PUBLICATIONS
Ethnomedicinal value of *Opuntia elatior* fruits and its effects in mice


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Cactus pear, *Opuntia elatior* Mill belongs to Cactaceae family is an important plant used as medicine for various ailments due to beneficial health promoting properties. The present study was undertaken to evaluate the nutritional components present in the cactus pear fruit. The ripened fruits of *Opuntia elatior* Mill were collected, authenticated, air dried, powered and subjected for ethanol extraction. The fruit extract was screened for its phytochemical components, which revealed the presence of alkaloids, carbohydrates, fats, oils, flavonoids, phenolics, tannins, steroids, and saponins. The oral administration of crude extract exhibited no toxic effect on the external morphology and body weight of the mice. Thus, the present investigation established scientific base for further use of *Opuntia elatior* for various pharmacological tests like antibacterial, antidiarrheal, anti-inflammatory, analgesic, antispermatogenic, antimicrobial and antidiabetic properties. The phytochemical research based on ethnopharmacological information is generally considered as an effective approach in the discovery of new anti infective agents, hence this study focused on the detection of phytochemical compounds present in *Opuntia elatior* fruits and its toxicity.

**Key Words:** Ethnomedicine, *Opuntia elatior*, phytochemicals, flavanoids, steroids

**INTRODUCTION**

India is a varietal emporium of medicinal plants and is one of the richest countries in the world in regard to genetic resources of medicinal plants. The cactus family is unusual among tropical plants. Cactus pear, produced by perennial Opuntia cactus, belongs to the Cactaceae family is well adapted to arid and semiarid climates, where water shall be a limiting factor for cultivation [1 & 2]. Cacti have been exploited as a cheap, alternate source of food suitable for humans and feed for animals and are cultivated as ornamental crops. Cacti exhibit many characteristics of a suitable crop model to achieve productivity and sustainability with minimal ecological or environmental impact to meet the growing demands for food and as a new source for meeting nutritional health requirements and thus deserves further investigations [3 & 4]. Due to agricultural problems linked to increasing arid zones and declining water resources, Opuntia sp. is gaining importance as an effective food source [5 & 6]. Many species of Opuntia cacti produce edible highly flavored fruit known as “Cactus pear”. Cactus pear fruit is a many seeded berry with a thick peel, enclosing a delicately flavored seedy pulp [7 & 2]. Cactus pear fruits are rich source of nutrients and vitamins [8 & 9] and are eaten fresh, dried or preserved in jams, syrups or processed into candy – like products [10 & 2].

In recent years, secondary plant metabolites (phytochemicals) previously with unknown pharmacological activities have been extensively investigated as a source of medicinal agents. There is increasing evidence that fruits and vegetables may protect against numerous chronic diseases, including cancer, cardio, cerebro vascular, ocular and neurological diseases [11, 12, 13 & 14]. The protective effect of fruit and vegetables has generally been attributed to their antioxidant constituents including vitamin C, vitamin E, carotenoids, glutathione, flavonoids and phenolic acids as well as other unidentified compounds [15 & 2]. Antioxidative effects of cactus fruit extract on lipid peroxidation inhibition in oils and emulsion model systems [16 & 4]. Prickly pear antioxidant actions could protect mammalian cells and organs and slow the aging process, illness and disease [2]. It could also lower low density lipoprotein (LDL) and cholesterol levels helping to check high blood pressure [17]. Concentrated juice in combination with the fruit is reported to be an aphrodisiac and also believed to be of value in spermatorrhoea and gonorrhea [14]. In Chinese medicine, cactus fruits are considered as weak poisons and used for treatment of inflammation, pain and detoxification agents for snake bites [18 & 4]. Since use of natural sweeteners other than sucrose is a priority area for the food industry [4].

In addition cactus fruits have been used as a raw material in the creation of natural sweeteners [19]. Hence screening of fruit extracts for various enzymes such as arylamidases, lipases, proteinases and glucosidases has been carried out. Amongst these enzymes, proteinases are the most important plant enzymes used in food, pharmaceuticals, detergent, leather and wood industries [20 & 4]. Generally, cactus pear fruit contains about 85% water, 11 – 14% fermentable sugar, 1.8% crude fiber, 0.1% lipids, 0.21 – 1.5% protein and 0.4 – 1.6% ash [21]. It also contains calcium and vitamin C upto 60 and 30 mg/100g and vitamin A 50 IU which are the main dietary essentials [21 & 4]. The phytochemical analysis of the Opuntia fruit showed presence of carbohydrates, flavonoids, phenolics, betalains; color pigment betacyanin as an active principle and high amount of sugar content. The low acidity of fruit makes it very sweet and delicious. The fruit juice also showed a haematinic, analgesic and anti-asthmatic activity [22]. But no information is available on total fruit extract components and its toxicity effect.

The phytochemical research based on ethnopharmacological information is generally considered an effective approach in the discovery of new anti infective agents, from plants. The literature study reveals that till today there is only one record of phytochemical composition and pharmacological study of the fruit juice of *Opuntia elatior* [22]. Thus the present study helps to further investigate about the phytochemical screening of the complete fruit extract.
which paves the way to know the pharmaceutical, nutraceutical and pharmacological applications of *Opuntia elatior* Mill fruits. Hence, this study focused for detection of phytochemical compounds present in the fruits of *O. elatior* Mill and toxicity effect on model organism mice.

**MATERIALS AND METHODS**

**Experimental materials**

**Collection and identification of the plant material:**

The fruits of *Opuntia elatior* Mill was collected from the field, Mysore district and authenticated by the Department of Studies in Botany, University of Mysore, Manasagangothri, Mysore.

**Animals:**

Twelve adult Swiss albino mice weighing 30 – 40 g were obtained from the Central animal facility, DOS 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. The animals had food (mice chow pellets) and water *ad libitum* during the period of experimentation. The protocols of the experiment approved by Institutional Animal Ethics Committee under the guidelines of CPCSEA, Government of India were followed for care and maintenance of animals.

**METHODS**

**Preparation of the plant extract:**

The ripened fruits were shade dried and powdered using an electrical grinder. This powder was dissolved in 500 ml of ethanol with continuous stirring for about 5 – 6 hrs. Then suspension filtered using Whatmann filter paper (No 1). This filtered solution was subjected to flash evaporator until the contents of ethanol completely evaporated and collected in receiving flask in the form of crude extract. Finally the ethanolic extract of total fruit was kept in incubator for drying at 38°C for 48 hrs. The resultant fruit crude extract was stored at 4°C until further use.

**Phytochemical Screening:**

Screening was done to identify the following phytochemicals in the extracts: alkaloids, carbohydrate, fat and oils, flavonoids, phenolics, saponins, steroids and tannins [23 & 24].

**Test for alkaloids:**

0.5 g of crude defatted with 5% ethyl ether for 15 minute. The defatted sample was extracted for 20 minute with 5 ml of aqueous HCL on a boiling water bath. The resulting mixture was centrifuged for 10 minute at 3000rpm. 1 ml of the filtrate was treated with few drops of Mayer’s reagent and a second 1 ml of Dragendorff’s reagent and turbidity was observed.

**Test for carbohydrates:**

0.5 g extract was shaken with Molish’s reagent, add conc. H₂SO₄ through sides of the test tube. Purple color appears between the two liquids. This is the indication of the presence of carbohydrates.

**Tests for fats and oils:**

a) A portion of the crude extract should be placed on a blotting paper. Appearance of stains after drying will be the indication of presence of fats and oils.

b) To 0.5 g of extract few drops of 0.5N alcoholic potassium hydroxide and a drop of phenolphthalein was added and heated on a water bath for 1-2 hrs. Formation of soap represents the presence of fats and oils.

**Test for flavonoids:** A portion of crude powder was heated with 10 ml of ethyl acetate over a stram bath for 3 minutes. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution and observed a yellow coloration.

**Test for saponins:**

0.5 g of crude powder was shaken with water in a test tube and it was warmed in a water bath and the persistent of froth indicates the presence of saponins.

**Test for steroids:**

0.5 g of crude powder was dissolved in 5 ml of methanol. 1 ml of the extract was treated with 0.5 ml of acetic acid anhydride and cooled in ice. This was mixed with 0.5 ml of chloroform and 1 ml of concentrated sulphuric acid was then added carefully by means of a pipette. At the separation level of the two liquids, a reddish – brown ring was formed, as indication of the presence of steroids.

**Test for tannins and phenolic compounds:**

0.5 g of the crude powder was stirred with 10 ml of distilled water. This was filtered and ferric chloride reagent was added to the filtrate, a blue- black precipitate was taken as evidence for the presence of tannin and phenolic compounds.

**Treatment:**

Toxicity test for fruit extract was performed using Swiss male albino mice. To this, animals were kept fasting for overnight providing only water. They were divided into four groups containing three animals each. One group maintained as control group and the other three treatment groups were then administered with the extract.

Control group animals received 0.2ml of distilled water whereas each animal in 2nd, 3rd and 4th group animals received 200mg (low dose), 400mg (medium dose), and 600mg (high dose) extract/ Kg body weight in 0.2ml distilled water respectively. The extract with the vehicle was administered to the animals through oral intubation by a smooth plastic tube attached to a syringe. The treatment period was fixed to 30 days. Each day after oral intubation the animals were observed for first 30mins and were kept for observation like sedation, convulsions, tremors, lethargy and death.

**Statistical analysis:**

The results are analyzed as Mean ± SE. Paired T test was used to determine the effect of treatment on the final body weight of the target animal.

**RESULTS AND DISCUSSION**

Medicinal plants have long been used to address human diseases due to their therapeutic values. In recent years natural products with high medicinal values are gaining much importance in the light of serious side effects posed by the medicinal derivatives from chemical origin [25]. Hence the present study was carried out to assess the important phytoconstituents present in the fruits of *O. elatior*, in support of traditional and folkloric use.

**Phytochemical screening of the crude extract of Opuntia elatior fruits**

The preliminary phytochemical screening of the fruit extract of *O. elatior* revealed the presence of alkaloids, carbohydrate, fat and oils, flavonoids, phenolics, saponins, steroids and tannins (Table 1) for the first time in ethanolic extract. Where the methanol extract of some flowers of medicinal
applications in cosmetics and pharmacology due to its biological properties.

Producer of oils. Hence, the unsaponifiable fraction of vegetable oil has a higher level of fat-soluble vitamins wherein, unsaponifiable content found in a significant amount of neutral lipid (87% of total lipids) and polar lipids. Thus the presence of alkaloids, flavonoids, phenolics, saponins, steroids and tannins have shown to inhibit particular enteropathogens [34 & 44].

Table 1. Phytochemical components present in the whole fruit extract of Opuntia elatior

<table>
<thead>
<tr>
<th>SI No</th>
<th>Compounds</th>
<th>Present/ Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>Present</td>
</tr>
<tr>
<td>2</td>
<td>Carbohydrates</td>
<td>Present</td>
</tr>
<tr>
<td>3</td>
<td>Fats and oils</td>
<td>Present</td>
</tr>
<tr>
<td>4</td>
<td>Flavonoids</td>
<td>Present</td>
</tr>
<tr>
<td>5</td>
<td>Phenolics</td>
<td>Present</td>
</tr>
<tr>
<td>6</td>
<td>Saponins</td>
<td>Present</td>
</tr>
<tr>
<td>7</td>
<td>Steroids</td>
<td>Present</td>
</tr>
<tr>
<td>8</td>
<td>Tannins</td>
<td>Present</td>
</tr>
</tbody>
</table>

Table 2. Initial and final body weight of the control and treatment group animals.

<table>
<thead>
<tr>
<th>Treatment groups (mg/Kg bw)</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>33.36±0.5602</td>
<td>39.06±0.887</td>
</tr>
<tr>
<td>Low dose (200 mg)</td>
<td>33.93±0.898</td>
<td>39.44±0.709</td>
</tr>
<tr>
<td>Medium dose (400 mg)</td>
<td>34.46±1.068</td>
<td>39.26±0.8950</td>
</tr>
<tr>
<td>High dose (600 mg)</td>
<td>34.73±0.8110</td>
<td>39.63±0.7965</td>
</tr>
</tbody>
</table>

Values represented as Mean±SE (n=3), P value >0.05, thus considered non significant.

The Opuntia elatior fruit with high sugar content make it highly delicious and sweet in taste. The sugar pattern in the fruit pulp is very simple and contains glucose and fructose virtually in equal amounts as has been reported by Fung et al (2006) in all cactus pears. However, directly absorbed high glucose concentrations in cactus fruits represents an energy source instantly available for brain and nerve cells, while ructose being sweeter may enhance the fruit's flavor [34 & 35]. The cactus mucilages are composed of polysaccharides such as arabinose, galactose, shaminose and galactouronic acid. Thus these mucilages have a high water holding capacity, so they could serve as thickening or emulsifying agents and form viscous or gelatinous colloids [36].

Figure 1: Effect of Opuntia fruit extract on body weight of mice

Flavonoids are recognised as a very important phytochemical mainly for their antioxidant activity, metal chelating properties and beneficial role in variety of cellular processes [36]. According to Gupta et al. (2002) a methanolic extract from Opuntia dillenii exerted antispermatogenic effects in animal tests on rats, due to the presence of flavonones derivatives, vitexin and myricetin. Also, flavonoids extracted from the seeds of Vitex negundo showed not only a significant drop in sperm count but also decreased motility upto 30% [40]. Thus, flavonoids reported from the O. elatior has some pharmaceutical applications.

Epidemiological studies have shown that consumption of food and beverages rich in phenolic content can reduce the risk of heart disease by slowing the progression of the atherosclerosis by acting as antioxidants towards LDL [41, 42 & 43]. Since, the nutritional and health benefits of cactus fruits are associated with their antioxidant properties related to phenolic compounds [34 & 44].

Table 2. Initial and final body weight of the control and treatment group animals.

Saponins extracted from Allbizza lebeck and Cestrum parqui reduced the sperm concentration of testes and decreased sperm motility significantly [45 & 46]. Thus saponins present in the O. elatior fruit extract might have an antispermatogenic effect.

Steroidal compounds observed in O. elatior have importance and interest in pharmacy. Because the leaves of C. rutidosperma are used as vegetable for expectant or breast feeding mothers to ensure their hormonal balance, since steroidal structure could serve as potent starting material in synthesis of these hormones [49]. Steroids in modern clinical studies have revealed their role as anti-inflammatory and analgesic agents [44 & 51].

Together, alkaloids, flavonoids, phenolics, saponins and tannins have been linked or suggested to be involved with antibacterial and antiviral activity, while tannins and flavonoids are thought to be responsible for antidiarrheal activity. Investigations on the mode of action indicate that tannins and flavonoids increase colonic water and electrolyte reabsorption and other phytochemicals act by inhibiting intestinal mobility, while some components have shown to inhibit particular enteropathogens [52 & 51].

Thus the presence of alkaloids, flavonoids, phenolics, saponins, steroids and tannins, in the fruit extract of O. elatior open ample scope for further inves-
tigations on antibacterial, antiviral, antidiarrheal and anti-inflammatory activity.

**Effect of Opuntia elatior fruit extract on mice**

Although several herbal preparations have effective medicinal properties, their use is often dictated by the extent of the toxicity they cause. Reduction in the body weight is the sign of toxicity of any chemical or herbal compound [53]. In view of this, the toxicity study of the fruit *O. elatior* extract was examined using mice. Interestingly, no variation in the external morphology and the body weight of mice after the treatment with the extract was observed (Table 1). There was a gradual increase in the final body weight of all control and treated group animals compared to the initial body weight. The extract had neither significant increase nor significant decrease in the final body weight of the treatment group. This clearly indicates that the extract do not cause any toxic effect on the target animal. Thus it can be used for further investigations.

**CONCLUSION**

In conclusion, as there is no information available on phytochemical composition of *O. elatior* and its pharmacological study, the present investigation was undertaken to know the valuable phytochemical components in *O. elatior* and their toxic effect on mice. Interestingly, the cheaply available fruit *O. elatior* is a potential source of many phytochemicals of medicinal values which do not posses toxic effects on the target animal mice. The procedure is simple and the resources required for the experiment are inexpensive. And *O. elatior* shall be used as a potential material for pharmaceutical and nutraceutical uses.

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**REFERENCES**


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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). Subsequently, 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 ooligospermia 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 methonil phylloclade extract of Opuntia dilleniia 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), Albizia 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 spasmylytic 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 as 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 (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 pellets 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 (Ramya Shee *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 mm² each was counted and multiplied by 5x10⁷ 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-Hamdan 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≥0.05) 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≥0.05) 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≥0.05) 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

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

Note: Mean values were compared by One-way ANOVA followed by Duncan’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 2: Effect of *Opuntia elatior* fruit extract treatment for 30 days on the total sperm count and abnormal spermatozoa count

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Total sperm count</th>
<th>Total number of abnormal spermatozoa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.5875±0.07465&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>25.00±1.35401&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>Low dose (250mg/ Kg bw)</td>
<td>5.4625±0.10078&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>25.500±1.32288&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>High dose (500mg/ Kg bw)</td>
<td>4.575±0.14506&lt;sup&gt;***&lt;/sup&gt;</td>
<td>54.500±3.59398&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>Recovery of high dose</td>
<td>5.2125±0.05543&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.000±1.95789&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: Mean values were compared by One-way ANOVA followed by Duncan’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

<table>
<thead>
<tr>
<th>Types of abnormalities</th>
<th>Control</th>
<th>Low dose (250mg/Kg bw)</th>
<th>High dose (500mg/Kg bw)</th>
<th>Recovery group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amorphous head</td>
<td>16.75±1.10868&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.75±0.85391&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.25±3.06526&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.25±0.47871&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hook less head</td>
<td>2.5±0.64550&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.0±0.70711&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.25±2.17466&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.25±0.62915&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pin head</td>
<td>1.5±0.64550&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0±0.40825&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.5±0.64550&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.75±0.95743&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Banana head</td>
<td>1.0±0.40825&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.75±0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.5±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.75±0.47871&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hammer head</td>
<td>3.25±0.85391&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.0±0.80182&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.75±1.10868&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.0±0.40825&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Double head</td>
<td>Nil</td>
<td>Nil</td>
<td>0.25±0.25&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>Nil</td>
</tr>
</tbody>
</table>

Note: Mean values were compared by One-way ANOVA followed by Duncan’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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Low dose (250mg/Kg bw)</th>
<th>High dose (500mg/Kg bw)</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertility index (m)</td>
<td>100 (4)</td>
<td>100 (4)</td>
<td>100 (4)</td>
<td>100 (4)</td>
</tr>
<tr>
<td>Fertility index (f)</td>
<td>100 (8)</td>
<td>100 (8)</td>
<td>100 (8)</td>
<td>100 (8)</td>
</tr>
<tr>
<td>Parturition index</td>
<td>100 (4)</td>
<td>100 (4)</td>
<td>100 (4)</td>
<td>100 (4)</td>
</tr>
<tr>
<td>Gestation index</td>
<td>100 (94)</td>
<td>100 (94)</td>
<td>100 (62)</td>
<td>100 (85)</td>
</tr>
<tr>
<td>Viability index</td>
<td>100 (94)</td>
<td>100 (94)</td>
<td>100 (62)</td>
<td>100 (85)</td>
</tr>
<tr>
<td>Lactation index</td>
<td>100 (94)</td>
<td>100 (94)</td>
<td>100 (62)</td>
<td>100 (85)</td>
</tr>
</tbody>
</table>

Note: Mean values were compared by One-way ANOVA followed by Duncan’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

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>No of males mated/ female</th>
<th>No of pregnant females</th>
<th>Litter size</th>
<th>Percentage fertility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12/250±0.125&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>12/250±0.125&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>97%</td>
<td>100%</td>
</tr>
<tr>
<td>Low dose (250 mg/ Kg bw)</td>
<td>11.7500±0.16366&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>11.7500±0.16366&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>96.91%</td>
<td>96.91%</td>
</tr>
<tr>
<td>High dose (500 mg/ Kg bw)</td>
<td>7.5000±0.26726&lt;sup&gt;***&lt;/sup&gt;</td>
<td>7.5000±0.26726&lt;sup&gt;***&lt;/sup&gt;</td>
<td>63.92%</td>
<td>63.92%</td>
</tr>
<tr>
<td>Recovery group</td>
<td>10.2500±0.16366&lt;sup&gt;***&lt;/sup&gt;</td>
<td>10.2500±0.16366&lt;sup&gt;***&lt;/sup&gt;</td>
<td>87.63%</td>
<td>87.63%</td>
</tr>
</tbody>
</table>

Note: Mean values were compared by One-way ANOVA followed by Duncan’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

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Litter size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12/250±0.125&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>Low dose (250 mg/ Kg bw)</td>
<td>11.7500±0.16366&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>High dose (500 mg/ Kg bw)</td>
<td>7.5000±0.26726&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>Recovery group</td>
<td>10.2500±0.16366&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Reproductive toxicity of opuntia fruit extract in male mice

Note: Mean values were compared by One-way ANOVA followed by Duncan’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 Ramyasheer 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 Opuntia elatior, offered further investigations.

The toxicity tests of Opuntia 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 (Ramyasheer et al., 2012). Thus dose levels less than LD50 was selected in the present study. The body weight of the treated animals remains unchanged which indicate no toxic effect of Opuntia 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 testosterione 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 cypemethrin 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 maintenance of fertilizing ability of the epididymal spermatozoa (Thejashwini et al., 2012). The ethanolic extract of Cyamopsis 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.

REFERENCES
Bajaj, V.K. and Gupta, R.S. (2011) Fertility suppression in male albino rats by administration of methanolic extract of Opuntia dillenii. Andrologia. 44; 530 – 537.
Reproductive toxicity of *opuntia* fruit extract in male mice


