Reversible Antifertility Effect of *Cassia tora* Linn in Male Rats

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ABSTRACT- **Background:** Plant *Cassia tora* has been used in traditional and modern medicines for different pharmacological activities.

**Objectives:** The present investigation has been taken to observe and evaluate effects of *Cassia tora* on the reproduction functions of male rats in search a safe, orally effective and reversible fertility regulating agent.

**Materials and Methods:** Fifty percent ethanolic extract of *Cassia tora* was prepared and administered orally in male Wistar rats at the doses of 50, 100 and 200 mg/kg.b.wt./rat/day dose levels respectively for a period of 60 days and some of the treated rats were kept 30 days for recovery of fertility to assessed reversibility effects. Hematological indices, serum clinical investigations were also performed to assess toxic effects if any caused in rats by treatment. Proteins, cholesterol, glycogen, ascorbic acid, sialic acid and fructose level were analyzed in rats. Serum FSH, LH and Testosterone levels were measured. Rats were castrated to evaluate effects on reproductive functions of hormones and mode of action of the *Cassia tora* treatment. For histopathological observations tissues were fixed in Bouin’s fluid, dehydrated, sectioned and stained with Hematoxylin and Eosin.

**Results:** Treatment of *Cassia tora* significantly reduced the weights of testes and accessory sex organs. Sperm density and motility were declined high significantly. Levels of Testosterone and FSH hormone were significantly decreased in rats. The protein, sialic acid, fructose, ascorbic acid and glycogen contents of reproductive accessory sex organs were decreased significantly. Germinal epithelium of testes degenerated and number of spermatocytes, spermatids and spermatooza in lumen of seminiferous tubules reduced.

**Conclusions:** The decreased testes and accessory sex organs weights, sperm motility, density and testosterone level in rats might be due to androgen suppression effects of *Cassia tora* treatment cause inhibition of spermatogenesis resulted reduction of fertility in treated male rats.

**Key-words-** *Cassia tora*, Contraception, Fertility, Sperm motility, Sperm density, Male rat

INTRODUCTION

The population explosion creates immense pressure on our natural and non-renewable resources leading to social economic imbalances and political tension [1-3]. Fertility regulation has therefore, become a major global concern [3-5]. Although, there are several types of contraceptive methods are available for fertility control in male and females, but all of these were find one or more side effects. Survey of literature enumerate that the use of plants products as antifertility agent cause minimal or no side effects as compared to currently available conventional contraceptive methods especially oral. Literature reveals that the plants and their products were used for fertility regulation since hazards of uncontrolled population were visualized [6-9].

World Health Organization have already been taken significant steps to carry out research aimed at finding new and effective antifertility agents from traditional medicinal plants [10-11]. The results obtained are encouraging and thus hoped that in near future an easily administrable and reversible oral contraceptive of plant origin would be available to common people. The present investigation is planned to evaluate contraceptive efficacy of *Cassia tora* fruits products to search a cheap, orally effective and reversible contraceptive from indigenous medicinal plants with emphasis of mode of action and side effects in rats with the objective to develop a cheap, safe, easily administrable, orally effective and reversible male fertility regulating agents from traditional medicinal plants.

MATERIALS AND METHODS

The Plant- The plant *Cassia tora* is belongs to family- Fabaceae and also known as *Cassia obtusifolia*, Foetid cassia, Sickle senna, Wild senna, Charota, Chakvat, Chakvat senna tora etc [12] have selected for the present study on the basis of literature survey of
The fruits of the plant were collected in the months of September and October of 2010 and study was carried out at Department of Zoology, University of Rajasthan Jaipur, India.

*Cassia tora* possess various medicinal and pharmacological activities these includes antihepatotoxic [13], antiallergic [14], antimutagenic [15], antifungal [16], radical scavenging and antimicrobial [16-28]. The chemical constituent of the plant *Cassia tora* are rubrofusarin triglucoside, non rubrofusarin gentobioside, demethyl-flavasperone, gentobioside, torachryson gentibioside, torachryson tetraglucoside, tory chryson apioglucoside, torachryson, toralactone aloemodin, rhein, emodin, naphthalene, anthaquinone, methicillin-resistant [23-26].

Preparation of Test material- Plant *Cassia tora* (RUBL20672) had been identified by deposit herbarium specimen stem at the Department of Botany, University of Rajasthan Jaipur, India. The fresh fruits of the plant *Cassia tora* were collected around the Jaipur district in the months of September and October of 2010. Washed, shade dried and crushed to make fine powder for further use. The 50% alcoholic extract of fresh fruits of this plant was prepared according to WHO protocol CG-04 [27].

Animal model- Colony-bred, healthy adult fertile male Wistar rats (*Rattus norvegicus*) weighing between 150-200gm about 50-60 days aged were used for the present study. The animals were kept in polycarbonate cages, measuring 430×270×150 mm and housed under controlled environment conditions with provision of 12hrs light: 12hrs dark conditions. The animals were fed with powdered rat diet and water was provided *ad libitum*. Body weight of each animal in all groups was measured weekly to see the possible changes in body weight throughout the experiment.

Experimental design and Protocol- Rats of similar body weight, size age were grouped as under. Whole study was divided into two experiments. Following experiments were carried out during the course of study, to observe antifertility effect and to observe mode of action/effects nature of the extract and reversibility effects.

The animals were divided into 5 treatment groups each consisting of 10 animals

**Group-A:** The animals were given sterile DW alone orally; serve as vehicle treated controls.

**Group-B:** The animals of this group were treated with *Cassia tora* (50%EtOH) at 50mg/kg.b.wt/day.

**Group-C:** The animals of this group were treated with *Cassia tora* (50%EtOH) at 100mg/kg.b.wt/day.

**Group-D:** The animals of this group were treated with *Cassia tora* (50%EtOH) at 200mg/kg.b.wt/day.

**Group-E:** The animals of this group were treated with *Cassia tora* (50%EtOH) 100mg/kg. wt/day for 60 days were kept for a recovery period of 30 days.

**Body organs weights-** The initial final body weights of the animals were recorded. Testes, epididymis, seminal vesicles ventral prostate were dissected out, freed from adherent tissues weighed to the nearest milligram on an electronic balance.

**Tissue Biochemistry-** The testis, epididymis, seminal vesicles ventral prostate were dissected out, freed from adherent tissues weighed at nearest milligram balance. Protein [29], glycogen [30], cholesterol [30], sialic acid [31], ascorbic acid [32], fructose [33] were estimated in right side of testis other accessory reproductive organs.

**Fertility Test-** Male rats were introduced to female, 200-300 gm (male: female ratio, 1:2) at 17:00 h after 55 days of treatment. The mated females were allowed to complete the gestation. The number of pups was recorded litter size percent fertility was calculated [27].

**Sperm Motility and Density-** For sperm motility density, 50 mg of cauda epididymis was minced in 1 ml of physiological saline, immediately within 5 min after sacrifice; 1 drop of evenly mixed sample was applied to a glass slide under a cover glass. The percent motility was determined by counting both motile immotile spermatozoa per unit area. After that cauda epididymal sperm density was made by routine procedure express as millions/mm³ suspension [34].

**Hormone Assay-** Blood samples were also collected for serum separation to estimate FSH, LH and testosterone by radioimmunooassay. Serum samples separated by standard procedures stored at -20°C for subsequent analysis. Serum levels of testosterone were assayed in duplicate using radioimmunooassay kit [35].

**Histopathological Study-** Contra lateral side of the testis, epididymis, vas deferens, seminal vesicle, ventral prostate, kidney, heart liver were fixed in Bouin's fluid, dehydrated in graded ethanol, cleared in xylene and free from adherent tissue and embedded in paraffin wax (Melting point 55°-62°C). Sections were made at 6 μ was stained with Harris's hematoxylin and eosin to observe histopathological changes.

**STATISTICAL ANALYSIS**

Statistical analysis is based on biological statistics. All the values of body organ weights, biochemical estimations histometry were averaged expressed as Mean ± Standard error (S.E.). Data are expressed as mean ± S.E. analyze for statistical significance by using student's “t” test. The data considered as significant and highly significant at p≤0.01 and p ≤ 0.001, respectively [36].

**Ethical Aspects-** The study was carried out under the supervision of ethical committee of the Department of Zoology, University of Rajasthan, Jaipur [Vide Letter No.Rs/98/10/7454 dated 19/8/2010] and CPSEA [37] guidelines were followed to maintain the experimental animals.
RESULTS
Changes in blood and serum profile
Cassia tora extract treatment in rats caused a non significant change in serum serum proteins, phospholipids, cholesterol, triglyceride, HDL, LDL and VLDL, acid phosphatase and alkaline phosphatase, LDH, SGOT, SGPT level after the treatment at different dose levels as compared to control rats. Cassia tora extract treated rats did not show any remarkable changes in blood and serum biochemistry and no significant alteration in hematological parameter (data not shown).

Effect on body and reproductive organ weight
Oral administration of extract of Cassia tora (fruit) for 60 days, caused no adverse effect on body weight; whereas the weight of testes and accessory sex organs decreased significantly (P≤0.001) might be due to decreased level of androgens required to maintain the growth and development of reproductive organs. The weight reduction was dose dependent i.e. high dose 200mg/kg.b.wt. (Group D) treated group drastically reduced followed by less in low dose (Group C) 100mg/kg.b.wt. group. Significant changes were observed in vas deferens, seminal vesicle and ventral prostate (Table 1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial b.wt. (gm)</th>
<th>Final b.wt. (gm)</th>
<th>Testes mg/100gm b.wt.</th>
<th>Epididymides mg/100gm b.wt.</th>
<th>Vas deferens mg/100gm b.wt.</th>
<th>Seminal vesicle mg/100gm b.wt.</th>
<th>Ventral prostate mg/100gm b.wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A Control</td>
<td>120.00±2.35</td>
<td>156.50±1.67</td>
<td>1405.79±12.58</td>
<td>595.26±10.70</td>
<td>148.26±2.10</td>
<td>492.42±3.31</td>
<td>76.50±1.72</td>
</tr>
<tr>
<td>Group B Cassia tora 50 mg/kg.b.wt</td>
<td>154.50±1.38</td>
<td>163.50±0.76</td>
<td>1399.51±18.63**</td>
<td>574.76±9.93 **</td>
<td>145.75±2.13 **</td>
<td>481.45±5.58 **</td>
<td>73.28±0.95 **</td>
</tr>
<tr>
<td>Group C Cassia tora 100mg/kg.b.wt</td>
<td>153.00±1.11</td>
<td>167.50±0.83</td>
<td>1381.06±5.86 **</td>
<td>574.30±3.88 **</td>
<td>142.83±2.30 **</td>
<td>479.26±7.80 **</td>
<td>72.74±1.17 **</td>
</tr>
<tr>
<td>Group D Cassia tora 200mg/kg.b.wt</td>
<td>114.00±2.08</td>
<td>166.50±0.76</td>
<td>1272.46±6.16 **</td>
<td>472.74±6.26 **</td>
<td>119.54±0.95 **</td>
<td>427.80±3.02 **</td>
<td>63.86±0.67 **</td>
</tr>
<tr>
<td>Group E Cassia tora 100 mg / kg. b.wt. recovery</td>
<td>112.00±2.00</td>
<td>160.50±1.17</td>
<td>1374.63±9.70 **</td>
<td>581.88±8.36 **</td>
<td>145.08±1.17 **</td>
<td>488.95±5.53 **</td>
<td>75.81±1.17 **</td>
</tr>
</tbody>
</table>

Data exposed as Mean ±S.E, ns = Non-Significant,* Significant (P<0.01), ** Highly Significant (P<0.001)

Biochemical changes
The ethanolic extract treatment of Cassia tora in rats were shown significantly decreased (P<0.001) level of protein, sialic acid, fructose, glycogen, cholesterol contents in reproductive organs. In recovery group (Group-E) after 30 days of withdrawal of treatment, it was altered up to non significant level (Table 2).

Table 1: Changes in level of protein, sialic acid, cholesterol, glycogen, ascorbic acid and fructose level in reproductive organs of rats following Cassia tora extract treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Protein (mg/gm)</th>
<th>Sialic acid (mg/gm)</th>
<th>Cholesterol (mg/gm)</th>
<th>Glycogen (mg/gm)</th>
<th>Ascorbic acid (mg/gm)</th>
<th>Fructose (mg/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cauda</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A Control</td>
<td>226.19±3.41</td>
<td>5.20±0.25</td>
<td>16.06±0.47</td>
<td>2.17±0.08</td>
<td>2.90±0.22</td>
<td>4.89±0.31</td>
</tr>
<tr>
<td>Group B Cassia tora 50 mg/kg.b.wt</td>
<td>226.19±3.41</td>
<td>5.20±0.25</td>
<td>16.06±0.47</td>
<td>2.17±0.08</td>
<td>2.90±0.22</td>
<td>4.89±0.31</td>
</tr>
</tbody>
</table>
Data exposed as Mean ±S.E, ns = non-significant, * Significant (P≤0.01), ** Highly significant (P≤0.001)

**Effect on Sperm motility and density**
Sperms motility and density in cauda epididymides decreased significantly (P≤0.001) in rats following extract treatment (Group B-D) in comparison to control rats (Group-A). Although, it was recover up to normal level in rats recovery (Group- E) (Fig. 1).

**Changes in hormone levels**
The level of testosterone, LH and FSH analysis shown the decreased level of these hormones in *Cassia tora* extracts treated rats (Group B-D) in dose dependent manner at dose levels of 50 mg/ kg b.wt.,100 mg/ kg b.wt.,200 mg/ kg b.wt., however, in recovery group (Group- E) it return up to normal level (Fig. 2-4).

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<table>
<thead>
<tr>
<th>Group</th>
<th>Cassia tora</th>
<th>Sperms motility (%)</th>
<th>Sperm Density</th>
<th>Testosterone (ng/ml)</th>
<th>LH (mIU/ml)</th>
<th>FSH (mIU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td></td>
<td>60.72±3.45 ns</td>
<td>70.42±4.56 ns</td>
<td>186.65±3.86**</td>
<td>191.98±3.91**</td>
<td>4.08±0.23**</td>
</tr>
<tr>
<td>Group B</td>
<td>100 mg/kg.b.wt</td>
<td>40.42±3.63**</td>
<td>20.50±4.60 ns</td>
<td>201.56±4.00*</td>
<td>14.12±0.63**</td>
<td>1.62±0.09**</td>
</tr>
<tr>
<td>Group C</td>
<td>200 mg/kg.b.wt</td>
<td>30.23±3.76**</td>
<td>15.50±4.60 ns</td>
<td>216.86±4.01 ns</td>
<td>219.09±5.05 ns</td>
<td>5.12±0.22**</td>
</tr>
<tr>
<td>Group D</td>
<td>100 mg/kg.b.wt</td>
<td>20.23±3.76**</td>
<td>15.50±4.60 ns</td>
<td>216.86±4.01 ns</td>
<td>219.09±5.05 ns</td>
<td>5.12±0.22**</td>
</tr>
<tr>
<td>Group E</td>
<td>200 mg/kg.b.wt</td>
<td>10.23±3.76**</td>
<td>5.50±4.60 ns</td>
<td>216.86±4.01 ns</td>
<td>219.09±5.05 ns</td>
<td>5.12±0.22**</td>
</tr>
</tbody>
</table>

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Fig. 1: Effect of *Cassia tora* extract treatment on sperm motility and density

Fig. 2: Effect of *Cassia tora* extract on testosterone level

Fig. 3: Effect of *Cassia tora* extract on LH level
Fig. 4: Effects of *Cassia tora* extract on FSH Level

**Effect on spermatogenesis**

Photomicrographs of the testes of *Cassia tora* treated rats at the dose levels of 50 mg/ kg b.wt., 100 mg/ kg b.wt. and 200 mg/ kg b.wt. caused degenerative changes. Germinal epithelium of seminiferous tubules was ruptured and wavy due to loosened connective tissue. Spermatogonia and sperm number were significantly reduced, cellular debris can be seen in lumen, inter tubular stroma was increased. However spermatogenesis alters up to normal level in rats of recovery group (Photomicrograph 1-5).

**Histopathological Observations of Testes**

Photomicrograph-1, Group-A (X 100 H.E.) testis of control rats showing a normal histoarchitecture, well developed germinal epithelium, seminiferous tubules filled with spermatocytes, spermatids and spermatozoa.

Photomicrograph-2, Group-B (X 100 H.E.) testes of rat treated at 50 mg/kg b.wt. showing degenerated epithelium, irregular and incomplete spermatogenesis.

Photomicrograph-3, Group-C (X 100 H.E.) testes of rat treated at 100 mg/kg b.wt. showing decreased spermatocytes and sperms in seminiferous tubules.

Photomicrograph-4, Group-D (X 100 H.E.) testes of rat treated at 200 mg/kg b.wt. showing incomplete spermatogenesis. Primary stages appear to be normal but later stages are absent.

Photomicrograph-5, Group-E (X 100 H.E.) testes of rat treated with recovery showing normal seminiferous tubule. Spermatocytes, spermatid and spermatozoa are clearly visible in the lumen. Sertoli cell and Leydig cell are also clearly seen.
DISCUSSION
Maintenance of structure and functional integrity of accessory reproductive organs requires continuous supply of androgen [8,25, 38-41]. Oral administration of alcoholic extract of Cassia tora (fruit) for 60 days brought a decrease in the weight of reproductive organs indicating that the circulating level of androgen was not enough to maintain the weights of reproductive organs, however the weights of vital organs were not affected. [42-46].

Seminal vesicles are androgen-dependent and this property may be used as biological marker of androgen activity [47-49]. Protein level is directly correlated with the secretory activity of the epididymis, which in turn depends on the androgen levels [50-51]. The low levels of testicular protein are usually indicative of inhibition of spermatogenesis [52-54]. The decreased sialic acid level in testes and accessory organs of male rats and the epididymis can be a causative factor for impaired sperm function [39,55-56]. A decrease in glycogen content of the testis reduces the energy source for spermatogenic activity which could affect protein synthesis [42,57]. Decrease in ascorbic acid cause hypofunctioning of testis and the degeneration of the germinal epithelium [58]. Fructose serves as source of energy for sperm, reduction in the fructose might be due to decreased secretory activity of seminal vesicle [59-60].

Sperm density and motility directly correlates with fertility chances and therefore decreased sperm density [50-54] and motility of spermatozoa might reduced fertility of rats followed extract treatment [61-63]. A large number of metaphasic cells in the germ epithelium of treated animals might be caused by cell cycle blockage or arrest at the spermatid level in the form of degenerative changes in the germinal cells together with few fragmented sperms in the lumen and acquired a thick, irregular basement membrane [72-74]. Spermatogenesis requires LH and FSH for initiation and maintenance in male rats. LH, through specific receptors found on the surface of Leydig cells, controls the production and secretion of testosterone. Normal testicular function is dependent on FSH and testosterone is absolutely required for normal spermatogenesis [75-80]. FSH establishes a quantitatively normal Sertoli cell population, whereas androgen initiates and maintains sperm production, thus both hormones co-operate via independent functions to enable maximal spermatogenic output [81-85].

CONCLUSIONS
It can concluded that treatment of Cassia tora fruits extract in male rats reduced levels of protein, fructose, ascorbic acid, glycogen, and sialic acid contents might be responsible to caused degenerative changes in germinal epithelium of seminiferous tubules of testes. The decreased level of testosterone further support anti-androgenic effects of the treatment resulted decreased sperm density and motility reduced fertility of extract treated rats.

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