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Antispermatogenic and antiandrogenic activities of various extracts of *Melia azedarach* Linn. seeds in albino rats

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SUMMARY

Petroleum ether, chloroform and ethanol extracts of the seeds of *Melia azedarach* Linn. administered orally to male rats at the dose level of 25 mg/100 g body weight for 48 days showed antispermatogenic activity, as the number of spermatocytes, spermatids and spermatozoa was decreased. The total cholesterol content was increased while protein and glycogen contents were decreased. The acid phosphatase content was also decreased while that of alkaline phosphatase increased. At the same time the weight of caput and cauda epididymis, prostate gland, seminal vesicle and Levator Ani muscle was decreased indicating its antiandrogenic property. Of the three extracts, the petroleum ether extract was more potent in its antispermatogenic and antiandrogenic activities and did not produce any sings of toxicity upto a dose of 25 mg/100 g body weight oral administration. After subjecting it to preliminary phytochemical screening the petroleum ether extract showed positive tests for steroids and saponins.

Key words: *Melia azedarach* Linn.; Antispermatogenic; Antiandrogenic; Antifertility

INTRODUCTION

In a UNICEF report it has been stated family planning could bring more benefits to more people than any other technology now available to the human race (Grant, 1992). In the last decade scientists working in India and worldwide have been trying to develop new strategies and technologies for better human reproductive health and fertility regulation. However, little information is available in regulation of male fertility. As an alternate approach to vasectomy, long-term contraception in the male is needed in this modern era. From Ayurvedic medicine, it is known that many plants serve as natural sources for antifertility substance, but only a few plants have so far been investigated for antispermatogenic activity in males (Chakraborthy *et al.*, 1991; Reddy *et al.*, 1997; Naseem *et al.*, 1998; Sharma *et al.*, 1999). The treatment of purified gossypol acetic acid which was isolated from cotton seeds resulted in severe oligospermia with impairment of sperm motility when administered alone or in combination with potassium chloride in langurs (Lohiya *et al.*, 1990; Sharma *et al.*, 1999). Neem oil extracted from the traditional Indian plant *Azadirachta indica* was reported to have antispermatogenic and spermicidal properties (Riar *et al.*, 1990; Purohit *et al.*, 1991; Manoranjitham *et al.*, 1993; Sharma *et al.*, 1996).

Melia azedarach Linn. (Maliaceae) which is an indigenous plant in most of the Asian countries, is cultivated extensively in India and China. Its different parts like roots, leaves, flowers, bark, seeds and their preparations have been used for medicinal purposes. In Ayurveda it is mentioned that the roots have the property of anthelmintic, removes kapha (secretion due to throat infection) and biliousness, pain in heart, blood impurities, uterine pains after delivery etc. The seeds have the property like antiseptic, antipyretic, insecticidal, repellent brain tonic, curing inflammation, emmenagogue etc. (Kirtikar and Basu, 1935; Ascher *et al.*, 1995; Valladares *et al.*, 1999). The important constituents of seeds are sterols, tannins, flavonoids, limonoids and liganans. (Lee *et al.*, 1991; Ascher *et al.*, 1995). The present study is carried out in order to explore

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the potentiality of various extracts of seeds of *M. azedarach* Linn. on male reproduction like spermatogenesis and development of accessory reproductive organs in rats.

MATERIALS AND METHODS

Plant material

M. azedarach Linn. (Maliaceae) seeds were collected from the fields of North Karnataka region during the month of March and April. This plant was authenticated in the Department of Botany, Gulbarga University, Gulbarga, India.

Preparation of the extract

The seeds were shade dried, powdered and subjected to Soxhlet extraction (1 kg) with solvents ranging from non-polar to polar, that is petroleum ether (60-80°C), chloroform and ethanol (95%) respectively. The extracts were concentrated to dryness in a flash evaporator under reduced pressure and controlled temperature. The petroleum ether extract yielded a yellow oily extract (20 g), chloroform extract yielded a brown gummy extract (15 g) and ethanol extract yielded a dark brown extract (40 g). All the extracts were prepared in tween-80 (1%) suspended in distilled water.

Phytochemical tests

The presence of various chemical constituents in petroleum ether, chloroform and ethanol extracts of *M. azedarach* Linn. were determined by preliminary phytochemical screening as described by Harborene (1973), Kokate (1985). Dregendroffs reagent was used for alkaloids, Libermann Buchard reagent for steroids, shinoda test for flavonoids, Keller kiliani test for glucosides and Ferric chloride reagent for phenols.

Mice toxicity studies

The acute toxicity study (Kattan *et al.*, 1994) of various extracts of *M. azedarach* L. were studied on 6-week old Swiss mice at a dose of 15 and 25 mg/100 g body weight/day. The extracts were prepared in Tween 80 (1%) and given orally. Tween 80 (1%) was used as control with the same oral volume as that used for the extracts. The dosing schedule used was once a day for 6 days and mice were weighed and observed for any morphological behaviour.

Animals

Colony bred male albino rats of Wistar strains, 60-70 days old were used for the experimentation. These rats were maintained under standard animal house conditions with a balanced food prescribed by CFTRI, Mysore, India, and water *ad libitum*.

Treatment

The animals were divided into four groups of five animals each. The various extracts of *M. azedarach* Linn. seeds were administered orally by using intragastric catheter at the dose level of 25 mg/100 g body weight and treated as follows: Group I received the vehicle, Tween-80 (1%) and served as control. Group II, III and IV received petroleum ether, chloroform and ethanol extracts respectively. The above treatment was given for 48 days.

Antifertility testing

All the experimental rats were sacrificed on the 49th day, one day after the last treatment. The body weight was recorded at autopsy. The testis, epididymis (caput and cauda), prostate gland, seminal vesicle, vas deferens and Levator Ani muscle were dissected out immediately after the sacrifice, trimmed off from fat and connective tissue and weighed to the nearest mg. The organs from one side of each animal were fixed in Bouin's fluid for histological studies. They were embedded in paraffin, sectioned at 5 μ, stained with Ehrlichs Haematoxylin and Eosine. The micrometric measurements like diameter of testis and seminiferous tubules were made from randomly chosen 20 sections from each group by using ocular and stage micrometer. The counting of spermatogenic elements like spermatogonia, spermatocytes and spermatids were made from these 20 round sections of each group. The sperm count from cauda epididymis was done by using haemocytometer (Kempinas and Lamano-carvaho, 1987).

Biochemical parameters

Organs from other side were processed for biochemical estimations like protein (Lowry *et al.*, 1951), cholesterol (Peters and Vanslyke, 1946), glycogen (Caroll *et al.*, 1956), acid phosphatase and alkaline phosphatase (Bassey *et al.*, 1946).

Statistical methods

The values were statistically analysed and means of different groups were compared using Students *t-test*. The values were judged as significant if $P< 0.01$ and $P< 0.001$.

RESULTS

Phytochemical screening (Table 1)

The petroleum ether and chloroform extracts have shown positive tests for steroids and saponins. The ethanolic extract has shown positive tests for steroids, flavonoids, phenolics, glycosides and saponins.

Mice toxicity study (Table 2)

The petroleum ether, chloroform extract showed no comparable weight loss after one week and appeared to be non-toxic as monitored by survival outcome. The ethanol extract exhibited comparable weight loss after one week and appeared to be presence of toxic compounds present in the extract.

Table 1. Phytochemical screening of various extracts of *Melia azedarach* Linn. seeds

Plant constituents	Petroleum ether extract	Chloroform extract	Ethanol extract
Alkaloids	-	-	+
Steroids	+	+	-
Flavonoids	-	-	+
Phenolics	-	-	+
Glycosides	-	-	+
Saponins	+	+	+

+, present; -, absent

Changes in the body weight (Table 3)

The body weight of the petroleum ether and chloroform extract treated animals shows no comparable change, but it was significant ($P< 0.01$) only in the ethanol extract treated animals.

Gravimetric changes in testis and accessory organs (Table 3)

The weight of the testis was decreased slightly in

Table 2. Effect of different extracts of *M. azedarach* Linn. seeds on toxicity of normal Swiss mice

Treatment	Dose (mg/100 g body wt.)	Initial body wt.	Final body wt.	Percentage change	Survival, alive/total (%)
Control	Tween-80 (1%)	28.60±0.82	32.00±0.48	11.11	5/5 (100%)
Petroleum ether	15	28.00±0.78	31.06±0.8	10.92	5/5 (100%)
Petroleum ether	25	28.50±0.62	30.50±0.75	7.01	5/5 (100%)
Chloroform	15	28.20±0.50	31.20±0.77	10.99	5/5 (100%)
Chloroform	25	28.00±0.70	30.10±0.81	7.50	5/5 (100%)
Ethanol	15	29.00±0.82	29.50±0.78	1.72	5/5 (100%)
Ethanol	25	28.70±0.80	27.50±0.891	-4.18	5/5 (100%)

Dose: mg/100 g body weight, Duration: 7 days, Values: mean±SE.

Table 3. Gravimetric changes in the testis and accessory organs due to administration of *M. azedarach* Linn. seeds extracts

Treatment	Body wt. (gm)	Testis	Weight of epididymis		Vas deferens	Prostate	Seminal vesicle	Levator ani
			Caput	Cauda				
Control	141.60± 2.16	1375.50± 23.08	224.80± 5.96	164.80± 4.50	89.90± 2.61	67.60± 2.83	338.20± 6.3	137.40± 4.93
Petroleum ether	137.00± 2.10	1282.60± 18.78**	146.60± 3.14**	105.00± 3.02**	54.40± 2.40**	24.40± 1.30**	124.80± 1.92**	106.50± 1.60**
Chloroform	140.00± 3.07	1310.00± 21.50	184.40± 4.00*	137.80± 3.50*	79.80± 2.86	54.00± 1.45*	298.40± 5.70*	128.00± 2.32
Ethanol	130.60*± 1.72	1296.40± 17.96	164.20± 3.60**	122.40± 2.50**	70.20± 2.80*	35.20± 1.40**	180.00± 3.64**	127.00± 2.53

Dose: 25 mg/100g body weight, Duration: 48 days, Organ weight: mg/100 g body weight. Values are mean±SE. Five animals were maintained in each group. *P<0.01, **P<0.001 when compared to control.

all treated groups, but it was significant only in the petroleum ether extract administered group. The weight of caput and cauda epididymis, prostate gland, seminal vesicle and Levator Ani muscle were reduced highly significantly with the administration of petroleum ether extract. Administrations of chloroform extract reduced the weight of both caput and cauda epidydimis, prostate gland and seminal vesicle significantly. Administration of ethanolic extract showed highly significant reduction in the weight of caput and cauda epidydimis prostate gland seminal vesicle, and significant reduction in vas deferens.

Biochemical changes in testis and accessory organs (Table 4 & 5)

Biochemical changes included decrease in the protein, glycogen and acid phospatase content and increase in the cholesterol and alkaline phosphatase content of the testis. The caput and cauda epididymal protein content was decreased and cholesterol content was increased in all the three extracts administered groups. In vas deferens the protein and glycogen content was decreased due to administration of all the extracts.

Micrometric and spermatogenic changes in testis (Table 6)

The diameter of the testis and seminiferous tubule decreased in propotion to its weight in all the extracts treated groups. But the testicular diameter was significantly decreased due to petroleum ether extract administration. The seminiferous tubules of testis treated with petroleum ether and ethanol extracts were significantly decreased in diameter. The number of spermatogenic elements like spermatogonia, spermatocytes and spermatids were decreased in all the groups received different extracts.

Sperm count (Table 6)

The cauda epididymal sperm count was decreased in all the extracts treated groups but it was significant due to petroleum ether and ethanol extract administration.

Table 4. Biochemical changes in the testis due to administration of various extracts of *M. azedarach* Linn. seeds

Treatment	Protein (mg/gm)	Cholesterol (μg/mg)	Glycogen (mg/gm)	Acid phosphatase (μmoles of p-nitro-phenol released/ 100mg/30 minutes)	Alkaline phosphatase (μmoles of p-nitro-phenol released/100mg/ 30 minutes)
Control	3.48±0.3	11.50±0.47	8.42±0.37	43.35±1.06	10.31±0.91
Petroleum ether	1.91±0.12*	33.00±2.50**	6.30±0.20*	34.39±1.09**	27.37±1.84**
Chloroform	2.32±0.22	21.70±2.12*	8.21±0.16	38.20±1.03*	19.51±1.72*
Ethanol	2.04±0.14*	28.20±2.11**	6.73±0.37*	34.57±1.04**	20.62±1.81

Dose: 25 mg/100 g body weight, Duration : 48 days. Values are mean ± SE, Five animals were maintained in each group. *P<0.01, **P<0.001 when compared to control.

Table 5. Biochemical changes in the epididymis and vas deference due to administration of various extracts of *M. azedarach* Linn. seeds

Treatment	Epididymis				Vas deference	
	Caput		Cauda			
	Protein (mg/100 gm)	Chlolesterol (μg/mg)	Protein (mg/100 gm)	Cholesterol (μg/mg)	Protein (mg/100 gm)	Glycogen (mg/100 mg)
Control	6.38±0.48	6.00±0.46	5.96±0.34	7.08±0.91	4.72±0.08	3.94±0.15
Petroleum ether	4.72±0.18*	29.40±1.60**	3.89±0.19*	24.51±2.50**	2.08±0.17*	3.15±0.11*
Chloroform	5.80±0.30	10.00±1.50*	3.86±0.19	12.91±1.81*	3.84±0.20*	3.51±0.15
Ethanol	4.54±0.04*	24.70±2.50**	3.58±0.18*	16.70±1.94**	2.90±0.15*	3.67±0.10

Dose: 25 mg/100 g body weight, Duration: 48 days, Values are mean±SE. Five animals were maintained in each group. *P<0.01, **P<0.001 when compared to control.

Table 6. Micrometric and spermatogenic changes in the testis due to administration of various extracts of *M. azedarach* Linn. seeds

Treatment	Diameter of testis (μm)	Diameter of seminiferous tubule (μm)	Spermatogonia	Spermatocytes	Spermatids	Sperm count (millions/cauda)
Control	4122.50±20.12	195.8±7.51	102.6±5.31	160.7±6.31	289.8±20.50	2.30±0.09
Petroleum ether	3273.8±18.70*	108.7±3.70*	82.3±3.90*	67.3±2.90*	38.5±2.50**	0.51±0.02*
Chloroform	3578.6±16.86	140.5±5.32	93.5±4.62	110.0±3.63	86.5±4.82*	0.98±0.07
Ethanol	3412.1±19.18	112.8±7.10*	76.5±4.61	80.7±3.83*	70.5±3.80*	0.78±0.07*

Dose: 25 mg/100 g body weight, Duration: 48 days, Values are mean±SE. Five animals were maintained in each group. *P<0.01, **P<0.001 when compared to control.

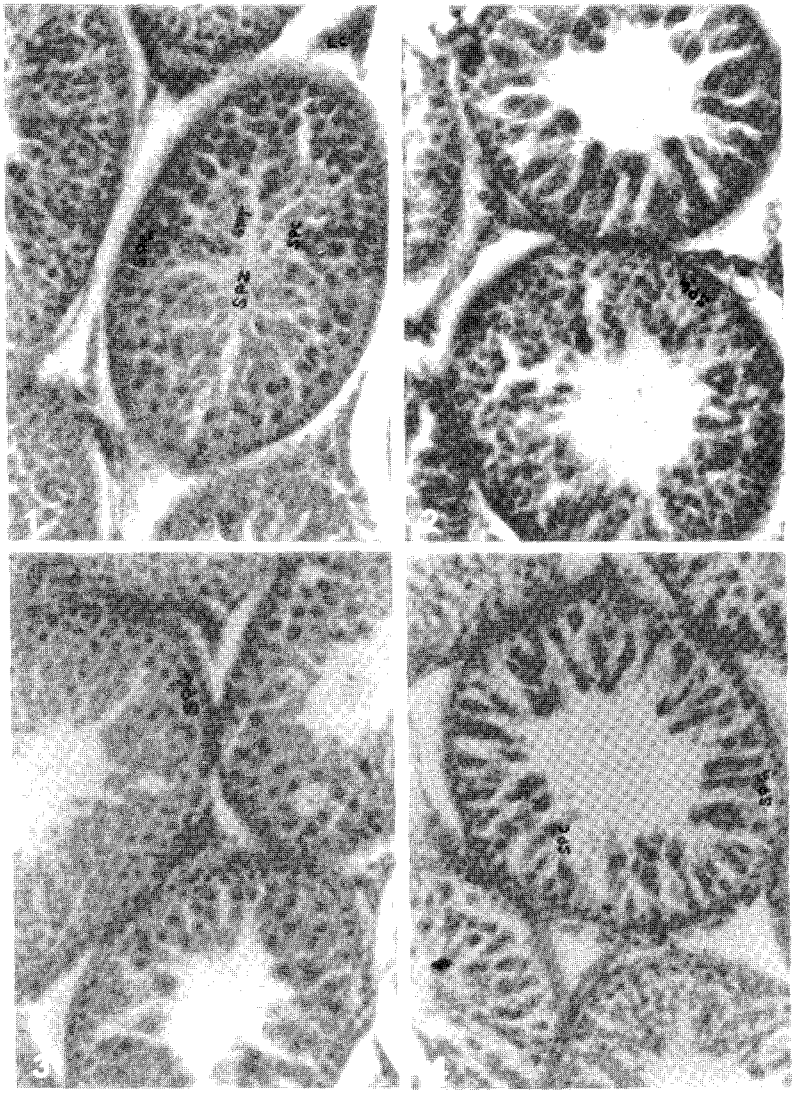

Fig. 1. Cross section of control rat testis showing normal spermatogenic activity. Leydig cells are normal (magnification 400). **Fig. 2.** Cross section of rat testis treated with 25 mg petroleum ether extract of *M. azedarach* Linn. seeds showing significant decrease in the spermatogenic elements. Leydig cells are degenerative (magnification 400). **Fig. 3.** Cross section of rat testis treated with 25 mg chloroform extract of *M. azedarach* Linn. seeds showing decrease in the spermatogenic elements (magnification 400). **Fig. 4.** Cross section of rat testis treated with 25 mg ethanol extract of *M. azedarach* Linn. seeds showing significant decrease in the spermatogenic elements. Leydig cells are degenerative (magnification 400). Spermatogonia: SPG, Spermatocytes: SPC, Spermatids: SPT, Spermatozoa: SPZ, Leydig cells: LC

DISCUSSION

The studies on a few plant extracts like *Carica papaya* (Chinoy *et al.*, 1996), *Habiscus rosa sinensis* (Reddy *et al.*, 1997), *Momordica charantia* (Naseem *et al.*, 1998), *Mentha arvensis* (Sharma *et al.*, 2001) and *Colebrookia oppositifolia* (Gupta *et al.*, 2001) have shown antifertility effects in male rats and mice. In the present study petroleum ether, chloroform and ethanol extracts of *M.azedarach* Linn. have reduced the weight of testis. The observed reduction of the testicular weight may be due to the altered production of seminiferous tubular fluid (Ghosh *et al.*, 1992) which is under the control of testosterone and FSH (Free *et al.*, 1980; Jegou *et al.*, 1983; Au *et al.*, 1986). Testosterone is known to regulate the growth and secretory activity of accessory sex organs (Ortiz, 1953; Jean-Faucher *et al.*, 1985; George and Wilson, 1988). Therefore, the observed reduction in accessory organs weight in the present study may be due to the non-availability of androgens. It is well established that the LH leutinises the cholesterol to produce pregnanolone which is subsequently metabolized to progesterone (Dorfmann, 1973; Hall, 1994). The increased level of cholesterol in the testis and accessory reproductive organs in the present study may be due to the hampered steroidogenesis, leading to reduced conversion of cholesterol to androgens. Whether this reduction is mediated through decreased availability of pituitary LH or directly due to its antiandrogenic activity on the accessory organs has to be tested.

Acid and alkaline phosphatases, which are associated with lysosomes have an important role in the metabolism of carbohydrates, phospholipids and nucleotides. Their presence in the testis and seminiferous tubules regulate the secretory activity of the testis and seminiferous tubules (Elkington and Blackshow, 1970). In the present study the acid phosphatase is decreased and alkaline phosphatase is increased due to all the three extracts treatment. This change has resulted in an altered physiological environment necessary for spermatogenesis (Mann, 1964). The reduced glycogen content indicates the low energy source of carbohydrates, which is dependent on availability of estrogens (Walaas, 1952). Therefore this decrease in the glycogen content indicates lack of energy source for spermatogenesis in the testis.

FSH is known to stimulate the conversion of spermatogonia to spermatids (Lostroch, 1963). Androgens are essential to induce meiosis and maintain spermatogenesis in response to FSH (Chemes *et al.*, 1979; Hanejii *et al.*, 1984; Russell *et al.*, 1987; Hall, 1994). In the present study reduction in the number of spermatogonial elements might be due to nonavailability of FSH and androgens after the administration of seed extracts of *M. azedarach* Linn. Out of the three extracts, petroleum ether extract was more effective in producing all these changes. It can be concluded that *M. azedarach* Linn. seed extracts have antispermatogenic and antiandrogenic effects in rats.

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