Research Article (Open access)

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

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ABSTRACT- Background: 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.

Methods: Body and organs weight of all treated animals was recorded; blood and serum were analyzed for hematological indices and clinical biochemistry. To observe the 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. The 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 the 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.

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

Key-Words: Antifertility, *Tephrosia purpurea*, Rat, Testosterone

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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[1]. However, different types of contraceptives are available to control human fertility.

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Currently available contraceptive are failed to check population and also have side effects[2]. Various adverse effects like hormonal imbalance, headache, depression, weight gain have been reported by different contraceptive users [3,4]. 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 [5,6]. In the 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 [7-9]. The plant *T. purpurea* also known as 'sharpunkha' has been used for treatment of various human diseases like, bilious febrile attack, bronchitis, boils, pimples, diarrhea, gonorrhea, heart and spleen diseases [10-12] but no intention has been paid for use of fertility regulating effects of *T. purpurea*, therefore, the present investigation was designed to observe effects on the reproductive functions and general

body metabolism of the ethanolic extract of the plant.

MATERIALS AND METHODS

Identification of the plant test material

Specimen voucher of *T. 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 plants were shade dried and then crushed mechanically. Their 50% ethanolic extract was prepared according to the WHO protocol CG-04[13].

Experimental animal model

Colony-bred, healthy, fertile male Wistar rats (*Rattus novergicus*) in the weight range of 150–200 gm were selected for 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 the Department of Zoology, University of Rajasthan, Jaipur guidelines were followed for the maintenance and experiments on animals[14].

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 was given the extract 100 mg/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 the testes, epididymides and expressed in million per ml[15].

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 cholesterol[16], serum alanine amino transaminase[17], aspartate amino transaminase[17], acid phosphatases[18] and alkaline phosphatases[19] analysis. FSH, LH and testosterone hormone level were assayed by radioimmunoassay[20].

Tissue Biochemistry

The testis, epididymis, seminal vesicles and ventral prostate were dissected out and analyzed for Protein[21], glycogen[22], cholesterol[23], sialic acid[24], ascorbic acid[25] and fructose[26] 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 hematology and serum biochemistry showed no significant changes, which mark the non-toxic action of the extract treatment on the metabolism of treated rats.

Effect on the body and reproductive organ 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 the body and organs found normal in the rat of recovery groups (Table 1).

Table 1: Effects of *T. purpurea* on Body and Organ weight on treated male rats

Treatment	Body Weight (gram)		Organ Weight (mg/100 gm.b.wt.)			
	Initial	Final	Testes	Seminal Vesicle	Cauda	Caput
Group-I	134.37±2.39	159.37±2.74	778.75±6.70	446.50±5.43	66.45±2.65	76.75±2.11
Group-II	138.75±2.63 ^{ns}	162.50±2.50 ^{ns}	661.87±5.17***	406.37±2.57***	62.86±1.90 ^{ns}	70.50±2.42
Group-III	147.90±2.83*	169.37±3.07 ^{ns}	594.50±2.27***	383.25±2.19***	47.60±1.02***	65.12±0.95**
Group-IV	138.12±1.87 ^{ns}	165.62±2.57 ^{ns}	588.91±2.21***	372.59±1.32***	42.93±0.88***	60.91±0.61***
Group-V	137.50±2.11 ^{ns}	155.00±2.31 ^{ns}	784.74± 4.54 ^{ns}	442.49±4.11 ^{ns}	62.87±0.69 ^{ns}	75.95±1.96 ^{ns}

(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 the recovery period in recovery group (Fig. 1-2).

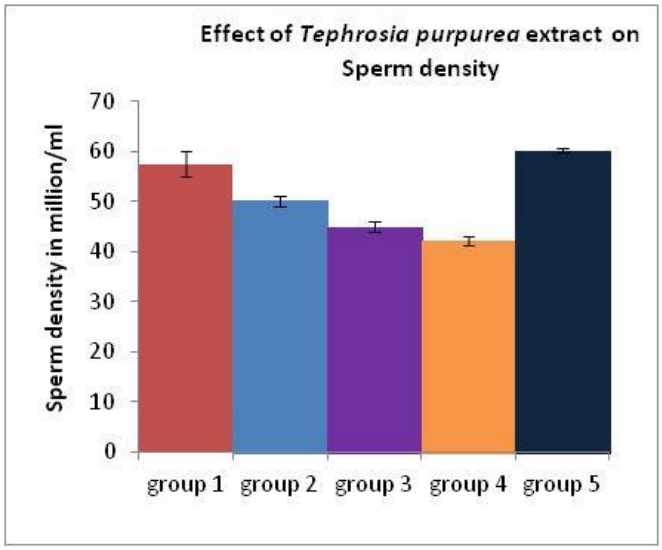


Fig. 1: Effects of *T. purpurea* extract on Sperm density

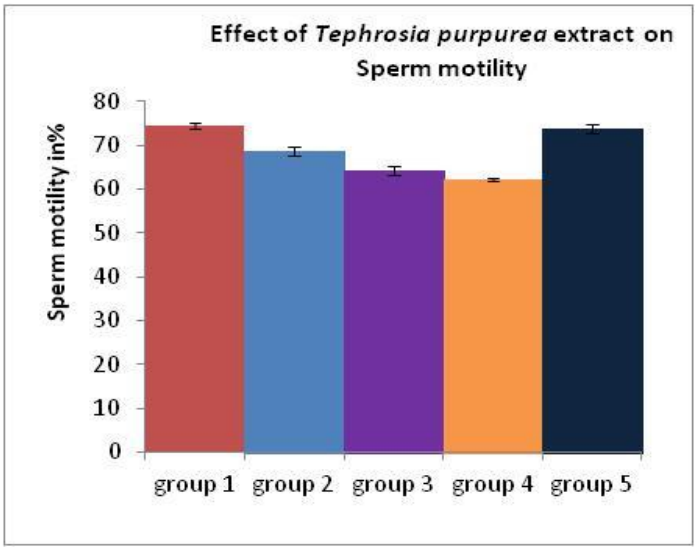


Fig. 2: Effects of *T. purpurea* extract on Sperm motility

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 2: Effects of *T.purpurea* on tissue biochemistry on treated male rats

	Protein (mg/gm)		Sialic Acid (mg/gm)		Cholesterol (mg/gm)	Fructose (mg/gm)	Ascorbic Acid (mg/gm)	Glycogen (mg/gm)
	Testis	Cauda	Testis	Cauda	Testis	Seminal Vesicle	Adrenal	Testes
Group-I	244.16±7.60	269.81±7.70	5.86±0.12	5.97±0.10	7.80±0.41	5.29±0.13	5.32±0.66	7.40± 0.52
Group-II	217.74±1.45*	238.99±3.80**	4.33±0.20***	5.32±0.13**	7.82±0.45^{ns}	4.94±0.26^{ns}	5.95±0.54^{ns}	6.86± 0.51^{ns}
Group-III	197.83±1.41***	236.17±2.05**	3.73±0.11***	4.46±0.13***	7.76±0.50^{ns}	4.54±0.26*	5.32±0.24^{ns}	7.39±0.38^{ns}
Group-IV	190.45±0.67***	230.18±0.93**	3.49±0.13***	4.35±0.08***	6.52±0.23*	4.30±0.10***	5.23±0.04^{ns}	6.23± 0.15^{ns}
Group-V	242.82±3.73^{ns}	268.24±0.65^{ns}	5.78±0.06^{ns}	5.68±0.15^{ns}	7.57±0.45^{ns}	5.31±0.06^{ns}	5.20±0.06^{ns}	6.76± 0.62^{ns}

(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 the 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 a 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 in recovery group (Fig: 3-5).

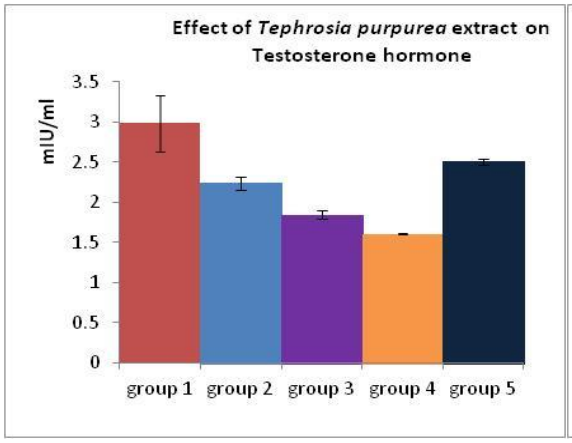


Fig. 3: Effects of *T. purpurea* extract on Testosterone hormone

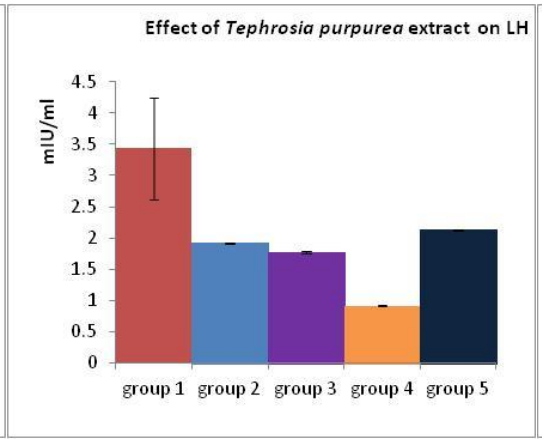


Fig. 4: Effects of *T. purpurea* extract on LH

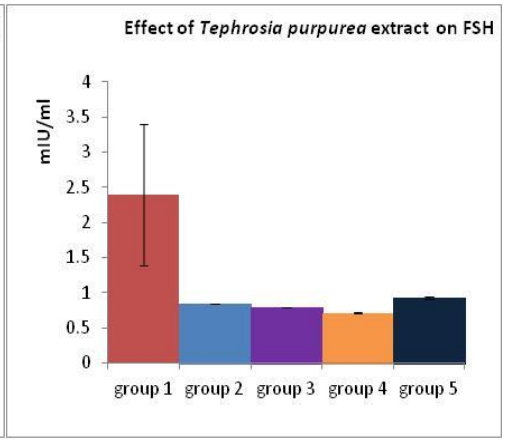
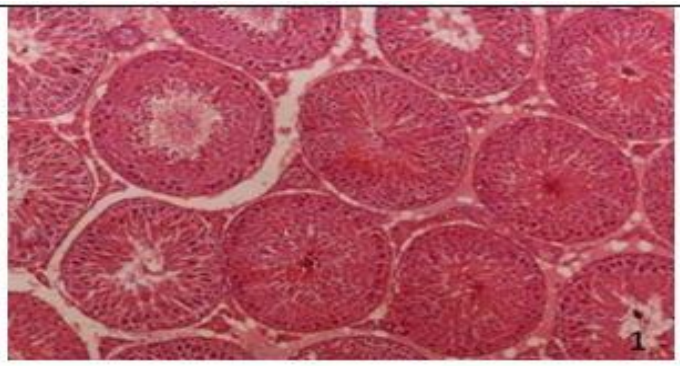


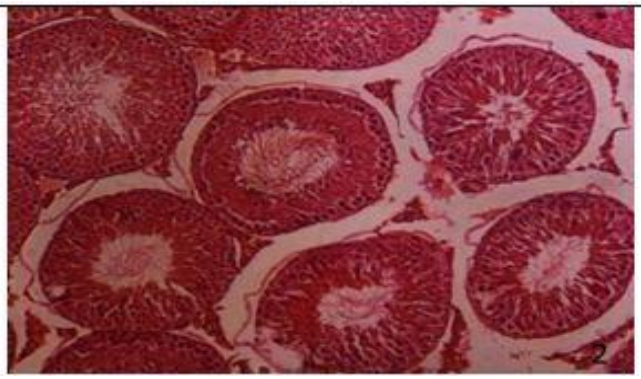
Fig. 5: Effects of *Tephrosia purpurea* extract on FSH

Effects on Histology of testes and spermatogenesis

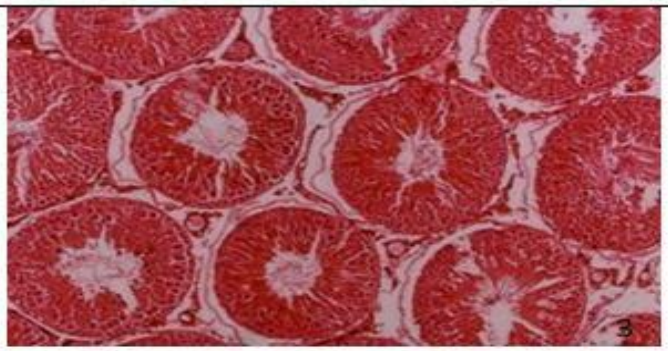
Histopathological observations of the testis after the *T. purpurea* treatment showed degenerated germinal epithelium of seminiferous tubules and reduced number of sperms in dose dependent manner. The histological study of control animals showed all successive stages of spermatogenesis in control animals (Photomicrograph-1).The lumen were filled with sperm, Leydig cells were present in between the tubules. *T. 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 the recovery period.



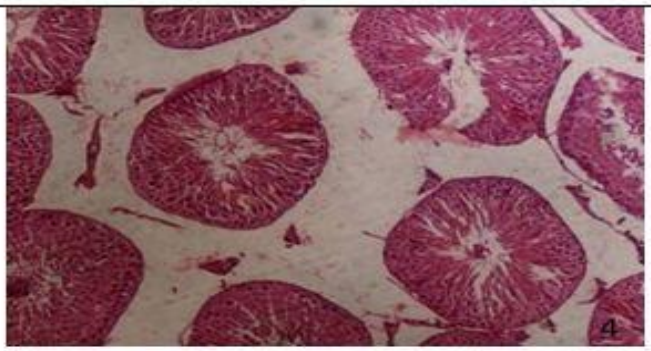
Photomicrograph-1,Group-I)(X100HE) testis of control rat showing normal spermatogenesis, lumen fill with sperms



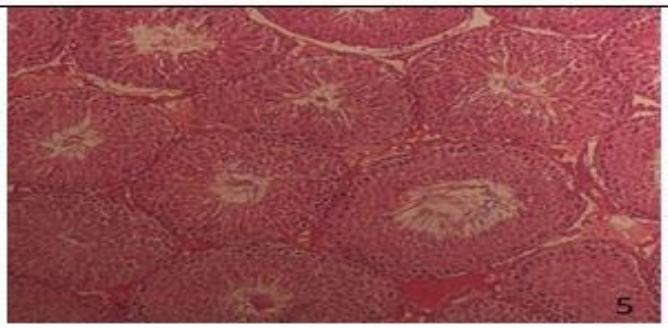
Photomicrograph-2,Group-II)(X100 HE) of testis of rat treated at 50 mg showing degenerative changes in epithelium



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 an ethanolic fruit extract of *T. 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 the 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 the lumen. Similar results have been reported in rats earlier by Chaturvedi *et al.* [27] 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 level of protein in testis and other reproductive organs indicate suppressed male hormone 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 the plant.

Since testosterone is the most crucial for the 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 the 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 of male sex hormone and spermatogenesis in testis[28]. Since testosterone hormone play key role in the male reproductive system therefore, decreased level of testosterone in rats after 60 days of *T. 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 by extract treatment possibly responsible to reduce 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 to the results of with the treatment of Withanolide- A in adult male albino rats[33].

CONCLUSIONS

It can be concluded that oral administration of 50% ethanolic extract of *T. purpurea* decreased fertility of male rats might be due to the decreased level of proteins, fructose and sialic acid contents; and decreased the levels 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 *T. purpurea*.

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