Editorial Article

EFFECT OF *TERMINALIA BELLIRICA* BARKS EXTRACTS ON ACTIVITIES OF ACCESSORY REPRODUCTIVE DUCTS IN MALE RATS.

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ABSTRACT

Adult male rats were administered with 10mg and 25mg/100g body weight of benzene and ethanol extracts of *Terminalia bellirica* barks orally for 50 days. Epididymis, vas deferens was dissected out, weighed and processed for biochemical estimation. The treatment with *T. bellirica* barks extracts resulted in decreased in the weight of accessory reproductive ducts. The total cholesterol content is increased while protein content and epididymal sperm count were significantly decreased. These changes may be due to non-availability of androgens in *T. bellirica* barks extracts treated rats.

Key words: *Terminalia bellirica,* Epididymis, Vas deferens, Rats

INTRODUCTION

The epididymis and the other accessory glands of reproduction in mammals depend on testicular androgens for the maintenance of their structural and functional integrity[1]. Cavazos and Melampy[2] have observed that epididymis of rat may need higher levels of androgens for maintaining its weight and secretary activity than the other accessory glands. Testosterone is necessary for the maintenance of cellular secretary activity of the epididymis[3].

The developmental changes of the sperm initiated in the testis continued during its sojourn in the epididymis[4,5], where they acquire the potentiality to fertilize the eggs. In the rat, this is achieved in the caput and corpus epididymis[6]. The vas deferens in addition to its role in transport of spermatozoa is also involved in maturation and survival of spermatozoa[7].

Various plants and their active principles have been extensively tested for spermatogenesis and accessory reproductive organs in different animals[8], *Terminalia bellirica* (Combertaceae) is used Indian Ayurvedic Medicine states where in its various parts have properties like analgesic, antidiuretic, anti-inflammatory, emmenogogue, abortifacient and also treatment for skin diseases[9,10]. 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[11-16]. The barks of this plant other species *Terminalia arjuna* has been reported to possess antifertility activity[17]. However, attention has been given in this modern era and attempts have been made to bring out safe, effective plant preparations as ideal contraceptive for males.

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 epididymis, vas deferens, prostate gland, seminal vesicle and Levator Ani were dissected out, blotted for of blood and carefully made free from surrounding fat and connective tissues they weighed up to the nearest milligram on electronic balance. The cauda epididymal sperm suspension was prepared in normal saline and sperm count from cauda epididymis were done by using haemocytometer[16] and the epididymis and vas deferens organs were processed for biochemical estimation like protein[17] and cholesterol[18].

Statistical analysis

The mean and standard error of mean (SEM) were calculated and the significance of difference analysed by applying "Student's *t*-test" as described by Snedchor[19].

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 Accessory Reproductive organs

Changes in the Epididymis

Gravimetric changes (Table 2.)

The weight of caput and cauda epididymis is decreased significantly (p<0.05) with the administration of 10 and 25mg of benzene extracts, whereas highly significantly (p<0.00) reduction with 10 and 25mg of ethanol extracts administration when compared to controls.

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

Table 2. Gravimetric changes in the accessory reproductive organs and sperm count of cauda epididymis due to the administration of various extracts of *Terminalia bellirica* barks.

Treatment	Dose (mg/100g body wt)	Epididymis		Vas deferens	Prostate	Seminal vesicle	Levator ani	Sperm count (Millions/cauda)
		Caput	Cauda					
Control	**0.1ml Tween-80 (1%)**	281.00 ± 5.01	273.30 ± 4.49	106.50 ± 2.40	71.00 ± 0.96	481.50 ± 8.64	250.50 ± 5.51	2.00 ± 0.40
Benzene extract	**10**	260.72 ± 10.24	258.42 ± 12.01	102.25 ± 8.42	68.04 ± 4.28	460.94 ± 12.40	244.61 ± 10.48	1.20 ± 0.96*
	25	259.16 ± 4.69*	260.66 ± 1.02*	99.50 ± 0.76*	62.83 ± 1.05**	450.33 ± 0.84*	232.83 ± 0.79*	0.98 ± 0.90*
Ethanol extract	**10**	254.01 ± 9.21*	240.19 ±4.21**	92.25 ±6.21**	58.28 ± 2.10**	448.72 ± 4.28*	226.42 ± 6.28*	0.85 ± 0.60***
	25	193.33±3..31***	173.00±3.31***	88.66±1.02***	42.50±3.05***	395.16±1.78***	202.00±4.15***	0.80 ± 0.12***

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 Sperm morphology and number (Table 2.)

The cauda epididymal sperms of control rats shows sickle shaped head and straight tailpiece. But in *T. bellirica* barks extracts administrated rat sperms are abnormal as their head region reduced and the tail is wrinkled or coiled. A significant reduction (p<0.05) in the sperm count of cauda epididymis with 10 and 25mg of benzene extract and highly significant (p<0.001) reduction with 10 and 25mg of ethanol extract is observed.

Changes in Vas deferens

Gravimetric changes (Table 2.)

A non significant decrease with both the doses of benzene extract and significant decrease ($p<0.01$) with both the doses of ethanol extract treated rats is observed in the weight of vas deferens.

Gravimetric changes of Prostate gland (Table 2.)

The weight of prostate gland showed non significant decrease with 10mg and significant ($p<0.01$) decrease with 25mg of benzene extract treated rats. Whereas, significantly ($p<0.01$) decreased with 10mg and highly significant ($p<0.001$) due to 25mg of ethanol extract administration.

Gravimetric changes of Seminal vesicle (Table 2.)

The weight of seminal vesicle showed non significant with 10mg and significant ($p<0.05$) reduction with the 25mg of benzene extract treatment. However, significant reduction observed with 10mg ($p<0.05$) and highly significant with 25mg ($p<0.001$) of treatment of ethanol extract.

Gravimetric changes of Levator Ani (Table 2.)

The weight of Levator ani showed non significant and significant ($p<0.05$) reduction is observed with the respective treatment of 10 and 25mg of benzene extract. More advanced reduction is observed with 10mg ($p<0.05$) and 25mg ($p<0.001$) of ethanol extract administration of *T. bellirica* barks.

Biochemical changes of Epididymis (Table 3.)

Protein: There is significant ($p<0.05$) decrease in the protein content of caput and cauda epididymis due to 10 and 25mg of benzene extracts treatment, but it is highly significant ($p<0.001$) due to administration of 10 and 25mg of ethanol extract.

Cholesterol: The total cholesterol content of caput and cauda epididymis is increased significantly ($p<0.05$) with cholesterol 10 and 25mg of benzene extracts and highly significant ($p<0.001$) with cholesterol 10 and 25mg of ethanol extract administration when compared to control group.

Table 3. Biochemical changes in the epididymis, due to the administration of various extracts of *Terminalia bellirica* barks.

Treatment	Dose mg/100g body weight)	Epididymis			
		Caput		Cauda	
		Protein (µg/mg)	Cholesterol (µg/mg)	Protein (µg/mg)	Cholesterol (µg/mg)
Control	0.1ml Tween-80 (1%)	60.03 ± 1.06	0.69 ± 0.20	57.40 ± 2.08	0.83 ± 0.02
Benzene extract	10	58.10 ± 2.90	0.72 ± 0.10	56.80 ± 1.12	0.98 ± 0.14
	25	52.40 ± 2.00*	1.04 ± 0.22	53.30 ± 1.08	1.20 ± 0.50
Ethanol extract	10	47.10 ± 4.03*	2.08 ± 0.25**	49.50 ±2.01*	2.10 ± 0.43*
	25	37.80 ± 2.08***	2.79 ± 0.40**	30.20 ± 2.03***	2.28 ± 0.29**

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

Biochemical changes of Vas deferens (Table 4.)

Protein: There is non significant decrease in the protein content of vas deferens due to benzene extracts treatment is observed, but it is almost significant ($p<0.05$) due to ethanol extract administration.

Cholesterol: Though the total cholesterol content of vas deferens is increased non significant with the administration of benzene extracts, but it is significant ($p<0.01$) with the treatment of ethanol extracts when compared to control.

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

Treatment	Dose mg/100g body weight)	Vas deferens	
		Protein (µg/mg)	Cholesterol (µg/mg)
Control	0.1ml Tween-80 (1%)	48.80 ± 2.05	1.02 ± 0.10
Benzene extract	10	47.90 ± 3.08	1.90 ± 0.48*
	25	43.00 ± 2.10	2.60 ± 0.76*
Ethanol extract	10	40.80 ± 1.12*	2.98 ± 0.60*
	25	32.80 ± 2.08**	3.24 ± 0.56**

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

DISCUSSION

Statistically significant reduction in the weight of accessory reproductive organs were observed in the rats treated with 10 and 25mg dose level of benzene and ethanol extract *Terminalia bellirica* barks. Structural and functional alteration in epididymis, vas deferens, prostate gland, seminal vesicle and Levator ani which is reflected by the gravimetric and biochemical results of treatment is also a clear indication of reduction in sperm count and deformation in structure. The relationship was also concluded by Vijaykumar et al.,[20-22] and Londonkar et al.,[23-25] in their treatment with various extracts and drugs in male albino mice and rats.

The male accessory reproductive organs play an important role in the sperm maturation, motility and formation of semen[4]. Spermatozoa formed in the seminiferous tubules are transported from the testis into epididymis and remain in the duct system for varying periods of time before being ejaculated[26]. During this period they acquire motility and fertilizing capacity in the epididymis[4].

The epididymis and the other accessory glands of reproduction depend on testicular androgens[1]. Testosterone being an important role for the maintenance of accessory sex organs[27]. In turn the synthesis and release of androgens depends on the availability of pituitary gonadotrophins like FSH and LH/ICHS[28, 29].

T. bellirica barks extract inhibits the release of FSH, LH and prolactin which are essential for the gonadal development and steroidogenesis in rats[30, 31]. Prolonged treatment of plant extracts decreases the weight of testis and imbalances the sperm production[32, 33]. In the present investigation, significant reduction in the weight of accessory reproductive organs is due to the non availability of androgen production by the testis of *T. bellirica* barks treated rats, as the androgen production depends on pituitary gonadotrophins. Decrease in the protein content of epididymis and vas deferens of both the extract treated rats indicates the poor growth rate. Decrease in the sperm count of cauda epididymis may be due to inhibition of the spermatogenesis due to extracts of plant treatment[20-22] which supports the above view. The increased cholesterol content observed in the epididymis and vas deferens also shown the reduction in the androgen production which depends on the pituitary gonadotrophins.

Further, it may be attributed to the lowered availability of pituitary LH/ICSH as the conversion of cholesterol to pregnanalone is dependent upon pituitary LH/ICSH[34, 35]. The reduction in the weight of prostate gland, seminal vesicle and Levator ani due to the administration of *Terminalia bellirica* barks extract may also attributed to the above reason. In conclusion, out of the two extracts tested, ethanolic extract at the dose level of 25mg/100g body weight is more prominent and effective in causing antispermatogenic and antisteroidogenic activities in male reproductive system.

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