CONTRACEPTIVE EFFECT OF *TERMINALIA BELLIRICA* (BARK) EXTRACT ON MALE ALBINO RATS

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Summary

To evaluate the contraceptive effect of *Terminalia bellirica* barks extract in male albino rats. The benzene and ethanol extract of *T. bellirica* barks at the doses of 10mg and 25mg/100g body weight was administered orally for 50days to adult male albino rats. On day 51, the rats were sacrificed and the testis and accessory reproductive organs were removed and weighed. The organs were processed for biochemical estimation and histological evaluation. The treatment with *T. bellirica* barks extract resulted in decrease in the weights of testis and accessory reproductive organs. The diameter of testis, somniferous tubules and Leydig cells nuclear were decreased. The spermatogenic elements like, spermatogonia, spermatocytes and spermatids in the testis were reduced significantly as well as sperm count in cauda epididymis. Total cholesterol content is increased while protein and glycogen content were significant reduction in the testis of extracts treated rats compared with the control. *T. bellirica* barks extract arrest spermatogenesis in male rats without side effects.

Keywords: *Terminalia bellirica*, Testis, Reproduction, Spermatogenesis, Rat.

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Introduction

Terminalia bellirica is large deciduous tree. It belongs to Combertaceae family and available in the forest of India, Burma and Ceylon expect in the dry and arid region of Sind and Rajputana. The bark has medicinal value in anemia and leucoderma [1]. The fruit is one of the three constituents of the important Indian Ayurvedic preparation 'triphala'. Antifertility effects of *Terminalia* species have been reported on mammals [2-5]. The present study was undertaken to evaluate the contraceptive effect of *T. bellirica* barks extract on testicular function of rats at an aim of developing on male fertility regulating agent of plant origin.

Material and methods

Plant material

The barks of the *Terminalia bellirica* were collected from around and near P.G centre of Gulbarga University, Gulbarga, Nandihalli camp, Sandur (Karnataka, INDIA)

Extraction

The barks were shade dried, powdered and subjected to soxhlet extraction using successively and separately non polar to polar solvents i.e., the benzene and ethanol (95%). The decoction obtained each time was evaporated under reduced pressure bellow 45° C. The dried mass was considered as the extract and individually screened for contraceptive effect in albino rats. For administration to test the animals the extract were macerated in Tween-80 (1%) and resuspended in distilled water for their complete dissolution.

Animals

Adult healthy virgin albino male rats (Wistar strain) of 60-70 days old and weighing 150-180g selected from the inbreed animal colony were used for the experiment. The animals were maintained under uniform husbandry conditions of light, temperature with free access to standard and diet as prescribed CFTRI, Mysore, INDIA and tap water *ad libitum*. The animals were divided in to five groups each group's six animals.

Treatment

After preliminary trials, 10mg and 25mg/100g body weight dose levels were selected for evaluating the effects of the crude drugs. The animals were divided into 5 groups each group contain six animals and treated orally using intragastric catheter every day for 50 days are shown below.

Group I: Treated with 0.1ml Tween-80 (1%) orally and served are controls.

Group II: Treated with 10mg/100g body weight of benzene extract in 0.1ml Tween-80(1%) orally.

Group III: Treated with 25mg/100g body weight of benzene extract in 0.1ml Tween-80 (1%) orally.

Group IV: Treated with 10mg/100g body weight ethanol extract in 0.1ml Tween-80 (1%) orally.

Group V: Treated with 25mg/100g body weight of ethanol extract inn 0.1ml Tween-80 (1%) orally.

Autopsy schedule

After 24h of last treatment of respective duration, the animals were weighed and sacrificed by cervical dislocation.

Data Collection

The testes were dissected out, blotted for of blood and carefully made free from surrounding fat and connective tissues they weighed upto the nearest milligram on electronic balance. The organs from one side of each animal were fixed in Bouin's fluid for histological evaluation. The tissues were embedded in paraffin section at 5mm stained with haematoxylin-eosin[6]. The organs from the other side were processed for biochemical estimation like protein[7], cholesterol[8] and glycogen[9].

The micrometric measurements such as diameter of testes and seminiferous tubules were calculated by the method described by Deb et al.,[10]. Spermatogenic elements count[11] was made from randomly chosen 20 round cross section taken from the middle part of the testis.

Statistical analysis

The mean and standard error of mean (SEM) were calculated and the significance of difference analyzed by applying Student's *t*-test as described by Snedchor[12].

Results

Effect on body weight (Table: 1)

During the period of experimental, the rat's kept healthy, growing at normal growth rate. The body weight gain was similar to that of control animals.

Changes in the testis

Gravimetric and Histometric changes (Table: 2 & 3)

Administration of the 10mg/100g body weight of benzene and ethanol extract decreased the weight of testis nonsignificantly, where 25mg/100g body weight of benzene and ethanol extract has reduced the weight of testis significantly ($P<0.05$).

Non significant reduction in he diameter of testis due to the administration of 10mg/100g body weight of benzene extract, whereas, 25mg of benzene extract and both the doses of ethanol extract reduced the diameter of testis significantly (P<0.01).
Similarly the diameter of semiriferius tubules was reduced nonsignificantly with the dose level of 10mg and almost significantly (P<0.05) with 25mg of benzene extract. However, 10 and 25mg of ethanol extract reduced the diameter of semniferous tubules significantly (P<0.01).
The diameter of Leydig cell nucleus is reduced non significant with 10mg and almost significant (P<0.05) with 25mg of benzene extract administration. However, the ethanol extract treated rats showed reduction in the diameter of Leydig cell nucleus almost significant (P<0.05) with 10mg and highly significant (P<0.01) with 25mg dose level.

Table 1. Changes in the body weight due to administration of various extracts of *Terminalia bellirica* barks.

Treatment	Dose (mg/100g body weight)	Initial body weight	Final body weight	Percent change
Control	**0.1ml Tween-80 (1%)**	155.05 ± 0.71	178.04 ± 0.39	15.34
Benzene extract	**10**	154.84 ± 1.02	172.02 ± 2.18	11.29
	25	156.05 ± 0.76	170.42 ± 1.93	9.20
Ethanol extract	**10**	152.09 ± 1.24	170.21 ± 2.18	9.44
	25	157.21 ± 1.98	168.48 ± 2.62	7.17

Duration: 50 days; Values are mean ± S.E.
Six animals were maintained each group,
*P<0.05, *P<0.01, **P<0.001, when compared to control

Changes in the spermatogenesis elements (Table: 3)

In the histological sections of the testis do not show any significant change after the treatment of 10mg benzene extract, where as 25mg of benzene extract reduced the number of spermatogonia (P<0.05), spermatocytes (P<0.05) and spermatids (P<0.01) significantly. The administration of 10mg ethanol extract reduced the number of spermtogonia (P<0.05), spermatocytes (P<0.01) and spermatids (P<0.01) significantly, where as with 25mg of ethanol extract reduced the number of spermatogonia (P<0.01), spermatocytes (P<0.001) and spermatids (P<0.001) significantly.

Table 2. Gravimetric and histometric changes in the testis due to the administration of various extracts of *Terminalia bellirica* barks.

Treatment	Dose (mg/100g body weight)	Testis (mg)	Diameter (µm)		
			Testis	Seminiferous tubules	Leydig cell nucleus
Control	**0.1ml Tween-80 (1%)**	1663.00 ± 43.46	6150.00 ± 20.00	308.62 ± 3.91	1.96 ± 0.04
Benzene extract	**10**	1628.08 ± 28.04	6097.00 ± 10.50	302.21 ± 5.62	1.82 ± 0.03
	25	1563.83 ± 27.66	5992.00 ± 21.80**	296.95 ± 0.73*	1.78 ± 0.06*
Ethanol extract	**10**	1605.46 ± 21.24	5965.00 ± 34.00**	280.26 ± 6.31**	1.76 ± 0.09*
	25	1545.16 ± 28.32*	5750.08 ± 77.39**	260.64 ± 2.08**	1.14 ± 0.10**

Duration: 50 days;
Values are mean ± S.E.
Six animals were maintained each group
*P<0.05, *P<0.01, **P<0.001, when compared to control

Table 3. Histological changes in the testis due to the administration of various extracts of *Terminalia bellirica* barks.

Treatment	Dose (mg/100g body weight)	Number of Spermatogonic Elements			No. of Leydig cells/section
		Spermatogonia	Spermatocytes	Spermatids	
Control	**0.1ml Tween-80 (1%)**	103.60 ± 5.30	162.70 ± 6.30	102.80v ± 2.50	208.33 ± 2.99
Benzene extract	**10**	92.21 ± 2.18	158.21 ± 4.29	92.91 ± 5.21	198.41 ± 1.62*
	25	87.16 ± 0.79*	146.00 ± 1.39*	80.80 ± 4.55**	195.00 ± 1.30**
Ethanol extract	**10**	82.29 ± 0.54*	132.18 ± 2.41**	72.86 ± 6.91**	182.00 ± 6.28**
	25	67.38 ± 5.51**	91.92 ± 1.11***	51.64 ± 3.42***	110.00 ± 10.20***

Duration: 50 days;
Values are mean ± S.E.
Six animals were maintained each group
*P<0.05, *P<0.01, **P<0.001, when compared to control

Change in the Leydig Cells (Table: 3)

The number of Leydig cells were reduced almost significantly ($P<0.05$) with the administration of 10mg and significantly ($P<0.01$) with 25mg of benzene extract, where as the ethanol extract administration reduced the number of Leydig cells significantly ($P<0.01$) with 10mg and highly significant ($P<0.001$) with 25mg of dose level.

Histological changes (Figure: 1-3)

In the histological sections of the testis, a significant reduction in the number of spermatogonia, spermatocytes and spermatids were observed. Necrosis in tubular epithelium, shrinkage of sertoli cells was recorded. No spermatozoa were observed in the lumen of seminiferous tubules in the ethanol extract treated rats. The Leydig cells in the benzene and ethanol extract of both the dose received groups were significantly degenerated.

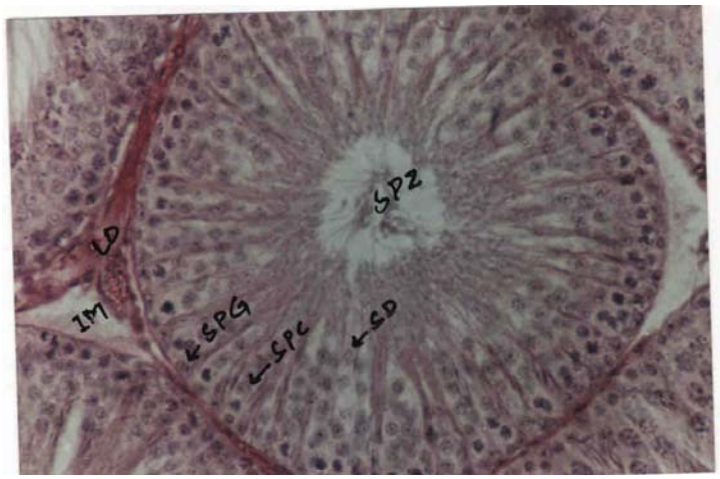

Fig: 1: Cross section of the testis of control rat showing normal spermatogenic activity and with the presence of all types of spermatogenic and Leydig cells.

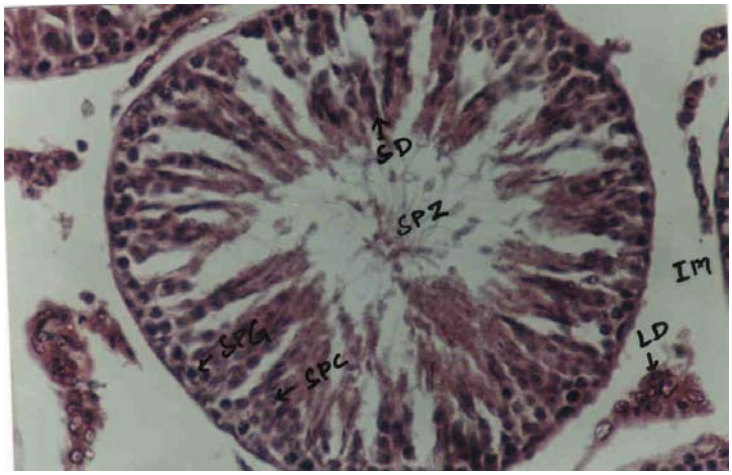

Fig: 2: Cross section of the testis rat treated with benzene extract of *Terminalia bellirica* barks showing reduction in the tubular diameter, number of spermatogenic elements and degenerating Leydig cells.

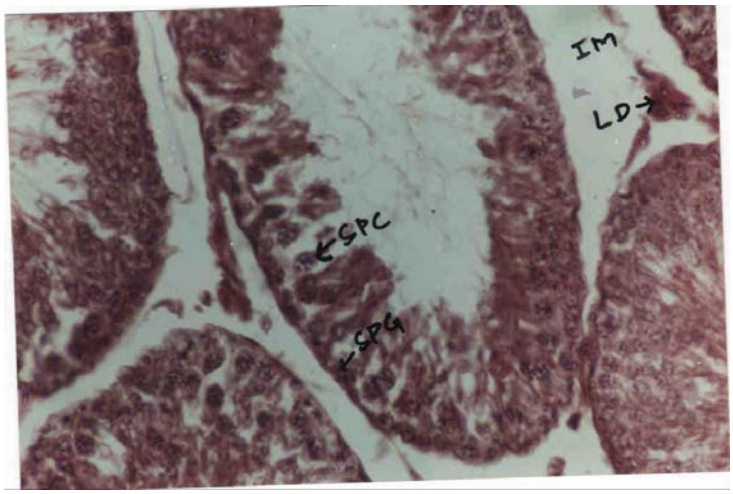

Fig: 3: Cross section of the testis of rat treated with ethanol extract *Terminalia bellirica* barks showing reduction in the tubular diameter, number of spermatogenic elements and enhanced degeneracy of Leydig cells.

IM: Interstititium; **LD**: Leydig cells; **SPG**: Spermatogonia;

SPC: Spermatocytes; **SD**: Spermatids; **SPZ**: Spermatozoas

Biochemical Changes (Table: 4)

There is significant (P<0.05) decrease in the protein and glycogen content of testis due to the 10 and 25mg of benzene extract, where cholesterol content is increased significantly (P<0.05) with both the dose of benzene extract administration. The administration of ethanol extract decreased significantly (P<0.01) protein and glycogen content of testis with 10mg and highly significant (P<0.001) with 25mg of dose level. The total cholesterol content is highly significant (P<0.001) increase with both the dose of ethanol extract treatment.

Discussion

Significant steps have been taken by the world health organization (WHO) to carry out research aimed of finding new and effective fertility regulating agents from plants. As mentioned earlier, most of the research mentioned in the literature is aimed toward male fertility regulation.

In the list provided by Bhargava[13], extracts from 19 plants have shown contraceptive effects in various animals.

The studies on *Crotalaria juncea* L., seeds have been shown to reduce circulating testosterone levels and secretary activity of the accessory organs resulting in the decrease in the volume of semen[14-16.] The effect of short administration of *Momordica charantia* seeds has brought reduction in sperm count and motility and sexual behaviors in male rats[17]. The treatment of various extracts of *Hibiscus rosa sinensis* flowers has shows antispermatogenic and antiandrogenic activity in male rats[18, 19]. These studies indicate that the extracts of these plants may inhibit the gonadotrophin release from the pituitary, so that the necessary gonadotrophin (FSH) which is essential for spermatogenesis is not released.

In the present study the contraceptive effect reveals the anispermatogenic activity has been indicated by the significant reduction in weight of testis and their diameter, number of spermatogonic elements like spermatogonia, spermatocytes and spermatids were decreased, suggesting indirectly the inhibition or non availability of pituitary gonadotrophins, specifically follicle stimulating hormone , which is essential for spermatogenesis[20, 21].

Increases in the cholesterol level and sudanophilic lipid indicates the non utilization of these precursors for steroidogenesis which may be due to an inhibition in the availability of gonadotrophins such our ICSH/LH or FSH that are necessary to stimulate the germinal epithelium[22].

Table 4. Biochemical changes in the testis due to the administration of various extracts of *Terminalia bellirica* barks.

Treatment	Dose (mg/100g body weight)	Testis (mg)	Protein (µg/mg)	Cholesterol	Glycogen
Control	**0.1ml Tween-80 (1%)**	1663.00 ± 43.46	64.02 ± 2.24	7.24 ± 0.81	10.28 ± 0.87
Benzene extract	**10**	1628.08 ± 28.04	56.40 ± 4.21*	8.42 ± 1.21*	9.21 ± 0.92*
	25	1563.83 ± 27.66	45.82 ± 6.63*	10.21 ± 1.72*	8.72 ± 0.62*
Ethanol extract	**10**	1605.46 ± 21.24	42.30 ± 9.21**	22.42 ± 4.50***	7.00 ± 0.18**
	25	1545.16 ± 28.32*	33.24 ± 5.88***	31.21 ± 0.25***	4.42 ± 0.39***

Duration: 50 days;
Values are mean ± S.E.
Six animals were maintained each group
*P<0.05, *P<0.01, **P<0.001, when compared to control

The glycogen content in the cells indicates energy storage sertoli cells and spermatogonia often contain glycogen, secrete substrates from the blood and provide source of reserve carbohydrate for seminiferous tubular cells, and the glycogen level has been found to be directly proportional to the steroid hormones[23]. The decreased glycogen content of the testis after the administration of *T. bellirica* bark extract might be correlated with decreased spermatogenic number due to reduced energy source for spermatogenic activity. Moreover, testosterone plays a pivotal role in sexual maturation, behavior and maintenance of accessory sex organs[24]. As the administration of bark extract has caused reduction in the spermatogenesis, steroidogenesis and androgen production it may alter the sexual behavior and may cause antifertility. Among the two extracts tested, ethanol extract at the dose level of 25mg/100g body weight is more effective in causing antispermatogenic and antisteroidogenic activities.

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