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Research Article

Reversible antifertility effect of *Opuntia elatior* Mill. fruit extract

Ramya MC*, Shivabasavaiah, Shivanandappa T

Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysuru-570006, Karnataka, India

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***Correspondence:**

Dr. Ramya MC,

E-mail: ramya.chamundaiah@gmail.com

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ABSTRACT

Background: This study was undertaken to evaluate the male antifertility potential of the fruit extract of *O. elatior* and its reversibility.

Methods: The methanol fruit extract was administered orally (300, 900 mg/kg bw) to male rats for 60 days, and fertility was assessed by analysing spermatogenesis, epididymal sperm count, serum testosterone levels and testicular hydroxysteroid dehydrogenase activity. Fertility was assessed by mating treated rats with normally cycling virgin females. Reversibility of fertility suppression was studied by withdrawal of treatment for two weeks.

Results: Epididymal sperm count and motility was markedly reduced up to 75-80% in rats treated for 60 days, without commensurate decline in serum testosterone levels. Testicular steroidogenesis was not affected as evident by the hydroxysteroid dehydrogenase activity. Fertility of the treated rats was suppressed when mated with normally cycling virgin female rats without affecting libido. The fertility suppression was dose-dependent being 100% in the highest dose. Withdrawal of the treatment for two weeks led to recovery of the epididymal sperm count, testicular HSDH activity, serum testosterone levels and the fertility.

Conclusions: The methanol extract of the fruit of *O. elatior* shows reversible male antifertility activity without affecting the serum testosterone levels and libido.

Keywords: Male antifertility, *Opuntia elatior*, Sperm analysis, Steroidogenesis, Reversibility

INTRODUCTION

Plants have been used in traditional medicine since ancient times, and several modern drugs have been discovered from herbal sources.¹ Although herbal medicines for fertility control have been in use in folk medicine, discovery of effective drugs for birth control from natural sources has remained elusive.²⁻⁴ There is recent spurt of interest in research on plants as potential sources to develop newer contraceptives. The need to develop male antifertility agents is well recognized and many plants have been investigated for their antifertility effects in the male.^{5,6} Studies on plant extracts have been shown to affect testicular function including spermatogenesis and steroidogenesis in laboratory animals.⁷⁻⁹ Many of the reports of plant extracts on

antifertility activity in the male, fall short of the requirements for a viable antifertility agent. An ideal, safe male antifertility agent should be able to selectively affect sperm production and function without altering androgen level or libido in addition to reversibility.¹⁰

Several species of the genus *Opuntia* (Cactaceae), occur in many parts of the world and they are also cultivated for their food and medicinal value. Fruits and phylloclade of *Opuntia* are considered natural health foods and recognized as valuable source of nutraceuticals.¹ The cactus *Opuntia dillenii* (Ker-Gawl.) Haw. grows wild and also cultivated in India; its fruits are edible and contain a variety of nutrients including minerals, salts, amino acids and vitamins.¹²⁻¹⁸ Phylloclade and fruits of *O. dillenii* are used in folk medicine for various ailments such as

allergies, rheumatism, ulcers and reported to possess hypolipidemic and hepatoprotective properties.¹⁹⁻²¹ Extracts of the phylloclade of *O. dillenii* have been reported to show antispermatic effect and fertility suppression in the laboratory rat.^{22,23} *O. elatior* is a common cactus in southern India and there is an unsubstantiated claim of its use in folk medicine for fertility control.²⁴ In view of the potential of *Opuntia* as a source of male antifertility agent, this study was undertaken to investigate the male antifertility activity of the fruit pulp of *O. elatior* with regard to sperm production, steroidogenesis, fertility and its reversibility.

METHODS

Chemicals

The fine chemicals, DHEA (5-androsten-3 β -ol-17one) and testosterone (17 β -hydroxyandrost-4-en-3-one) were obtained from Sigma chemical co. (USA); Nicotinamide Adenine Dinucleotide sodium salt (NAD), Nicotinamide Adenine Dinucleotide reduced (NADH), Iodonitro Tetrazolium chloride (INT) and phenazine methosulphate (PMS) were purchased from Sisco research labs, Mumbai.; testosterone ELISA kit from labor Diagnostika Nord GmbH & Co. KG, Germany.

Preparation of the extract

The taxonomic identity of the plant, *Opuntia elatior*, was ascertained by a taxonomic expert (Prof. G. R. Shivamurthy) and a voucher specimen is available at the department of Botany, University of Mysore, Mysuru (Authentic herbarium catalogue number of the species from India, K000100500, Kew botanical gardens, UK). The fruits collected from the fields of Kolar (Karnataka State, Southern India) during the months of Jan-Mar (2011, 2012), were cut into pieces, seeds removed manually, air dried and powdered in a blender.

The dry powder (150 g) was subjected to sequential extraction for 36 h using the solvents, petroleum ether, ethyl acetate and methanol.

The final methanol extract was evaporated to dryness in a flash evaporator which yielded 38 g of solid residue. The dried extract was dissolved in water (100 mg/ml) for experiments.

Animals

90 day old male albino rats of wistar strain (weighing 180-200 g) were housed in poly propylene cages, maintained under standard conditions (25-26°C, 70% relative humidity, 12:12 light/dark) in the animal house facility, and provided with the laboratory chow and water *ad libitum*. Guidelines of the institutional animal ethical committee (Approval No. 841/b/04/CPCSEA) were followed.

Experimental design

Rats were divided into three groups of ten animals each. Group I (control) received only water, group II and III were administered orally with the methanol extract at 300 mg and 900 mg/kg bw respectively for a period of 60 days. The dosages were selected based on a preliminary study showing effectiveness of the extract.²⁵ On 61st day, autopsy was performed on half of the animals in each group and the remaining animals were maintained without treatment (withdrawal) for a period of two weeks before autopsy.

Autopsy

At the end of the respective treatment, the animals were sacrificed by ether anesthesia. The reproductive organs (testis, epididymis, seminal vesicle, ventral prostate) were dissected out and weighed. Blood was collected from the heart and allowed to clot to obtain serum for assaying testosterone.

Sperm analysis

Sperms were obtained from the cauda epididymis by mincing in physiological saline, and centrifuged at 3000 rpm. The supernatant was used for sperm count using Naeubauer's chamber. The sperm motility was assessed by standard protocols.²⁶

Histology

Testes, epididymides, seminal vesicles and ventral prostate were fixed in Bouin's fluid for 24 hours and processed for paraffin embedding. Paraffin sections (5 μ m thick) were stained with haematoxylin and eosin and observed under microscope.

Testosterone assay

Serum testosterone levels were measured by ELISA using a commercial kit (LDN, Germany). The sensitivity of the assay was 0.022 ng/ml.

Hydroxysteroid dehydrogenase assay

Testes were homogenised in 0.1M tris buffer (pH 7.4) and centrifuged at 5000 rpm for 15 min in a cold centrifuge and the supernatant was used for enzyme assay. Activity of Δ^5 3 β and 17 β hydroxysteroid dehydrogenases were assayed using pregnenolone and testosterone, respectively, as the substrates.²⁷ Protein was determined using bovine serum albumin as the standard.²⁸

Reversibility study

After a period of 60 days, the treatment was withdrawn for a recovery period of two weeks, and the animals were sacrificed. Investigations on sperm count, motility and fertility were done.

Fertility

After 60 days of the treatment period, the treated and control male rats were allowed to mate with normally cycling (untreated) virgin females in the ratio of 1:2.²⁶ The vaginal smear was checked for sperms for positive mating. The mated female rats were kept for observation for pregnancy and allowed to litter. The males were autopsied, the reproductive organs and blood were collected for sperm analysis, testosterone measurement and histology as described earlier.

Statistical analyses

The data were analyzed by one-way analysis of variance (ANOVA) using the statistical software (SPSS, version 14). The significance was determined at P <0.05.

RESULTS

Body weight and reproductive organ weights

Body weights of rats were not significantly affected in the treatment groups and were comparable to their control groups.

The relative weights of testes in the treatment groups were marginally lower but not statistically significant.

The weights of epididymis were marginally higher whereas that of ventral prostate and seminal vesicle slightly lower, but the differences were not statistically significant (Table 1).

Table 1: Antifertility effect of *O. elatior* fruit extract in the male rat: reproductive organ weights.

Group	Final body weight (g)	Relative organ weight (g/100 g bw)			
		Testis	Epididymis	Seminal vesicle	Ventral prostate
Control	222.00 ± 3.54 ^a	1.04 ± 0.034 ^a	0.36 ± 0.03 ^a	0.21 ± 0.017 ^a	0.06 ± 0.045 ^a
300 mg/kg bw	233.11 ± 3.06 ^{ab}	0.98 ± 0.036 ^a	0.32 ± 0.01 ^{ab}	0.10 ± 0.006 ^b	0.05 ± 0.004 ^a
900mg/ kg bw	216.22 ± 3.34 ^c	1.00 ± 0.035 ^a	0.32 ± 0.02 ^{ab}	0.12 ± 0.027 ^{ab}	0.05 ± 0.01 ^a
Recovery-300 mg/kg bw	240.00 ± 5.50 ^{ab}	0.96 ± 0.01 ^a	0.42 ± 0.01 ^b	0.51 ± 0.03 ^c	0.09 ± 0.01 ^a
Recovery-900 mg/kg bw	35.0 ± 6.10 ^{ab}	1.07 ± 0.02 ^a	0.44 ± 0.04 ^b	0.49 ± 0.02 ^c	0.11 ± 0.01 ^a

Values (Mean ± SE) denoted by different alphabets differ significantly (P <0.05, DMRT), n=5

Sperm analysis

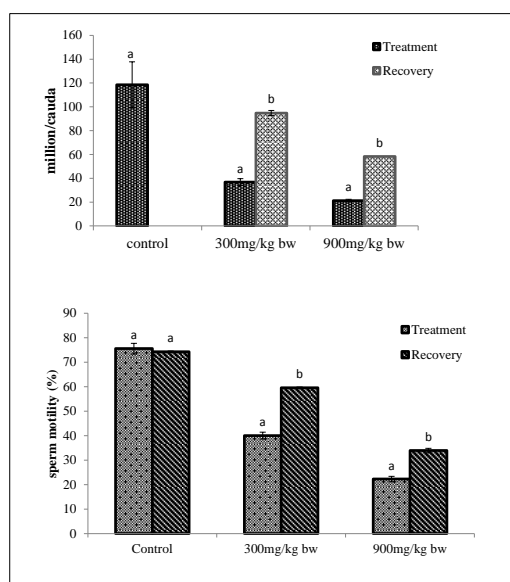


Figure 1: Antifertility effect of *Opuntia elatior* fruit extract in the male rat: (a) Sperm count (b) Sperm motility.

Values (Mean ± SE) denoted by different alphabets differ significantly (P <0.05 DMRT) n=5

Sperm count from the cauda epididymis was markedly reduced by 80% in treatment groups, when compared to the control groups. The progressive sperm motility was also significantly declined by 46% and 73% in the treated rats at 300 and 900 mg/kg bw, respectively. Both sperm count and motility were recovered up on the withdrawal of treatment (Figure 1).

Histology

The testicular volume, the seminiferous tubule diameter and seminiferous epithelium of the treated rats of epididymis were comparable to that of control group showing no signs of testicular degeneration or spermatogenic arrest. The Leydig cells also appeared normal in the testis of the treated rats. The histological features of the epididymis of treated rats showed no visible changes in the tubule diameter, epithelial cell height and were comparable to those of control animals. Similarly, the histological profile of the seminal vesicle and ventral prostate of treated rats showed no significant changes from that of control animals (Figure 2).

Hydroxysteroid dehydrogenase activity

The enzyme activities of Δ⁵ 3β and 17β HSDHs were not significantly affected in the testis of treated rats. However, 17β HSDH activity was marginally lower in

the testis of treated animals but was not significant (Figure 3).

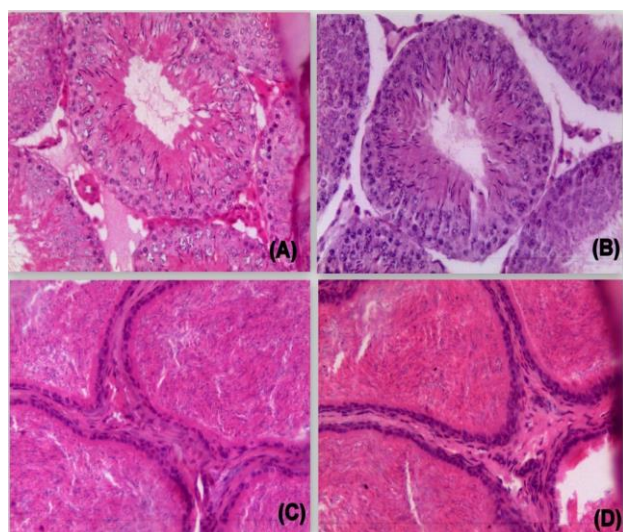


Figure 2: Histology of the testis and cauda epididymis of male rats treated with *O. elatior* fruit extract for 60 days. Testis (A - Control & B - Treated) and cauda epididymis (C - Control & D - Treated); (Stain - H & E; magnification x200).

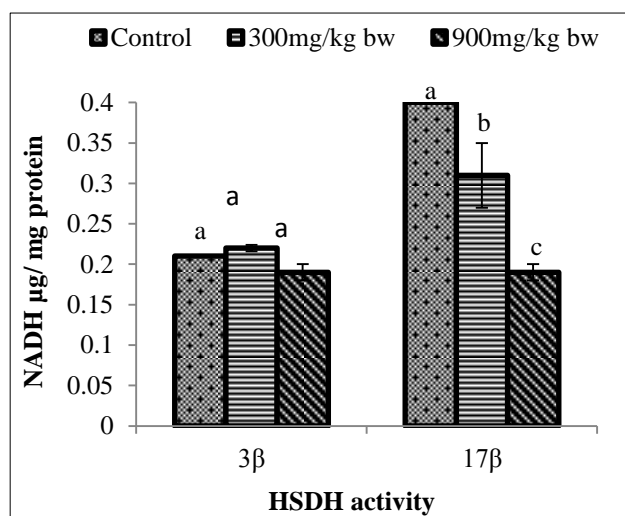


Figure 3: Antifertility effect of *Opuntia elatior* fruit extract in the male rat treated for 60 days: Testicular hydroxysteroid dehydrogenase activity.

Values (Mean ± SE) denoted by different alphabets differ significantly (P <0.05 DMRT) n=5

Serum testosterone

Serum testosterone levels were not significantly affected in the treated rats, and were comparable to the control groups. However, the serum testosterone levels were found to be slightly higher than that of control group after withdrawal of treatment (Table 2).

Table 2: Antifertility effect of *Opuntia elatior* fruit extract in the male rat: serum testosterone.

Group	Testosterone (ng/ml)
Control	594 ± 2.68 ^c
300 mg/kg bw	506 ± 8.72 ^b
900 mg/kg bw	383 ± 10.0 ^a
Recovery-300 mg/kg bw	736 ± 0.40 ^d
Recovery-900 mg/kg bw	733 ± 0.64 ^d

Values (Mean ± SE) denoted by different alphabets differ significantly (P <0.05, DMRT), n=5

Fertility

Fertility of male rats treated with the extract for 60 days when mated with untreated females was markedly affected. The vaginal smears of the mated female rats showed sperms indicating that the male rats from the treated group mated successfully and the libido was not affected. The pregnancy was 50% in the lower dose (300 mg/kg bw) group, whereas, there was no pregnancy at the higher dose (900 mg/kg bw). Fertility of the treated male rats was restored after the withdrawal of treatment for a period of two weeks. However, litter size was reduced in rats treated with the high dose (Table 3).

Table 3: Effect of *O. elatior* fruit extract on fertility of male rats.

Group	Mating success (vaginal sperm)	No. of rats pregnant (%)	
		60 days treatment	Withdrawal of treatment
I (Control)	+	100	100
II (300 mg/kg bw)	+	0	33.3
III (900 mg/kg bw)	+	0	16.6

DISCUSSION

Several medicinal plants have been shown to have varied degree of antifertility effects in the male, mainly affecting spermatogenesis and often affecting the androgen production in the testis.^{1, 29-35} The medicinal value of the cactus, *Opuntia*, is widely known in many parts of the world and several studies have reported the nutraceutical value, hepatoprotective properties and anti-inflammatory potential in experimental studies.^{11,21,36} The medicinal properties of *Opuntia* have been attributed to a variety of phytochemicals present in the cladode and fruits.¹¹ Male antifertility activity of the phylloclade extracts of *O. dillenii* has been investigated by Gupta et al., (2002), Bajaj & Gupta, (2012). Their studies showed decline in testicular weight, suppression of spermatogenic activity, reduction in serum testosterone, lower sperm count and partial fertility. However, their studies did not show 100% efficacy of the extract on fertility suppression and reversibility.²³ However, in most of the studies, the effect of the plant extract on fertility was only partial and,

reduction in serum testosterone is not a desirable feature since it could affect the libido.

In our study, treatment of the extract of *O. elatior* has shown marked decline in epididymal sperm count and motility in rats which was dose-dependent being 80% at the highest dose. The fertility of the treated male rats was suppressed in both dosage groups, being 100% at the high dose. Our results show that the weights of testes and the accessory organs, epididymis, prostate and seminal vesicle, were not significantly affected. Histological examination of the testes showed no discernible effect on spermatogenesis and, the Leydig cells appeared normal. However, several tubules in the epididymis showed low density of sperm but normal secretions. The serum testosterone levels were marginally lower in treated rats but not significant enough to affect the libido as evident from fertility studies. Therefore, the lack of effect of the *O. elatior* extract on accessory organs or libido could be attributed to the absence of significant effect on serum testosterone levels in treated rats. The results of serum testosterone levels are consistent with the testicular enzyme activities of $\Delta^5 3\beta$ HSDH, a key enzyme in androgen biosynthesis, which was not significantly affected in the testis of treated rats. However, 17β HSDH, although showed reduced activity in the testis of treated rats, was not reflected in the serum testosterone levels.

Our results of reversibility studies showed that withdrawal of treatment after 60 days for a period of two weeks was enough to induce recovery of the sperm count, testicular HSDH activity and serum testosterone levels, which are consistent with the results of fertility. Recovery of fertility as revealed from pregnancy and litter was partial which could be attributed to the short period (2 weeks) of withdrawal of treatment. Further studies are needed to know if longer recovery period would lead to complete recovery of fertility. The chemical ingredients present in the methanol extract of the fruit of *O. elatior* are being investigated, and may contain many phytochemicals including the active principle(s) which need to be identified.

Overall, our results show that the methanol extract of the fruit of *O. elatior* was quite effective in suppressing the male fertility without affecting the libido and it was totally reversible. The mode of action of the antifertility effect appears to involve extragonadal targets since epididymal sperm count and motility were suppressed without affecting the sperm production.

In conclusion, our study has shown that the methanol extract of the fruit pulp of *O. elatior* could be a promising source of a natural male antifertility agent which is effective in suppressing fertility without affecting libido and, was reversible after the withdrawal of treatment. Efforts are underway to isolate the bioactive compound in the extract, responsible for the antifertility effect.

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Conflict of interest: None declared

Ethical approval: The study was approved by the institutional animal ethics committee (Approval No. 841/b/04/CPCSEA)

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