weight) | Cauda epididymal epithelial cell height (μm) |
| Control (G1)    | 13.3 ± 3.3      | 376 ± 55         | 39.3 ± 1.14 |
| Intact + Solasodine (G2) | 14.5 ± 4.0      | 207 ± 13(a)      | 21.2 ± 0.45(b) |
| Castration (G3) | 10.1 ± 2.9      | 217 ± 19(b)      | 22.74 ± 0.45(b) |
| Castration + Solasodine (G4) | 12.6 ± 3.5      | 215 ± 5(a)       | 19.16 ± 0.31(b) |
| Castration + TP (G5) | 11.15 ± 3.2     | 277 ± 7(a)       | 32.46 ± 0.63(b) |
| Castration + Solasodine + TP (G6) | 13.5 ± 2.6      | 241 ± 29(b)      | 27.6 ± 0.60(b) |

*P Values: *P<0.05; **P<0.001; ++P<0.001; NS non significant
a,b: compared with G1
*,: compared with G3
**: compared with G5
denudation of lining epithelium with normal vasculature (capillaries always appeared normal). The fact that solasodine does not act so severely on the empty epididymal tract (castrated dogs) suggests that it has a direct cytotoxic effect on epididymal cells which are involved with their functional capacity to absorb seminal fluid. Such effects were also reported earlier in rats and dogs receiving α-chlorohydrin\textsuperscript{18,19}.

Low levels of protein in epididymis after solasodine treatment reflects an inhibiting effect of solasodine treatment.

Figs 1-6—(1) —Cauda epididymis from control dog. (Normal luminal epithelium presenting stereocilia.); (2) —Cauda epididymis after solasodine treatment. (Reduction in the epithelial cell height.); (3) —Cauda epididymis following castration. (Empty lumen with associated reduction in epithelial cell height.); (4) —Cauda epididymis following castration and solasodine treatment. (Note the shrunken and pyknotic appearance of the epithelium with massive increase in intertubular stroma.); (5) —Cauda epididymis following castration and T.P. treatment. (Note the increase in tubular dimensions and cell height.), and (6) —Cauda epididymis following castration and simultaneous T.P. and Solasodine treatment. (Restoration of epithelial cell height was affected by solasodine treatment). [All figures ×100, H & E.]
on androgen from expressing its activity at target sites. The principal cells of the epididymal epithelium synthesize protein which is androgen dependent and forms one of the constituents that ensure the maturation of sperm.

The epididymis is androgen dependent and the action of solasodine is accompanied by a considerable reduction of androgen support from the testes. This is reflected by the reduced sialic acid concentration in the epididymis of intact and solasodine treated dogs, comparable with similar reduction occurring after castration alone. Peyre and Laporte reported that in the rat castration causes a decrease in the epididymal sialic acid concentration. According to them this could be due to the absence of spermatozoa or to a direct action of the drug on the epididymal epithelium. The present examination also revealed the absence of spermatozoa in the lumen and denudation of the lining.

Glycogen contents of the epididymides reduced after solasodine treatment alone or with the conjuction of TP. The manifestation of these changes probably attribute to the anti androgenic nature of solasodine. The reduced acid phosphatase activity of cauda epididymides after solasodine treatment may be due to the suppressed hydrolytic and secretory processes. Castration results in a significant decrease in acid phosphatase activity of epididymides.

It can be concluded that solasodine possesses an antiandrogenic nature as it brought about epididymal dysfunction.

References