



Phytochemical screening and evaluation of antifertility activities of *Rhus mysorensis* in female albino rats

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It is well known that the plant kingdom contains numerous bioactive substances affecting the regulation of reproduction. This study was aimed at screening the phytochemicals and antifertility activity of methanolic extract of root *Rhus mysorensis*. The phytochemical analysis was carried out using standard methods. Antifertility activity was carried out using female albino rats. Two dose level (200 and 400 mg/kg, orally) was evaluated for the Effects on mating success, fertility index, gestation length, litter size and body weight. Phytochemical screening showed the presence of Alkaloids, carbohydrates, flavonoids, phenols, saponins, tannins, and phytosterols. *Rhus Mysorensis* at 200 and 400mg/Kg had a 20% and 0% reduction in mating success respectively with a significant ($P<0.01$; $P<0.001$) reduction in fertility index at 33% and 20 % respectively *Rhus Mysorensis* at 200mg/Kg caused a significantly ($P<0.05$) prolonged gestation length compared to the control and also caused a significant reduction ($P<0.001$) in litter size compared to the control. The results suggest that methanolic extract of *Rhus mysorensis* possess significant antifertility activity, which is consistent with the literature report in folk medicine of this plant in fertility regulation.

INTRODUCTION

Origin of humanity led to phototherapy belonging to the field of medicine. Medicinal plants are the richest bio resource for the development of traditional medicine. Recent research has been shifted towards the development of ethnomedicine that provide eco friendly nature and non-hazardous and cost affordable to the human beings [1]. In spite of the evidence that oral contraceptive agents have improved the rate of infertility, their unusual side effects limit the use.

The adverse effects caused by oral and injectable contraceptive agents include increased blood transaminase, increased cholesterol, hormonal imbalance, hypertension, indigestion, headache, depression, fatigue, intermenorrhoeal bleeding, and increased risk of cancer and weight gain [2]. The increase in population is becoming a comprehensive dilemma, causing much pressure on economic, social and natural assets. In the present scenario world's population has amplified at an alarming rate and is the main cause of poverty.

Rhus mysorensis belonging to the family of Anacardiaceae, used in treatment of diabetes [3]. Young shoots made into paste, and applied externally on spots to treat psoriasis [4] and pharmacologically used identified as hepatoprotective activity [5,6]. The plant is aromatic, often gregarious shrub commonly distributed in North West India to the peninsular India. With a thin brown bark and spiny branches. Root is divided into 3 rootlets. Rootlets are deeply toothed, or lobed, the middle

one 1-1.5 in long, the lateral ones smaller. The rootlets are nearly stalkless. Flowers are small, white, or greenish, borne in panicles at the end of branches or in root axils. Sepal is small, 4-5-parted, persisting even in fruit. Petals are 5, ovate, falling off early. Disk fleshy, obscurely 5-lobed. Ovary 1-celled; styles 3. Fruit is a small, dry, compressed drupe, 3 mm in diameter. The wood which is hard, reddish-yellow, close-grained and heavy is only used for fuel, and the branches for fencing fields. [7]

In the context of above mentioned medicinal properties of *Rhus mysorensis*, the present work was framed out to carry out the detection of various phytochemicals and antifertility activities of crude extract of this plant.

MATERIALS AND METHODS

Collection of plant material

The whole plants of *Rhus mysorensis* was collected from hilly region of Tirupati, A.P, India. The plant was authenticated by Dr. K. Madhav Chetty, Asst. Professor, Dept. of Botany, Sri Venkateswara University, Tirupati.

Extraction of plant material

The roots were removed and air dried under shade, powdered mechanically and stored in airtight containers. About 500gm of the powdered material was extracted with methanol (80%) by Soxhlet process. The filtrate was concentrated at reduced pressure by Rotary Vacuum Evaporator [8].

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Table 1 Phytochemical screening of the Methanolic extracts of *Rhus mysorensis*

TEST	REAGENTS	RESULTS
Test for alkaloids	Dragendorff's	+
	Mayer's	+
Test for steroidal Compounds	Acetic anhydride and conc. sulfuric acid	+
	Chloroform and conc. sulfuric acid	+
Test for Phenolic Compounds	Ferric chloride and potassium ferrocyanide	+
Test for flavonoids	10% Lead acetate	+
	Sodium hydroxide	+
	Ethyl acetate	+
Test for Saponins	Froth test	±
	Ferric chloride	±
	Aqueous hydrochloric acid	±
Test for tannins	Test for tannins	±
Test for anthraquinones	Test for free anthraquinones	-
	Test for o-anthraquinone glycosides	-

Key: + :present; ± - trace; ++ ;strong; - negative: absent

Phytochemical –Analysis

The methanolic root extract of *Rhus mysorensis* was subjected to preliminary phytochemical screening as per reported methods.

Animals and housing

The rats were housed in pairs in standard rodent cages consisted of a plastic tray like bottom covered in dry non-treated wood shavings for bedding, and a wire cage top with a food hopper seated through a hole in the roof of the cage. The rats were kept under a 12-h reverse light cycle, in a temperature controlled environment of (22 ±1°C). Food and water were available at libitum, and water was replenished daily. The Institutional Animal Ethics Committee approved the experimental protocol and the conditions in the animal house approved by Committee for Supervision on Experiments on Animals. The study was conducted in accordance with IAEC guidelines (Registration no-1696/PO/a/13/CPCSEA/IAEC/22.06. 2013).

Dose preparation of the test animals

The dose of extract was reconstituted by suspending the required quantity of *Rhus mysorensis* in Tween 80 (5% v/v in saline) freshly before use and was injected per orally (p.o.). Vehicle control groups received equal volume of Tween 80 (5 % v/v in saline).

Toxicity Studies

The limit test (2000mg/kg) of methanolic extract of *Rhus mysorensis* was performed as per the OECD guidelines, the Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h and daily thereafter for a period of 14 days for changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, salivation, lacrimation, urinary incontinence and defecation and central nervous system (tremors and convulsion) changes and Mortality was determined over a period of 2 weeks . We did not find any changes including morbidity and mortality in rats.^[9]

Antifertility studies

Effects on mating success, fertility index, gestation length, litter size and body weight

The effect of *Rhus mysorensis* methanolic root extracts on reproductive parameters namely, mating success, fertility index, gestation length, litter size and body weight was evaluated using various treatment regimes on normocyclic female rats aged between 50-60 days. Male rats were kept in the same room but in different cages and were

introduced into female cages at the ratio of 1 male per 2 females at the appropriate time. A total of 36 rats were used which was divided into 3 groups (1, 2 and 3) with 12 rats each. The 12 rats in each group were further divided into 2 subgroups (A, B) with 6 rats each. Group I, sub group A and B received 200 and 400 mg/Kg *Rhus mysorensis* respectively. These doses were administered for 14 days through intra-abdominal gavage after which the rats were mated. The first day of gestation was taken to be the day spermatozoa were detected in the vaginal smear under the light microscope. Group II animals were first mated after which sub group A and B received 200 and 400 mg/Kg of *Rhus mysorensis* aqueous extract respectively. Group III sub group A, B, were treated in a similar manner as group 1 except extract administration was continued after mating until end of gestation. Control groups consisted of eighteen negative control rats that received 0.5ml physiological saline through intra-abdominal gavage. Six positive control rats received a subcutaneous injection of estrogen/ progesterone combination (15µg estradiol / 0.15 mg progestrin).Both negative and positive control animals were then mated. Gestation length, litter sizes as well as body weights of all animals were recorded.

Statistical analysis

The results were expressed as mean± S.E.M tested with one way analysis of variance (ANOVA) followed by the Dunnett's multiple comparison tests using Graph Pad Prism Version 3.0 for Windows (Graph Pad Software).

RESULTS & DISCUSSION

Clinical assessment of Female antifertility agents should include acceptability, safety and efficacy during and after the treatment. The present study was therefore carried out to evaluate the claimed antifertility effect of *Rhus mysorensis* root using different aspects of reproductive physiology in female wistar rats. Phytochemical screening was done to generate preliminary data on the constituents of the plant extracts. The chemical tests revealed the presence of major secondary metabolites such as alkaloids, flavonoids, tannin, saponins and sterols. The results obtained from the tests were summarized in Table: 1. Acute toxicity studies revealed that the administration of graded doses of Methanol root extracts of *Rhus mysorensis* (up to a dose of 2000 mg/kg) did not produce significant changes in behaviors such as alertness, motor activity, breathing, restlessness, diarrhea, convulsions, coma and appearance of the animals.

Table 2 Effect of *Rhus mysorensis* extract on reproductive parameters (before mating)

Groups	Mating success	Fertility Index	Gestation length (days)	Litter size	Body weight
Negative control	100 %	100 %	21± 0.05	10 ± 0.12	231± 0.01
Positive control	Nil	Nil	Nil	Nil	262.3 ± 0.11
RM 200mg/Kg	70 %	29 %**	23± 0.22*	4 ± 0.12***	269.5± 0.25
RM400mg/Kg	90 %	21 % ***	22± 0.03	3 ± 0.42***	242.8 ± 0.21

Table: 3 Effect of *Rhus mysorensis* extract on reproductive parameters (after mating)

Groups	Mating success	Fertility Index	Gestation length (days)	Litter size	Body weight
Negative Control	100 %	100 %	22± 0.1	10 ± 0.12	231± 0.01
Positive control	Nil	Nil	Nil	Nil	242± 0.05
RM 200mg/kg	100%	92%	28± 0.21**	7± 0.16	265± 0.18**
RM 400mg/kg	100%	90%	23± 0.14	7± 0.22	248± 0.13*

Table 4 Effect of *Rhus mysorensis* extract on reproductive parameters ("before and after mating")

Groups	Mating success	Fertility Index	Gestation length (days)	Litter size	Body weight
Negative Control	100 %	100 %	21-23	8 ± 0.02	234± 0.05
Positive control	Nil	Nil	Nil	Nil	248± 0.47
RM 200mg/kg	0%	0%	Nil	Nil	221± 0.5
RM 400mg/kg	0%	0%	Nil	Nil	207± 0.13

Rhus Mysorensis at 200 and 400mg/Kg had a 30% and 10% reduction in mating success respectively with a significant ($P<0.01$; $P<0.001$) reduction in fertility index at 33% and 20 % respectively (Table:2). The reduction in fertility index was dose dependent, with a significant reduction at 200mg/Kg ($P<0.01$) and 400mg/Kg ($P<0.001$) for *Rhus Mysorensis*. *Rhus Mysorensis* at 200mg/Kg caused a significantly ($P<0.05$) prolonged gestation length compared to the control. *Rhus Mysorensis* root extract at 200 and 400mg/Kg caused a significant reduction ($P<0.001$) in litter size compared to the control. The rats gained weight during the 14 days pre mating extract administration. $P<0.05$ * $P<0.01$ ** $P<0.001$ ***

Rhus Mysorensis root extract at 200and 400mg/Kg also had no effect on mating success with a non-significant reduction in fertility index at 92% and 90 % respectively compared to the negative control (Table:3). *Rhus Mysorensis* at 200mg/Kg caused a significantly ($P<0.01$) prolonged gestation length compared to the negative control. *Rhus Mysorensis* plant extract caused a non-significant alteration of the litter size compared to the negative control. *Rhus Mysorensis* plant extracts caused significant ($P<0.05$; $P<0.01$) increase in body weight compared to the negative control. 200and 400mg/Kg *Rhus Mysorensis* extract had a 100% reduction in mating success and fertility index with none of the mated rats littering. There was significant loss in weight in all rats.

CONCLUSION

The results of the present study conclude that *Rhus Mysorensis* when administered orally possess significant antifertility activity. The mechanism of action should however further be explored and the

responsible phytochemical compounds isolated in order to come up with novel chemical compounds that will increase the contraceptive pool.

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