Antifertility and Antispermatic Effects of Ethanolic Extract of *Tephrosia purpurea* Fruits in Albino Rats

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Received: 18 March 2016/Revised: 03 April 2016/Accepted: 01 May 2016

ABSTRACT- Objective: In this experiment adult male albino rats were treated with 50% ethanolic extract of *Tephrosia purpurea* fruits at the dose levels of 50, 100 and 200 mg/kg body weight for 60 days, to evaluate antifertility effects in search of a reversible male contraceptive agent from medicinal plants.

Materials and Methods: Body and organs weight of all treated animals was recorded, blood and serum were analyzed for hematological indices and clinical biochemistry. To observe effects on reproductive system of animal’s protein, fructose, sialic acid, ascorbic acid, and glycogen contents were estimated in their testes and sex accessory organs. The treated male rats were mated with proestrous females and sperm motility, sperm density was determined and FSH, LH and testosterone hormones were measured to evaluate the effects on fertility. For histopathological observation testes were fixed in Bouin’s fluid, sections were cut at 6 µ and stained with Harris’s Haematoxylin and eosin.

Results: Analysis of blood and serum revealed no significant effect after 60 days of the extract treatment. Body weight of the extract treated rat had no significant alteration, whereas the weight of reproductive organs was decreased significantly as compared to animals of control group. Protein, sialic acid, fructose contents and level of LH and testosterone hormones was decreased significantly after treatment in extract treated rats as compared to control.

Conclusions: The fertility, sperm density and motility were declined significantly in rats treated with the ethanolic extract of *Tephrosia purpurea* fruits. It is concluded that it might be due to androgen inhibition effects.

Key-words- Antifertility, *Tephrosia purpurea*, Rat, Testosterone

INTRODUCTION

Rapidly increasing population now becomes a global concern since it creates negative impact on social, economic development and health of human being. Uncontrolled population is the major reason behind poverty, unemployment and environmental pollution. However, different types of contraceptives are available to control human fertility.

Currently available contraceptive are failed to check population and also have side effects. Various adverse effects like hormonal imbalance, headache, depression, weight gain have been reported by different contraceptive users. This situation demands the search of safe, cheap, orally effective and reversible new contraceptives.

The plants have been a source of folk medicine since ancient times. In last decades several plant species have been explored for the antifertility activities in many animal models including non human primates to develop a safe reversible male contraceptive agent for human use. The plant *Tephrosia purpurea* also known as ‘sharpunka’ has been used for treatment of various human diseases like, bilious febrile attack, bronchitis, boils, pimples, diarrhea, gonorrhea, heart and spleen diseases but no intention has been paid on use of fertility regulating effects of *Tephrosia purpurea*, therefore, the present investigation was designed to observe effects on the reproductive systems of the treated rats.
functions and general body metabolism of the ethanolic extract of the plant.

MATERIALS AND METHODS

Identification of the plant test material
Specimen voucher of *Tephrosia purpurea* was submitted to the taxonomist for the identification of the plant at the Department of Botany, University of Rajasthan, Jaipur (RUBL 211331).

Preparation of plant test material
The fruits of plant were shade dried and then crushed mechanically. Their 50% ethanolic extract was prepared according to the WHO protocol CG-0413.

Experimental animal model
Colonby-bred healthy fertile male Wistar rats (*Rattus norvegicus*) in the weight range of 150-200 gm were selected to the study. The animals were housed in polypropylene cages, measuring 430×270×150 mm. They were maintained under laboratory condition of temperature, humidity (60% ±1%) and 12 h light/ dark cycle. They fed rat pallated rats feed and water was provided *ad libitum*.

Ethical aspects
The CPCSEA (2006) and Ethical committee of Department of Zoology, University of Rajasthan, Jaipur guidelines were followed for the maintenance and experiments on animals14.

Experimental design
The animals were randomly divided into five treatment groups each consisting of 8 animals. Group- I served as a control and treated with distilled water for 60 days. The three animal Groups- II, III, IV were given extract at dose levels of 50, 100 and 200 mg/kg/body wt/day respectively for 60 days dissolved in distill water. Animals of Group-V were given the extract 100mg/kg/body wt/day dissolved in distilled water for 60 days followed by 30 days of recovery period. This group served as recovery group.

Sperm motility and density
To determine sperm motility and density the cauda epididymis was immediately removed after the autopsy. The results were determined by counting both motile and immotile sperm in Neubaur chamber. The sperm density was calculated in testes, epididymides and expressed in million per ml15.

Fertility Test
To check the fertility of all rats the fertility test was performed prior to the experiment and during 55 to 60 days. Male rats were cohabited with proestrous females in ratio of 1:2. The female rats were allowed to complete gestation period. Their vaginal smears were checked for positive mating. The inseminated female rats were separated and the numbers of litters delivered were recorded and litter size, fertility percentage was calculated.

Body and Organ Weights
The initial and final body weights of the animals were recorded. Reproductive and vital organs viz, liver, kidney, heart were dissected out, freed from adherent tissue and weighed accurately up to milligram level.

Histopathology
The testis was fixed in Bouin's fluid and processed, sectioned at 6 µ and stained with Harris's Haematoxylin and eosin and observed under a light microscope.

Serum Biochemistry
Serum was separated and stored at -20°C for total cholesterol16, serum alanine amino transaminase17, aspartate amino transaminase17, acid phosphatases18 and alkaline phosphatases19 analysis. FSH, LH and testosterone hormones level were assayed by radioimmunoassay20.

Tissue Biochemistry
The testis, epididymis, seminal vesicles and ventral prostrate were dissected out and analyzed for Protein21, glycogen22, cholesterol23, sialic acid24, ascorbic acid25 and fructose26 contents.

STATISTICAL ANALYSIS
The data obtained from the above experiments were expressed in terms of mean ± SEM. The data were analyzed statistically by using Student’s “t” test and the significance of the differences was set as significant at p<0.05 and highly significant at p<0.001.

RESULTS
The blood hematoloy and serum biochemistry showed no significant changes which marks the non-toxic action of the extract treatment on metabolism of treated rats.

Effect on the body and reproductive organs weight
No dose regimen showed any significant change in the body weight of the rats in comparisons to control (Group-I) animals. However, weight of reproductive organs was decreased significantly while vital organs and body weight showed no significant changes (non significant data are not shown). The weight of body and organs found normal in the rat of recovery groups (Table-1).
Table I- Effects of *Tephrosia purpurea* on Body and Organ weight on treated male rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body Weight (gram)</th>
<th>Organ Weight (mg/100 gm.b.wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group-I</td>
<td>134.37±2.39</td>
<td>159.37±2.74</td>
</tr>
<tr>
<td>Group-II</td>
<td>138.75±2.63**</td>
<td>162.50±2.50 ns</td>
</tr>
<tr>
<td>Group-III</td>
<td>147.90±2.83***</td>
<td>169.37±3.07***</td>
</tr>
<tr>
<td>Group-IV</td>
<td>138.12±1.87**</td>
<td>165.62±2.57**</td>
</tr>
<tr>
<td>Group-V</td>
<td>137.50±2.11ns</td>
<td>155.00±2.21s</td>
</tr>
</tbody>
</table>

(Mean ± SEM ) Group II, III, IV and V Compared with Group I.

**=Highly significant (p≤0.001), *=Significant (p≤0.01), *=Significant (p≤0.05 ),ns= Non significant

**Effect on sperm motility and density**
The sperm density and motility decreased significantly (p<0.001) after treatment of the dose of plant. They were found normal after recovery period in recovery group (Fig. 1-2).

**Biochemical changes**
The ethanolic extract treatment of *Tephrosia purpurea* decreased levels of protein (p<0.001), sialic acid (p<0.001), fructose (p<0.001), glycogen (p<0.001) and cholesterol (p<0.05) levels in reproductive organs however, no significant change observed in vital organs. There was no significant change observed in recovery group (Table 2).
Table II- Effects of *Tephrosia purpurea* on tissue biochemistry on treated male rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Protein (mg/gm)</th>
<th>Sialic Acid (mg/gm)</th>
<th>Cholesterol (mg/gm)</th>
<th>Fructose (mg/gm)</th>
<th>Ascorbic Acid (mg/gm)</th>
<th>Glycogen (mg/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Testis</td>
<td>Cauda</td>
<td>Testis</td>
<td>Cauda</td>
<td>Testis</td>
<td>Seminal Vesicle</td>
</tr>
<tr>
<td>Group-I</td>
<td>244.16±7.60</td>
<td>269.81±7.70</td>
<td>5.86±0.12</td>
<td>5.97±0.10</td>
<td>7.80±0.41</td>
<td>5.29±0.13</td>
</tr>
<tr>
<td>Group-II</td>
<td>217.74±1.45*</td>
<td>238.99±3.80**</td>
<td>4.33±0.20**</td>
<td>5.32±0.13**</td>
<td>7.82±0.45**</td>
<td>4.94±0.26**</td>
</tr>
<tr>
<td>Group-III</td>
<td>197.83±1.41***</td>
<td>236.17±2.05**</td>
<td>3.73±0.11***</td>
<td>4.46±0.13***</td>
<td>7.76±0.50**</td>
<td>4.54±0.26*</td>
</tr>
<tr>
<td>Group-IV</td>
<td>190.45±0.67***</td>
<td>230.18±0.93***</td>
<td>3.49±0.13***</td>
<td>4.35±0.08***</td>
<td>6.52±0.23*</td>
<td>4.30±0.10***</td>
</tr>
<tr>
<td>Group-V</td>
<td>242.82±3.73ns</td>
<td>268.24±0.65ns</td>
<td>5.78±0.06ns</td>
<td>5.68±0.15ns</td>
<td>7.57±0.45ns</td>
<td>5.31±0.06ns</td>
</tr>
</tbody>
</table>

(Mean ± SEM ) Group II, III, IV and V Compared with Group I. 
* =Highly significant (p≤0.001), ** =Significant (p≤0.01), * =Significant (p≤0.05 ), ns= Non significant

**Blood and Serum profile of animals after the treatment**

No significant change was observed in total cholesterol, serum alanine amino transaminase, aspartate amino transaminase, acid phosphatases and alkaline phosphatases in serum of all rats after the treatment at different dose levels in comparison to control rats (data are not shown).

**Changes in Hormones level**

The extract treatment caused significantly low level of testosterone hormone (p<0.05) and LH (p<0.05) in dose dependent manner. However, no significant change was observed in rats after the treatment in FSH as compared to control. The hormone levels were found normal in rats of recovery group (Fig: 3-5).

**Effects on Histology of testes and spermatogenesis**

Histopathological observations of the testis after the *Tephrosia purpurea* treatment showed degenerated germinal epithelium of seminiferous tubules and reduced number of sperms in dose dependent manner. The histological study of control animals showed all successive stages of spermatogenesis in control animals (Photomicrograph-1). The lumen were filled with sperm, Leydig cells were present in between the tubules. *Tephrosia purpurea* treatment at 50 mg/kg (Photomicrograph-2) showed a few lesions affecting in tubules, while rats treated with 100 and 200 mg/kg/body wt/day (Photomicrograph-3,4) affected almost all tubules, however, spermatogenesis alters up to normal level in rats of recovery Groups-V (Photomicrograph-5) after recovery period.
Photomicrograph-1, Group-I (X100HE) testis of control rat showing normal spermatogenesis, lumen fill with sperms.

Photomicrograph-2, Group-II (X100 HE) of testis of rat treated at 50 mg showing degenerative changes in epithelium.

Photomicrograph-3, Group-III (X100 HE) of testis of rat treated at 100 mg showing less spermatocytes and sperms.

Photomicrograph-4, Group-IV (X100HE) of testis of rat treated at 200 mg showing reduced tubules and sperms in lumen.

Photomicrograph-5, Group-V (X 100HE) of testis of rat kept for recovery showing normal germinal epithelium.
DISCUSSION

The weight of testes and accessory reproductive organs was significantly decreased by the treatment of ethanolic fruit extract of Tephrosia purpurea as compared to control rats. In tissue biochemistry, the level of sialic acid was found significantly decreased. Sialic acid is essential for the structural integrity of acrosomal membrane of sperm. Therefore, the significantly decreased levels of sialic acid might affect the structure of spermatozoa and this may be the reason of decreased motility and fertilizing ability of sperm. The significantly declined glycogen content in testis reflects possibly decreased number of post meiotic germ cells, reflects reduced number of mature sperm in lumen. Similar results have been reported in rats earlier by\(^2\) with different plant extract treatment.

A marked reduction in sperm motility and density was observed in treated rats when compared to control animals. In mating experiments the fertility of male rats was reduced and this might be due to decreased sperm motility and density of treated rats. The decreased protein level in testis and other reproductive organs indicate suppressed male hormones level especially of androgens. A decreased level of cholesterol indicates the low synthesis of cholesterol in reproductive organs. This may be the reason behind the decreased synthesis of testosterone in testis after the treatment with different doses of plant.

Since testosterone is the most crucial for initiation, continuation of spermatogenesis and also to maintain accessory sex organs. The decreased level of testosterone indicates that the treatment suppress the synthesis of androgen level in treated animals. The testosterone level and spermatozoa production are regulated by LH and FSH. The testosterone is main androgen produced by Leydig cells under the influence of LH, LH together with testicular autocrine and paracrine factors responsible for the regulation and production male sex hormone and spermatogenesis in testis\(^2\). Since testosterone hormone play key role in male reproductive system therefore, decreased level of testosterone in rats after 60 days of Tephrosia purpurea treatment suggests antiandrogenic effects of the treatment resulted decreased no. of mature sperm due to degenerative changes in germinal epithelium and germ cells. The decreased level of testosterone in rats followed with extract treatment possibly responsible to reduced proteins, fructose and sialic acid contents\(^29,31\) in testis, epididymis and seminal vesicle; and inhibition of spermatogenesis can occur due to altered Leydig cell functions\(^32\). These results are similar with the results of with the treatment of Withanolide- A in adult male albino rats\(^33\).

CONCLUSION

It can be concluded that oral administration of 50% ethanolic extract of Tephrosia purpurea decreased fertility of male rats might be due to the decreased level of proteins, fructose and sialic acid contents; and decreased level of testosterone and LH hormones leads to degenerative changes in testis and accessory reproductive organs resulted inhibition of sperm production and motility. Further study is needed in higher animal models to observe effects and to develop a male contraceptive from Tephrosia purpurea.

ACKNOWLEDGMENT

The Head and Coordinator, Centre for Advanced Studies, Department of Zoology, University of Rajasthan, Jaipur for laboratory facilities and UGC, New Delhi for financial support are greatly acknowledged.

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