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REPRODUCTIVE TOXICITY OF *OPUNTIA* FRUIT EXTRACT IN MALE SWISS ALBINO MICE

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ABSTRACT
To examine the contraceptive efficacy of *Opuntia elatior* fruits on male mice two treatment groups of 250 and 500 mg/ Kg bw, was selected along with control. The final body weight, weight of testis and accessory reproductive organs of mice along with total sperm count and sperm abnormalities were recorded after treatment. A significant reduction in the weight of the testis and epididymis was noticed in 500 mg/ Kg bw compared to control. In 500 mg/ Kg bw treated animals a 18.12% reduction in the sperm count and 17.82% increase in the total percentage of abnormal spermatozoa was found to be significant count. The fertility indices with 36.08% decrease in the litter size indicate the contraceptive effect of the fruit extract on mice. The weight of the testis and epididymis, total sperm count and the percentage of the abnormal spermatozoa were returning to the normal levels after the cessation of the treatment for 30 days. Thus the extract from *O. elatior* fruit shall be an effective contraceptive agent to regulate male fertility.

KEY WORDS: Reproductive toxicity, *Opuntia elatior*, contraception, fertility regulation *etc.*

INTRODUCTION

India, having 1.2 billion people, might overtake China in about a decade as the world's most populous country. The population dynamics fueling India's growth and changing age structure are the combined impact of increasing life expectancy and declining fertility (Population reference bureau, 2012). India is first among the countries which adopted an official family planning programme, as early as 1950, but has not prevented the population touching one billion mark (Rao, 2001). Subsquently, WHO has taken up an important step on the methods for the regulation of male fertility (De Kretser, 1978), which include suppression of sperm production, disruption of sperm maturation and function and interruption of sperm transport (Thakur *et al.*, 2010). Research in the field of male contraception remains as a challenging task due to its shortcomings in safety and efficiency of the drugs (De Kretser, 1978; Vogelsong, 2005). Antifertility drugs acceptable for men remain difficult to produce which possess complete azoospermia over a long period. This can only be effective and safe while the residual sperm produced by men whose spermatogenesis has been suppressed by antifertility drugs to oligospermia are incapable of fertilizing ova (Waites, 1986), while non hormonal male contraceptives lead to total spermatogenesis arrest and ultimately to irreversible sterility (Thakur *et al*., 2010). The hormonal contraceptives affect the metabolism pathways, secondary sexual characters, behavioral characteristics and finally libido potency (De Kretser, 1978; Waites, 1986), that results in gynaecomastia (Waites, 1986). The synthetic and hormonal contraceptives side effects prompted scientists to examine herbal contraception and identify suitable antifertility inducing biomolecules. The major sources of the alternative medicine since ancient period are the potential use of biologically active components from plant origin (Joshi *et al.*, 2004). Scientific studies using different plants on male contraception have shown promising results as safe and effective contraceptive. The methonolic phylloclade extract of *Opuntia dillenii* caused antispermatogenic effect in mice (Gupta *et al*., 2002; Bajaj and Gupta, 2011), reduced sperm count and decreased sperm motility as has reported using seed extract of *Vitex negundo* (Das *et al*., 2004), *Albizzia lebbeck* (Gupta *et al*., 2005), *Cestrum parqui* (Souad et al., 2007), leaf extract of *Aegel marmelos* (Kumar *et al*., 2011), seed extract of *Thespesia populnea* (Nagashree, 2010), *Madhuca indica* (Shivabasavaiah *et al*., 2011), and *Cyamposis psoralioides* (Thejashwini *et al*., 2009a, b, 2012), but no work has been carried out using *O. elatior* fruit extract. *Opuntia elatior* belongs to the family Cactaceae is usually grown in arid and semiarid regions. The uses of *O. elatior* whole plant and other species of Opuntia are enormous (Ramyashree *et al*., 2012). The fruit being considered to be edible (Tiwari *et al*., 2010) has been documented as a medicinal plant in Vijayanagar forest (Vegda *et al*., 2012). In addition fruits of *O. elatior* are used for haematinic, anti-asthmatic and spasmolytic action by tribal people of Saurashtra region of Gujarat state, and have been successfully controlled the disease as well (Chauhan, 2010). The *O. elatior* fruits have been used for whooping cough, diabetes, high blood cholesterol, obesity, as a blood purifier (Kshirsagar *et al*., 2012). The fruit pulp is also fed for the infant's stomach ache (Patil and Biradar, 2011) and to cure asthma (Patil *et al*., 2008), rheumatism (Patil and Ahirrao, 2011) burning sensation in the stomach (Kumar *et al*., 2008) and diphtheria in livestock (Kumar and Bharathi, 2012). Interestingly, *O. elatior* fruits have been used since date back as a source of contraceptive medicine by tribal women mixing it with jaggery and taken orally for 2-3 days for complete sterility (Jain *et al*., 2007). Since there is no scientific data available on the antifertility effect of this

plant except tribal knowledge we have examined the antifertility effect of *O. elatior* using male Swiss albino mice.

MATERIALS AND METHODS

Collection and identification of the plant material

The fruits of *Opuntia elatior* Mill collected from the field were authenticated by the department of studies in botany, University of Mysore, Mysore.

Animals

Adult male and female Swiss albino mice weighing 30 – 40 g were obtained from the Central animal facility, Department of Studies in Zoology, University of Mysore, Mysore. They were housed in polypropylene cages (3 animals/ cage) containing husk as the bedding material under 12 h light and 12 h dark schedule at 27±2°C and 70% humidity. They were fed with mice chow pelletes and water *ad libitum* during the period of experimentation. The protocols for the maintenance of animals followed were approved by Institutional Animal Ethics Committee CPCSEA, Government of India.

Preparation of the plant extract

The procedure for extraction from fruit was detailed elsewhere (Ramyashree *et al*., 2012).

Treatment

250 and 500 mg/Kg bw selected were treated for 30 days at an interval of 24 hours with a recovery period of 30 days. The adult mice were divided into three groups, while control group received 0.2 ml distilled water, the other two groups received 250 and 500 mg/Kg bw of the fruit extract in 0.2 ml of distilled water respectively per mouse. For recovery, few animals were kept without treatment for 30 days, with food and water *ad libitum* for another 30 days.

Weight of the body and reproductive organs

The body weight of each animal of the entire group was noted before autopsy. Eight mice in each group were autopsied and weights of the testes, epididymis, vas deferens, seminal vesicle and ventral prostate were recorded. During autopsy epididymis of each mouse was carefully separated from the testis and used for sperm count.

Sperm count

For the total sperm count, cauda region of the epididymis was minced in 1 ml of buffered saline and filtered through muslin cloth. The filtrate was taken in a leukocyte pipette up to 0.5 and make up to the mark 11 with buffered saline. The suspension was well mixed and charged to the Neubauer's chamber. The total number of spermatozoa present in 8 squares of 1 mm^2 each was counted and multiplied by 5×10^4 to express the number (millions) of spermatozoa/ epididymis (Vega *et al*., 1988).

For the abnormal sperm count, the sperm suspension obtained for total sperm count was mixed with aqueous eosin and kept for 30 min. A drop of the spermatozoa suspension was taken on a clean slide as uniform smear and dried. One thousand spermatozoa were screened per mouse for abnormal head shape like amorphous head, hookless head, pin head, banana head, hammer head, folded head and double head (Vega *et al*., 1988).

Fertility test

Eight adult female mice with proven fertility were used after each treatment period for the fertility test. Four animals were taken from each group and each male mouse from the experimental and control groups were kept with female mice for two weeks. The female was examined for the presence of spermatozoa in the smear every day and presence of spermatozoa in the vaginal plug confirmed the mating (Al-Hamdani and Yajurvedi, 2010; Thejashwini *et al*., 2012).

Statistical analysis

All the data were computed following Duncan's multiple range test of One way ANNOVA.

RESULTS

Weight of body and reproductive organs

The ethanolic fruit extract of *O. elatior* did not show any significant changes in the body weight of the treated mice. No toxic effect of the fruit extract was observed neither in low dose (250mg/ Kg bw) nor in high dose (500mg/ Kg bw) treated animals with final body weight of the recovery group animals remains same. Significant ($P\geq0.05$) reduction in the weight of the testis (561.62mg/100g bw) and epididymis (180.25mg/100g bw) was recorded at high dose (500mg/ Kg bw) treated animals. No such reduction in the weights of the vas deferens, seminal vesicle and ventral prostate was obvious, whereas low dose (250mg/ Kg bw) does not induced any significant reduction in the weight of testis and other accessory reproductive organs of treated animals compared to control (Table 1).

Sperm count

The total sperm count in high dose (500mg/ Kg bw) was significantly ($P\geq0.05$) reduced to 81.87%, when compared to the control. There was no such significant reduction in the total sperm count in the low dose (250mg/ Kg bw) treated animals when compared to the control group. The total sperm count was recovered to 93.28% after the cessation of the treatment for 30 days (Table 2).

Sperm abnormalities

A significant ($P\geq0.05$) increase of 17.82% abnormal sperms were observed in high dose (500mg / Kg bw) treated animals when compared to control. 68.65% of amorphous sperm was found high compared to other head abnormalities like hook less head, pin head, banana head and double head. No significant alteration in the percentage of the abnormal sperms in low dose animals. Percentage of abnormal sperms was reduced to 2.98% in the recovery group animals after the cessation of treatment (Table 3).

Fertility test

The fertility test clearly indicates the result of the extract on the fertility indices of the animal. There was no difference in the litter size of the low dose (250 mg/ Kg bw) treated animals when compared to that of the control group animals. There was a significant drop in the litter size of the animals in the high dose (500 mg/ Kg bw) treated animals when compared to that of the control group animals. But a recovery in the number of the litter size was observed in the animals after the cessation of the treatment for 30 days. There was no difference in the fertility index of male and female, gestation index, lactation index, parturition index and viability index in both control and treatment groups (Table 4, 5, 6).

TABLE 1: Effect of *Opuntia elatior* fruit extract treatment for 30 days on the body and reproductive organs weight of mice

Treatment groups	Initial body weight	Final body weight	Testis	Epididymis	Vas deferens	Seminal vesicle	Ventral prostate
Control	27.93±0.33^{NS}	38.18±0.47^{NS}	698.5±22.61^{NS}	241.75±3.39^{NS}	74.12±2.97^{ab}	531.25±27.77^{NS}	16.87±2.22^{NS}
Low dose (250mg /Kg bw)	28.18±0.25^{NS}	39.06±0.33^{NS}	696.25±17.81^{NS}	254.12±5.62^{NS}	75.87±6.25^{ab}	500.00±30.91^{NS}	13.25±1.91^{NS}
High dose (500mg /Kg bw)	27.96±0.30^{NS}	38.93±0.42^{NS}	561.62±8.08***	180.25±7.22***	65.62±3.43^{a}	429.87±51.54^{NS}	15.72±1.19^{NS}
Recovery of 500mg /Kg bw	27.75±0.27^{NS}	38.56±0.59^{NS}	667.25±36.48^{NS}	209.37±11.96^{NS}	79.25±2.17^{b}	466.37±52.46^{NS}	14.12±1.86^{NS}

Note: Mean values were compared by One- way ANNOVA followed by Duncun's multiple range test. Values with same superscript are not significantly different whereas those with different superscript are significantly different from each other. NS = Non significant, *** = Highly significant (P<0.001).

TABLE 2: Effect of *Opuntia elatior* fruit extract treatment for 30 days on the total sperm count and abnormal spermatozoa count

Treatment groups	Total sperm count	Total number of abnormal spermatozoa
Control	5.5875±0.07465 ^{NS}	25.00±1.35401 ^{NS}
Low dose (250 mg/ Kg bw)	5.4625±0.10078 ^{NS}	25.5000±1.32288 ^{NS}
High dose (500 mg/ Kg bw)	4.575±0.14506 **	54.5000±3.59398 **
Recovery of high dose	5.2125±0.05543 *	29.0000±1.95789 ^{NS}

Note: Mean values were compared by One- way ANNOVA followed by Duncun's multiple range test. Values with same superscript are not significantly different whereas those with different superscript are significantly different from each other. NS = Non significant, * = Significant (P<0.05), ** = Highly significant (P<0.01), *** = Highly significant (P<0.001).

TABLE 3: Effect of *Opuntia elatior* fruit extracts treatment for 30 days on the count of abnormal spermatozoa

Types of abnormalities	Control	Low dose (250mg/Kg bw)	High dose (500mg/Kg bw)	Recovery group
Amorphous head	16.75±1.10868 ^{a}	16.75±0.85391 ^{a}	28.25±3.06526 ^{b}	17.25±0.47871 ^{a}
Hook less head	2.5±0.64550 ^{a}	4.0±0.70711 ^{a}	13.25±2.17466 ^{b}	3.25±0.62915 ^{a}
Pin head	1.5±0.64550 ^{a}	1.0±0.40825 ^{a}	3.5±0.64550 ^{b}	2.75±0.95743 ^{ab}
Banana head	1.0±0.40825 ^{a}	0.75±0.25 ^{a}	2.5±0.5 ^{b}	1.75±0.47871 ^{ab}
Hammer head	3.25±0.85391 ^{a}	3.0±1.08012 ^{a}	6.75±1.10868 ^{b}	4.0±0.40825 ^{ab}
Double head	Nil	Nil	0.25±0.25 ^{NS}	Nil

Note: Mean values were compared by One- way ANNOVA followed by Duncun's multiple range test. Values with same superscript are not significantly different whereas those with different superscript are significantly different from each other. NS = Non significant, * = Significant (P<0.05), ** = Highly significant (P<0.01), *** = Highly significant (P<0.001).

TABLE 4: Effect of *Opuntia elatior* fruit extracts treatment for 30 days on the fertility parameter of mice

Parameters	Control	Low dose	High dose	Recovery
Fertility index (m)	100 (4)	100 (4)	100 (4)	100 (4)
Fertility index (f)	100 (8)	100 (8)	100 (8)	100 (8)
Parturition index	100 (4)	100 (4)	100 (4)	100 (4)
Gestation index	100 (94)	100 (94)	100 (62)	100 (85)
Viability index	100 (94)	100 (94)	100 (62)	100 (85)
Lactation index	100 (94)	100 (94)	100 (62)	100 (85)

Note: Mean values were compared by One- way ANNOVA followed by Duncun's multiple range test. Values with same superscript are not significantly different whereas those with different superscript are significantly different from each other. NS = Non significant, * = Significant (P<0.05), ** = Highly significant (P<0.01), *** = Highly significant (P<0.001).

TABLE 5: Effect of *Opuntia elatior* fruit extracts treatment for 30 days on the litter size of mice

Treatment groups	No of males mated/ female	No of pregnant females	Litter size	Percentage fertility
Control	4/8	8	97	100%
Low dose (250 mg/ Kg bw)	4/8	8	94	96.91%
High dose (500 mg/ Kg bw)	4/8	8	62	63.92%
Recovery for high dose	4/8	8	85	87.63%

Note: Mean values were compared by One- way ANNOVA followed by Duncun's multiple range test. Values with same superscript are not significantly different whereas those with different superscript are significantly different from each other. NS = Non significant, * = Significant (P<0.05), ** = Highly significant (P<0.01), *** = Highly significant (P<0.001).

TABLE 6: Effect of *Opuntia elatior* fruit extracts treatment for 30 days on the litter size of mice

Treatment groups	Litter size
Control	12.1250±0.125 ^{NS}
Low dose (250mg/ Kg bw)	11.7500±0.16366 ^{NS}
High dose (500mg/ Kg bw)	7.500±0.26726 ***
Recovery group	10.2500±0.16366 ***

Note: Mean values were compared by One- way ANNOVA followed by Duncun's multiple range test. Values with same superscript are not significantly different whereas those with different superscript are significantly different from each other. NS = Non significant, * = Significant (P<0.05), ** = Highly significant (P<0.01), *** = Highly significant (P<0.001).

DISCUSSION

Medicinal plants have been of age long remedies for human diseases because they contain components of therapeutic values (Nostro *et al*., 2000; Britto and Gracelin, 2011). Therapeutic properties of the green parts of the Opuntia plant the cladodes, have very long been known in the traditional medicine (Cornett, 2000, Knishinsky, 1971). Recently the potential activity of the fruit and the nutritional benefits has been explored recently, that made the cactus pear fruits a health promoting food and food supplements. However a systematic research is in need to confirm the benefits of these fruits to document health effects and claims (Livrea and Tesoriere, 2006). Preliminary phytochemical screening revealed the presence of tannins, phenolics, saponins, alkaloids and flavonoids (Chauhan, 2010 and Ramyashree *et al*., 2012). As these compounds are associated with nutritional and health promoting aspects, the fruits of Opuntia are also considered to be of therapeutic value. (Stintzing *et al*., 2001) The presence of different phytoconstituents of pharmacological importance of fruits of *O. elatior*, offered further investigations.

The toxicity tests of *O. elatior* fruit extract revealed no toxic side effect on the external morphology and the body weights of the mice upto 600 mg/ Kg body weight (Ramyashree *et al*., 2012). Thus dose levels less than LD_{50} was selected in the present study. The body weight of the treated animals remains unchanged which indicate no toxic effect of *O. elatior* on growth and metabolic processes of the treated animals which in conformity with the observation of D'Cruz and Mathur (2005), where piperine treated mice did not show any significant changes in the body weight. However, a significant difference in the weight of the reproductive organs in treated group animals compared to that of the control was obvious, wherein weight of the testes and epididymis was declined to 561.62 mg/100g bw and 180.25 mg/100g bw in the high dose (500 mg/ Kg bw) treated animals compared to that of control. But no such significant changes observed in weight of vas deferens, seminal vesicle and ventral prostate. No reductions in the weight of the testes and other accessory reproductive organs was observed in the low dose (250 mg/Kg bw) treated animals compared to control. Reduction in the weight of the reproductive organs is correlated to reduced circulating androgen level according to Gupta (2006), where the methanolic extract of *Strychnos potatorum* seeds was responsible for reduced weights of testis and accessory reproductive organs, which might be due to low levels of androgen. Similar impairment in the reproductive activity of testis and accessory reproductive organs was also mainly because of circulating androgen (Raji, 2006) where methanolic seed extract of *Ricinus communis* caused a significant decrease

in the weight of the reproductive organs, which is mainly due to decreased level of testosterone. The reproductive organ weight reduction is the clear indication of structural and functional alteration in the testes and epididymis due to drug (Singh *et al*., 2011), as ethanolic extract of *Tinospora cordifolia* stem induced reduced reproductive organs weight. For the normal functioning, growth and development of reproductive organs testosterone is a key element, whereas 50% ethanolic extract of *Calendula officinalis* flower showed weight loss of reproductive organs (Kushwaha *et al*., 2007).

Depletion in the sperm count may be one of the reasons for altered spermatogenesis and reduced fertility. Spermatogenesis is the process of male gamete production, wherein the spermatogonia transform into highly specialized matured spermatozoa within testis (Wistuba *et al*., 2007) which is regulated by gonadotrophins and testosterone in mammals (Jones., 1991). Alteration in any step of the spermatogenesis may result in reduced sperm count and increased number of abnormal sperms. A marked reduction in the sperm counts of cauda epididymis in 500 mg/ Kg bw treatment may be due to alteration in sperm production in the testis and interference in testicular spermatogenesis, which is in accordance with Parveen and co workers (2003), where they explain the reproductive toxicity of *Quassia amara* in male rats. Adhikary (1990) and Sarkar (2000) co workers demonstrates that the reduced testis weight and decreased testosterone level may also be one of the reason for the reduction in the sperm count in rats treated with ethanolic extract of *Piper betle* stalk. Maturation of sperm is also one of the important events which take place in epididymis where the sperm is nurtured by epididymal secretion (Jones, 1991 & Cooper, 1999). The ethanolic extract of *Piper betle* Linn stalk responsible for the reduced sperm count which is mainly because of the alteration in the sperm maturation (Sarkar *et al.,* 2000). Thus reduced sperm count might be due to impairment in hormonal regulation of spermatogenic process wherein testosterone is a major precursor which stimulates certain phases of spermatogenesis (Jones, 1991). Treatment with cypermethrin to mice induced reduction in the sperm count which may be due to reduced testosterone level (Al-Hamdani and Yajurvedi, 2010). Hence the reduced sperm count may be due to altered spermatogenesis which may be mainly because of reduced testosterone level and reduced testis and epididymis weight. An increase in the number of sperm count in the recovery group after 30 days may be noted. 30% reduction in the litter size in 500 mg/ Kg bw treated animals, due to low androgen concentration (Dohle *et al.,* 2003), which might be sufficient for the normal mating behavior, but insufficient for the maintainance of fertilizing ability of the epididymal spermatozoa (Thejashwini *et al*., 2012). The ethanolic extract of *Cyamposis psoralioides* caused upto 50% reduction in the litter size, which was mainly due to reduced testosterone level (Thejashwini *et al*., 2012). All these factors thus brought about functional sterility in 500 mg/ Kg bw treated mice. However, the induced infertility was completely reversed after withdrawal of treatment of another period of 30 days. The present study shows that treatment with *O. elatior* fruit extract had no impact on libido of extract-treated males, though, the number of live implants decreased significantly in females impregnated

by males treated with 500 mg/kg bw. Low sperm count and high percentage abnormal spermatozoa level each have been associated with reduced fertility (Raji, 2006) as obvious in the present study.

CONCLUSION

The present study explains the contraceptive efficacy of the *Opuntia elatior* fruits. The fertility regulating effect of the fruit is only at higher dosage *i.e.*, at 500 mg/ Kg bw. The fruit extract have also brought down the litter size to almost 50%, which is the clear indication of the effect of the extract on male fertility. Thus the present investigation provides an ample of opportunities for the future study of the *Opuntia elatior* fruits as they contain many of the biologically active components which are of potential health benefits to mankind and also acts as an efficient contraceptive agent to regulate fertility in male mice.

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