Effects of Ethanol Extract of Caesalpinia digyna on Testicular Steroidogenesis in Rats

SARATHCHANDIRAN T*, PAVAN KUMAR B, KARIMULLA SK, NARAYANAN V, VINOTH KUMAR S
Gokula Krishna College of Pharmacy, Sullurpet-524 121, Nellore (DT), A.P, India

ABSTRACT
Ethanol extract of Caesalpinia digyna (Rottler) root (EECD) was evaluated for possible testicular antisteroidogenic activity in mature male rat. The ethanol extract at the doses of 50,100, and 200 mg kg⁻¹ body weight (i.p) arrested the testicular steroidogenises. The cholesterol and ascorbic acid content in testis were significantly elevated in treated rats. The extract also significantly inhibited the activity of Δ⁵-3β-hydroxy steroid dehydrogenase (Δ⁵-3β-HSD) and glucose-6-phosphate dehydrogenase (G-6-PD), the two key enzymes involved in testicular steroidogenesis. Results of this study suggested that the ethanol extract of Caesalpinia digyna (Rottler) root acts as a testicular antisteroidogenic agent.

KEY WORDS: Caesalpinia digyna (Rottler), antifertility, Δ⁵-3β-HSD, testicular steroidogenesis G-6-PD, cholesterol, ascorbic acid.

Introduction
During the past few decades sporadic attempts have been made by various investigators to develop male contraceptive agents from various antifertility plants available in their locality or in the market. Various medicinal plant extracts have been tested for their antifertility activity both in male and female (Kamboj, 1988). Some of these plants had spermicidal effects; others caused reduction in the sperm counts and altered the mobility of the sperm. Some of them caused testicular change and altered hormone levels (Bhargava, 1984; Reddy et al., 1997). But so far no significant lead has been obtained by these studies. These results prompted us to screen various plants in our locality. Based on these trials one plant, Caesalpinia digyna (Rottler) was selected for detailed studies. (Anon., 1992)

*Corresponding Author
Sarath1974@rediffmail.com
0091 90004 36245

Materials and methods

Plant material
The Root of Caesalpinia digyna (Rottler) was purchased from Abhirami botanicals, Tuticorin, Tamilnadu, India. Taxonomical identification was made from botanical survey of medicinal plant unit, Government Siddha Medical College, Government of India. Palayamkottai, Tamilnadu, India. The root was dried at room temperature, powdered by the mechanical grinder, sieved and stored for further used. The powder was soxhlatled with 90% ethanol at 60-70°C. The extract was filtered and concentrated to dry mass by vacuum distillation. The semi dried material was diluted with n-butanol and water 1:1, the n-butanol soluble material was separated by separating funnel, dried under room temperature and powdered for further use.

Animals
Three months old Wister strain male albino rat of 150-200 g. body weight was procured from the

Department of Pharmaceutics, Rural college of

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Pharmacy, Devanahalli, Bangalore Rural District, Karnataka, India. Rats were fed with standard pelleted feed from Gold Mohur Laboratory animal’s feeds, Bangalore, India and water ad libitum. The experiment was performed under the guidance of the Ethical Committee, Rural college of Pharmacy, Devanahalli. The animals were housed in polypropylene cage under control environmental condition with provision of 12 h light and 12 h dark.

Materials

The Ethanolic extract (dissolved in normal saline) of *Caesalpinia digyna* (Rottler) root, digitonin, cholesterol, nicotinamide adenine dinucleotide (NAD), Ascorbic acid, Dehydroepiandrosterone (DHEA), Necotinamide adenine dinucleotide phosphate(NADP), Glucose-6-phosphate (G-6-P) (SIGMA CHEMICALS, USA), Chloroform LR, Acetone AR, Diethyl ether LR, Potassium hydroxide LR, Disodium hydrogen orthophosphate LR, Potassium dihydrogen phosphate LR, Glacial acetic acid, Acetic anhydride, Ethylacetate, Sodium acetate (S.D.Chemicals, India), Ethanol (Bengal Chemicals and Pharmaceuticals Ltd, India), Phenolphthalein, Thiourea (Sarabhai M Chemicals Ltd, India), Sodium chloride(Basynth, India), Tris-HCl buffer(SRL, India), Metaphosphoric acid (E.Mark,Germany), 2,4-Dinitrophenyl hydrazine (Loba Chemicals, India), Concentrated bromine solution, Concentrated sulphuric acid (International Chemical Industry, February 20,2007 India).

Experimental design

Treatment of Animals: Twenty four healthy Wistar albino male rats were selected for present study. The animals were equally divided into 4 groups containing 6 rats each and treated as follows:

Group I: Phosphate buffer saline (PBS) (5 ml kg⁻¹ body weight).

Group II: Ethanolic extract of *Caesalpinia digyna* (Rottler) (EECD) dissolved in phosphate buffer saline (PBS) (50 ml kg⁻¹ body weight).

Group III: Ethanolic extract of *Caesalpinia digyna* (Rottler) dissolved in phosphate buffer saline (PBS) (100 ml kg⁻¹ body weight).

Group IV: Ethanolic extract of *Caesalpinia digyna* (Rottler) dissolved in phosphate buffer saline (PBS) (200 ml kg⁻¹ body weight).

The phosphate buffer saline (PBS) and the different doses of Ethanolic extract were administered intraperitoneally on daily for 18 days after 18 hr. of fasting. The rats were weighed before and after the commencement of the experiment. All the animals were sacrificed 24 hr. after the last dose and 18 hr. of fasting. Testis, cauda epididymis and adrenal glands were immediately dissected out, trimmed off from adherent fats and weighed. Sperm from cauda epididymis were released in phosphate buffer saline (PBS).

Biochemical estimation

Testicular tissues about 3 mg weight carefully homogenized in Potter Ekevhjem homogenizer using chloroform: Ethanol mixture (2:1) and non-polar part was extracted out and total cholesterol content was estimated Abell *et al.* (1952).

About 5 mg of tissue was homogenized in Potter Ekevhjem homogenizer using 2.5ml ice cold 5% Metaphosphoric acid and centrifuged for 20min at 355 rpm then ascorbic acid content was calculated (Sierralta *et al.*, 1978).

About 3 mg of testicular tissues was again homogenized in Potter Ekevhjem homogenizer using 0.5M Tris-HCl (Ph 8.3) and centrifuged. The activity of G-6-PD was estimated as described by BergMeyer (1965).

About 2 mg of testicular tissues was homogenized in Potter Ekevhjem homogenizer using 1 ml of normal saline and 1ml of 0.1 M phosphate buffer (pH 7.4) and centrifuged. The activity of Δⁿ⁻³Β-HSD was estimated as described by Nakajin *et al.* (1995).

Protein was estimated with Folin’s phenol reagent and the activities of enzymes were expressed in unit per mg of protein as described by Lowry *et al.* (1951). Statistical analysis: Statistical analysis was done by student’’t-test.
Results and discussion

The results are summarized in the table 1. The ethanolic extract of root of *Caesalpinia digyna* (Rottler) (EECD) at all the doses of 50, 100 and 200 mg kg\(^{-1}\) body weight significantly increased the level of total cholesterol and ascorbic acid contents of testicular tissues in treated rats. The activities of \(\Delta^\text{3}\)-β-HSD were inhibited significantly (p<0.05 by 50 mg and p<0.001 by both 100 and 200 mg). Similarly, the activities of G-6-PD were inhibited significantly (p<0.01 by 50 mg and p<0.001 by both 100 and 200 mg) by all the doses of root of *Caesalpinia digyna* (Rottler) (EECD). From the data, it is evident that the drug treated rats resulted in, the cholesterol and ascorbic acid content is more than the Phosphate Buffer Saline (PBS) control treated rats and decrease in the \(\Delta^\text{3}\)-β-HSD and glucose-6-phosphate dehydrogenase activities in comparison to phosphate buffer saline (PBS) treated rats.

In our earlier studies, the Ethanolic extract of root of *Caesalpinia digyna* (Rottler) showed reduction in the number of spermatozoa (sperm count) and their motility, which is due to inhibition of androgenic synthesis and as a result there is reduction in weights of testis and accessory reproductive organs. This idea was further strengthened by the accumulation of cholesterol and ascorbic acid, the principal precursor for the formation of androgens in biogenic pathway in the testes. The further support to this proposition obtained from the diminished values of \(\Delta^\text{3}\)-β-HSD and glucose-6-phosphate dehydrogenase activities in testis. It is well documented that \(\Delta^\text{3}\)-β-HSD is a key enzyme involved in androgen biogenesis. Knorr successfully established that \(\Delta^\text{3}\)-β-HSD is an important enzyme in the production of steroid hormone. McKerns have shown that gonadotrophins through the activation of glucose-6-phosphate dehydrogenase metabolism in pentose phosphate pathway increased the rate of formation of NADPH essential for hydroxylation reaction in the formation of the steroid hormones from cholesterol.

### Table 1:

**Effect of ethanolic extract of *Caesalpinia digyna* (Rottler) (EECD) root on testicular cholesterol, ascorbic acid, \(\Delta^\text{3}\)-β-Hydroxy steroid dehydrogenase (\(\Delta^\text{3}\)-β-HSD) and glucose-6-phosphate dehydrogenase (G-6PD) contents in rats (mean±SD,n=6)**

<table>
<thead>
<tr>
<th>Treatment Design</th>
<th>No. of treatment days</th>
<th>Cholesterol (mg g(^{-1}) tissue)</th>
<th>ascorbic acid (mg g(^{-1}) tissue)</th>
<th>(\Delta^\text{3})-β-HSD (\mu) mg(^{-1})</th>
<th>G-6-PD (\mu) mg(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS (5 ml kg(^{-1})b.w i.p)</td>
<td>18</td>
<td>80.31±8.1</td>
<td>140.32±32</td>
<td>8.2±0.81</td>
<td>23.32±0.32</td>
</tr>
<tr>
<td>EECD (50mg kg(^{-1})b.w i.p)</td>
<td>18</td>
<td>98.20±5.3**</td>
<td>175.81±12</td>
<td>7.01±0.22*</td>
<td>20.81±0.81*</td>
</tr>
<tr>
<td>EECD (100mg kg(^{-1})b.w i.p)</td>
<td>18</td>
<td>29.20±6.10</td>
<td>179.83±48**</td>
<td>6.31±0.81**</td>
<td>16.72±0.16**</td>
</tr>
<tr>
<td>EECD (200mg kg(^{-1})b.w i.p)</td>
<td>18</td>
<td>150.80±8.20***</td>
<td>198.68±51***</td>
<td>5.52±0.21***</td>
<td>12.78±0.12***</td>
</tr>
</tbody>
</table>

PBS - Phosphate buffer saline, BW - Body Weight, i.p. - intra peritonal, EECD - Ethanolic extract of *Caesalpinia digyna* (Rottler) root, * - P<0.05, ** - P<0.01, *** - P<0.001, Significantly different from PBS Control

### Conclusion

On the basis of present findings and experimental data, it may be concluded that the Ethanolic extract of *Caesalpinia digyna* (Rottler) root exhibited inhibition of testicular steroidogenesis in male rats thereby acting as an antifertility agent.

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References


