

# Research Paper: Testicular Activity of Mice Treated With MeOH Extract of *Achyranthes aspera* Leaves



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## ABSTRACT

**Objectives:** In India control of fertility is based on the folk use of numerous Indian Traditional Plants that has been practiced for contraception from many years. Present investigation aims that, to evaluate the contraceptive potential of *Achyranthes aspera* leaves MeOH extract in male albino mice and has shown promising results of antispermatogenic, antiandrogenic and hypolipidaemic activities.

**Materials & Methods:** MeOH extract of *A. aspera* leaves at the doses of 25 mg and 50 mg/100 gm body weight was administered orally for 30 days to adult male albino mice. On day 31st, the rats were sacrificed and the testis were removed and weighed for gravimetric analysis. Organs were processed for biochemical estimation, histological evaluation and histomorphometry for testicular analysis.

**Results:** Treatment with *A. aspera* leaves extract resulted in decrease the weight of testis  $0.925 \pm 0.015$  significantly ( $P < 0.05$ ). Morphometric analysis like diameter of testis  $2554.22 \pm 18.545$  seminiferous tubules  $247.074 \pm 8.051$  and Leydig cells  $7.842 \pm 0.248$  were decreased significantly ( $P < 0.05$ ) at 50mg/100gm body weight when compare to control. Spermatogenic elements like, spermatogonia, spermatocytes and spermatids in the testis were reduced significantly ( $P < 0.05$ ) as well as Leydig cells count in testis when compared to control. Biochemical analysis resulted as, protein, glycogen, and cholesterol content were decreased significantly ( $P < 0.05$ ) in testis of extracts treated mice, whereas alkaline phosphatase increased and acid phosphatase decreased when compared with that of control.

**Conclusion:** MeOH extract of *A. aspera* leaves arrest the spermatogenesis and inhibited testicular function in male mice without side effects to act as potential contraceptive.

## 1. Introduction

Indian traditional herbal medicines have a strong conventional or conceptual base and

the potential to be useful as drugs in terms of safety and effectiveness leads for treating different diseases. World Health Organization has made an attempt to identify all medicinal plants used globally and listed more than

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20,000 species [1]. According to the WHO more than 80 % of the world's population relies on traditional herbal medicine for their primary health care [2]. Fertility control through medicinal plants and natural products is being given great attention by WHO these days. A large number of plants are known to have antifertility activity and contraceptive potential [3-5].

The present investigation focused on MeOH extract of *Achyranthes aspera* leaves to evaluate some indicators for contraception such as testicular activities like anti-spermatogenic/ spermatogenic and antiandrogenic/ androgenic in male albino mice. Current literature of this plant shows that, as a indigenous system of medicine act as emmenagogue, antiarthritic, antifertility, laxative, ecboic, abortifacient, antihelminthic, aphrodisiac, antiviral, antiplasmodic, antihypertensive, anticoagulant, diuretic and antitumor [6, 7]. Shibeshi et al. [8] reported MeOH extract at dose level of 1 gm/kg and 1.6 gm/kg body weight of *A. aspera* leaves has antifertility effect and is safer contraceptive doses and also reported abortifacient activity with hormonal profile at dose level of 3 gm/kg and 5.5 gm/kg body weight [9]. Similarly, Paul et al. [10] extracts of *A. aspera* roots have been reported to possess spermicidal activity in human and rat sperm. And also study was made on hydroethanolic, n-hexane and chloroform extracts, which were found to be most effective for sperm immobilization, sperm viability, acrosome status, 5'-nucleotidase activity and nuclear chromatin decondensation. Vasudeva and Sharma [11] reported that, ethanolic extract of the root of *A. aspera* shows post coital antifertility activity in female albino rats. But so far, no one reported systematic investigation on leaves of *A. aspera* MeOH extract to know the testicular activities at lower doses on albino mice.

## 2. Materials and Methods

### Plant material

Fresh leaves of *Achyranthes aspera* were collected from in and around Gulbarga University, Gulbarga (Karnataka, INDIA) during August-October 2015 and authenticated at the herbarium, Department of Botany, Gulbarga University, Gulbarga, where voucher specimens are deposited.

### Extraction

Leaves were shade dried, powdered and subjected to soxhlet extraction of MeOH for 18-20 hours. The decoction so obtained was evaporated under reduced pressure and controlled temperature (50-60°C). Dried mass

considered as the extract, preserved at 6°C in refrigerator until used and diluted as required for experimental studies. 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 mice (Wister strain) of 6-8 weeks old and weighing 25-30 gm selected from inbred 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 five animals.

### Treatment

After preliminary trials, 25 mg and 50 mg/kg body weight dose levels were selected for evaluating the effects of the crude extracts. Animals were divided into 3 groups each group contain five animals and treated orally using intragastric catheter every day for 30 days as shown below:

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

Group II: Treated with 25 mg/kg body weight of MeOH extract in 0.1 ml Tween-80 (1%) orally.

Group III: Treated with 50 mg/1kg body weight of MeOH extract in 0.1 ml 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 and they weighed up to the nearest milligram on electronic balance (Anamed Electronic Balance, India). 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 5 mm stained with haematoxylin-eosin [12]. The organs from the other side were processed for biochemical estimation like protein [13], glycogen [14], cholesterol [15] and acid and alkaline phosphatase [16]. The micrometric measurements such as diameter of testes and seminiferous tubules were calculat-

ed by the method described by Deb et al. [17]. Spermatogenic element counts were made by randomly chosen 20 cross section taken from middle part of testis [18].

### Statistical analysis

Statistical analyses were carried out by using students t-test to find significance. Results were -judged significant if  $P < 0.05$  [19].

## 3. Results

After 30 days of MeOH extract *Achyranthes aspera* leaves treatment the mice did not show any changes in general behavior and morphology. But changes were observed in the gravimetric studies, biochemical, histological studies and kinetics of spermatogenic elements.

### Gravimetric changes in testis

Administration of the 25 mg/kg body weight of MeOH extract decreased ( $1.090 \pm 0.007$ ) the weight of testis nonsignificantly, whereas 50 mg/kg body weight of MeOH extract has reduced ( $0.925 \pm 0.015$ ) the weight of testis significantly ( $P < 0.05$ ) when compared to control ( $1.190 \pm 0.172$ ).

### Biochemical studies of testis

#### Changes in testicular protein content

In testis of the control rats, the protein content was  $1.190 \pm 0.172$   $\mu\text{g}/100\text{gm}$ . Non significant change was  $14.19 \pm 0.456$   $\mu\text{g}/\text{gm}$  observed in 25 mg/100gm body weight treated rats. Where as in 50 mg/100gm body weight treated rats shows  $11.50 \pm 0.153$   $\mu\text{g}/\text{gm}$  significant decrease ( $P < 0.05$ ) in protein content was noticed when compared to the controls (Table 1).

#### Changes in testicular glycogen content

In the testis of the control rats, the glycogen content was  $1.09 \pm 0.023$   $\mu\text{g}/100\text{gm}$ . In 25 mg/kg body weight treated rats, the glycogen content decreased nonsignificantly. However, in 50 mg/kg body weight treated rats decreased significantly ( $P < 0.05$ ) when compared to the controls (Table 1).

#### Changes in testicular cholesterol content

In the testis of the control rats, the cholesterol content was  $1.456 \pm 0.097$   $\mu\text{g}/100\text{gm}$ . In 25 mg/kg body weight treated rats, the cholesterol content decreased. However, in 50 mg mg/100gm body weight treated rats decreased

significantly ( $P < 0.05$ ) when compared to the controls (Table 1).

#### Changes in testicular alkaline and acid phosphatase content

In testis of the control rats, Alkaline phosphatase content was  $13.45 \pm 1.234$   $\mu\text{M}/\text{min}/\text{mg}$ . In 25 mg/kg body weight treated rats, Alkaline phosphatase content increased nonsignificantly. However, in 50 mg/kg body weight treated rats increased significantly ( $P < 0.05$ ) when compared to controls (Table 1).

In testis of the control rats, Acid phosphatase content was  $10.63 \pm 1.209$   $\mu\text{M}/\text{min}/100\text{mg}$ . In 25 mg/kg body weight treated rats, the Acid phosphatase content decreased nonsignificantly. However, in 50 mg mg/100gm body weight treated rats decreased significantly ( $P < 0.05$ ) when compared to controls (Table 1).

#### Changes in histopathological/metrical studies

The histometric studies of testis of control mice has shown  $2668.76 \pm 60.345$  ( $\mu\text{m}$ ), whereas in 25 mg/kg treated mice were shows  $2676.18 \pm 27.278$  ( $\mu\text{m}$ ) nonsignificant change in the diameter of the testis, but in 50 mg/kg treated mice were shows  $2554.22 \pm 18.545^*$  significant reduction ( $P < 0.05$ ) in the diameter of the testis when compared to control (Figure 1, 2, 3).

#### Diameter of seminiferous tubules

The histometric studies of seminiferous tubules of control mice has shown  $289.17 \pm 0.06$   $\mu\text{m}$ , however, diameter of seminiferous tubules were significantly reduced ( $P < 0.05$ ) in 25 mg/kg treated mice  $258.091 \pm 10.39$  ( $\mu\text{m}$ ) and 50 mg/kg treated mice  $247.074 \pm 8.051$  ( $\mu\text{m}$ ) respectively, when compared to control.

#### Diameter of leydig cells

The histometric studies of Leydig cells of control mice has shown  $10.601 \pm 0.501$   $\mu\text{m}$ , however, diameter of Leydig cells were significantly reduced ( $P < 0.05$ ) in 25 mg/kg treated mice  $8.117 \pm 0.365$  ( $\mu\text{m}$ ) and 50 mg/kg treated mice  $7.842 \pm 0.248$  ( $\mu\text{m}$ ) respectively, when compared to control.

#### Changes in spermatogenic elements

It is evident form Table 3 and Figures 1, 2, and 3, that there is significant reduction ( $P < 0.05$ ) in the number of spermatogenic elements like, spermatogonia, spermatocytes and spermatids in both the doses when compared to control mice, similarly number of Leydig cells also significantly reduced ( $P < 0.05$ ) in both the doses of MeOH extract of *A. aspera* which indicates the reduction in the

**Table 1.** Gravimetrical and biochemical changes in the testis after treatment with MeOH extract of *A. aspera* leaves for 30 days

Treatment	Dose (mg/100 mg)	Testis (g/100 mg)	Protein (µg/100 mg)	Glycogen (µg/100mg)	Cholesterol (µg/100 mg)	ALP (µM/min/100 mg)	ACP (µM/min/100 mg)
MeOH <i>A. aspera</i>	0.2	1.190±0.172	15.40±0.087	1.09±0.023	1.456±0.097	13.45±1.234	10.63±1.209
MeOH <i>A. aspera</i>	25	1.090±0.007	14.19±0.456	1.01±0.106	1.312±0.090	13.61±0.176	9.75±0.645
MeOH <i>A. aspera</i>	50	0.925±0.015*	11.50±0.153*	0.808±0.119*	0.891±0.0353*	15.25±1.456*	7.72±0.787*

\*: Significant compared to control (P&lt;0.05)

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**Table 2.** Morphometrical changes of the testis on administration of MeOH extract of *A. aspera* leaves for 30 days

Treatment	Diameter in µm		
	Testis (µm)	Seminiferous Tubules (µm)	Leydig Cells (µm)
Control	2668.76±60.345	289.17±0.06	10.601±0.501
MeOH <i>A. aspera</i> (25 mg/kg)	2676.18±27.278	258.091±10.39*	8.117±0.365*
MeOH <i>A. aspera</i> (50 mg/kg)	2554.22±18.545*	247.074±8.051*	7.842±0.248*

\*: Significant compared to control (P&lt;0.05)

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spermatozoid production by inhibiting at the stage of spermatogenesis. It is marked from the results that, extract has shown antispermatogenic, antiandrogenic and hypolipidaemic properties. Antispermatogenic activity was reflected by the cessation of spermatogenesis and arrested the development of spermatogenic elements.

#### 4. Discussion

The reduction recorded in the weight of the testis characterize that both the dose of MeOH *A. aspera* leaves extract act as toxicant described by Da Silveira et al. [20] also suggest the possibility of depletion in the level of testosterone in circulation in the experimental mice [21]. This reduction may be due to the decreased production of seminiferous tubular fluid, which contributes to the weight of testis [22].

The reduced protein content may also be another reason as the growth rate of any organ is proportional to its protein content [23]. Since evidently FSH stimulates the development of spermatogonia to spermatocytes and also maintains the spermatogenic process [24]. It shows the presence of enough energy stores for spermatogenic activity [25] and a trend towards a decrease in the glycogen content in experimental mice fed on a high dose extract of MeOH *A. aspera* leaves indicates interference in glucose metabolism affecting probably the process of spermatogenesis [26].

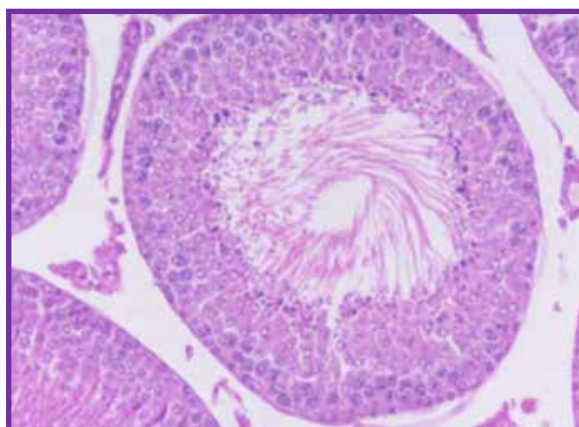
The lowered concentration cholesterol might be due to higher activity of steroid synthesis. Cholesterol the originator for androgen production in the testis is present in Leydig cells and spermatogenic cells, including spermatocytes. The elevated cholesterol level has been

**Table 3.** Effect of administration of MeOH extract of *A. aspera* leaves on the total number of spermatogenic elements and Leydig cells

Treatment	Dose (mg/kg)	Spermatogenic Elements			Leydig Cells
		Spermatogonia	Spermatocytes	Spermatids	
Control	0.2	106±2.526	121.2±3.112	72.67±8.165	22±0.108
MeOH <i>A. aspera</i>	25	90±0.312*	116.6±3.125*	30.52±4.109*	20±0.860*
MeOH <i>A. aspera</i>	50	86.3±1.142*	111±3.312*	21.75±0.793*	17.3±0.139*

\*: Significant compared to control (P&lt;0.05)

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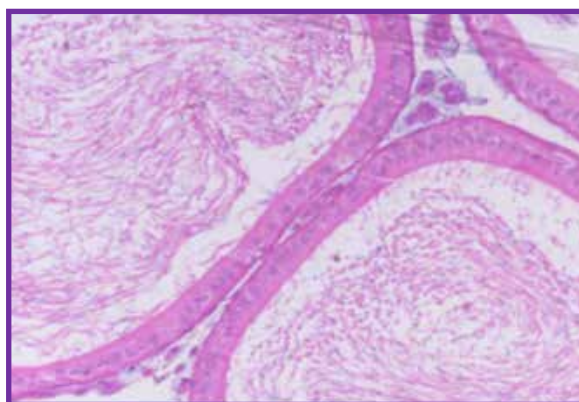


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**Figure 1.** Photomicrographs of testis histology (a) control rat showing normal seminiferous tubules with active spermatogenesis, interstitium and normal Leydig cells (×400)

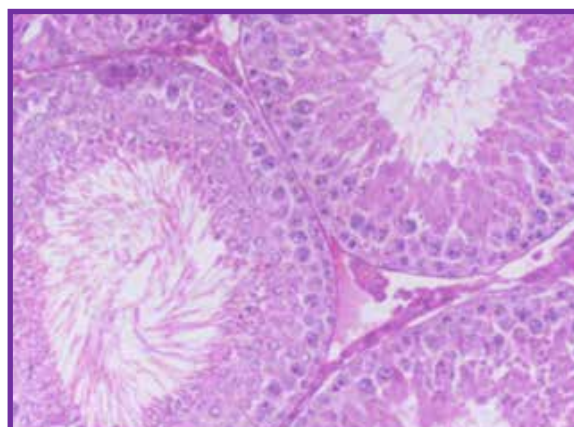
attributed to its decreased utilization for steroidogenesis which may be due to pituitary repression or a direct inhibitory action of target tissue [27]. There was a significant ( $P < 0.05$ ) reduction in the level of cholesterol in treated mice, which indicates that this drug has hypolipidaemic activity [28].

A decrease in the acid phosphatase in free state would thus reflect decreased testicular steroidogenesis in the treated rats and this may be correlated with the reduced secretion of gonadotrophins [29]. Acid and alkaline phosphatases distributed widely in the testis are important in the physiology of sperm [30]. Changes in the phosphatase activity may be an indicative of suppression of spermatogenic processes or an exchange of materials between germinal and Sertoli cells [31].



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**Figure 3.** Photomicrographs of testis histology treated with 25 mg of MeOH extract of *Achyranthes aspera* leaves note the shrinkage of seminiferous tubules, significant decrease in the number of spermatogonia, spermatocytes & spermatids and note the abnormal of spermatozoa in lumen. The germinal epithelium also showed degenerative changes and in spermatocytes nuclear pyknosis is seen (×400)



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**Figure 2.** Photomicrographs of testis histology treated with 50mg of MeOH extract of *Achyranthes aspera* leaves showing partial shrinkage of seminiferous tubules, decreased interstitium and Leydig cells. Note the nonsignificant decrease in the spermatogonial elements

These reductions in the diameter of the testis, seminiferous tubules and primary spermatogonial cells (Table 2) may be attributed to a decline in the testosterone production [32] suggesting that the extract of MeOH *A. aspera* leaves possesses antiandrogenic properties as the decrease in the number of spermatocytes and spermatids are completely androgen dependent and also arrested the production of sperm cells [33, 34]. The atrophic state of Leydig cells in the testis of treated animals may be due to declined LH secretion [35]. The number of mature Leydig cells has a direct bearing on spermatogenesis [36]. Deformation of Leydig cells further indicates the inefficiency of these cells to synthesize testosterone [37].

It is evident that FSH stimulates the development of spermatogonia to spermatocytes and also maintains the spermatogenic process [38] and both FSH and LH/ICSH are necessary for meiosis and development of spermatids [39]. The androgens are necessary to induce meiosis, formation and development of spermatids in response to FSH [40]. The observed reduction in the number of spermatogonia, spermatocytes and spermatids may indicate lowered availability of FSH and LH/ICSH, which are essential for initiation and maintenance of spermatogenesis.

## 5. Conclusion

As the administration of MeOH *A. aspera* leaves extracts has caused reduction in spermatogenesis, steroidogenesis and androgen production, it may alter the sexual behavior and may cause antifertility. Out of the two doses tested, 50 mg/kg body weight extract is more effective and potential in causing antispermatogenic, antiandrogenic and hypolipidaemic activities in albino mice.

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## Conflict of Interest

The authors declared no conflicts of interest.

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