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Effect of Nelumbo nucifera seeds on the reproductive organs of female rats

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Abstract

Background: Nelumbo nucifera has been used as antifertility agent in females by the local tribals of Rajasthan especially of Udaipur district India.

Objective: The present study was conducted to investigate the effect of Nelumbo nucifera on the fertility and general physiology in female rats.

Materials and Methods: 20 female albino rats were taken and divided into two groups. Group 1 served as control and group 2 received ethanolic extract at dose of 800mg/kg b.wt. for investigating the nature of the drug and antifertility effect. Vaginal smear was monitored everyday in the morning to study the estrous cycle. On day 41st all the animals were sacrificed and various haematological and biochemical parameters were estimated.

Results: Data revealed that oral administration of Nelumbo nucifera extract brought about a significant decline in the weight of Ovary; Control (43±4.75mg), Nelumbo extract treated (25±3.86mg), Uterus; Control (236±0.004mg), Nelumbo extract treated (214±0.007mg) and Vagina; Control (221±0.002mg), Nelumbo extract treated (178±0.003mg) as well as protein and glycogen level, however cholesterol level increased significantly. In addition, the diestrous phase of the estrous cycle was found to be prolonged; Control (1.81±0.21) days, Nelumbo extract treated (3.62±0.42) days.

Conclusion: These results suggest that Nelumbo nucifera has the anti-estrogenic nature without altering the general physiology of the female rats.

Key words: *Anti-estrogenic, Female rats, Nelumbo nucifera, Estrous cycle.*

Introduction

The development of new fertility regulating drug from medicinal plants is an attractive preposition. A wide variety of synthetic contraceptive agents are available but these are not without side effects. Plants like Prangos ferulacia has been reported to have abortificient effect on the pregnant rats (1).

Antifertility effect has been studied in the plant Woodfordia fructicosa (2). Antisteroidogenic activity of two Indian medicinal plants, Croton roxburghii and Zizyphus jujuba has been observed in mice (3). Nelumbo nucifera commonly known as Lotus and Kamala in Hindi belongs to family Nymphaeacea. Presences of various alkaloids have been reported from the entire plant including nuciferine, neferine, lotusine and isoliensinine. The seeds of Nelumbo nucifera contain 2-3% oil comprised of myristic, palmitic, oleic and linoleic acid. Mukherjee et al (4) showed that the rhizome extract of Nelumbo nucifera has antipyretic activity. Gupta et al (5) showed that the seed extract of Nelumbo nucifera has antisteroidogenic effect in the testis and ovary of the rat and Mazumdar et al (6) reported antifertility effect in mice. The present study was therefore carried out to study its effect on the reproductive organs and blood profile including total erythrocyte count, total leucocyte count, haematocrit, haemoglobin

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and blood sugar for studying toxic side effects in female albino rats.

Materials and methods

Preparation of extract

The seeds of Nelumbo nucifera were purchased from the local market and authenticated by the Department of Botany, University of Rajasthan. The extract was prepared according to WHO protocol CG-04 (7) for the preparation of an alcoholic extract. The seeds were dried, powdered and then subjected to soxhelet apparatus for extraction with 50% ethanol. The extract obtained was filtered and then evaporated to dryness under reduced pressure which yielded about 8.5% of solid residue.

Animal study

Antifertility experiments were performed on inbred Wistar strain cyclic female albino rats weighing 200-210g. Twenty animals were maintained under standard husbandry conditions with food and water ad libitum.

Vaginal smear for each rat was monitored daily in the morning. Only rats with normal estrous cycle were selected for the experiment. To study the effect of ethanolic extract of Nelumbo nucifera on the estrous cycle, the selected animals were divided into two groups; group 1 served as control and group 2 received ethanolic extract at dose of 800 mg/kg b.wt.

The treatment was continued for 40 days. During this period the vaginal smear of the rats were examined daily in the morning hours. On day 41st the rats were sacrificed under light ether anesthesia. Ovary, uterus and vagina were dissected out, weighed and kept at -20°C for biochemical estimations. Blood was collected by cardiac puncture for haematological studies.

All the experiments were performed according to the guidelines of University Animal Ethics Committee.

Acute toxicity Test

To determine the accurate dose level, acute toxicity test was performed and for this 3 separate animal groups were taken.

The study was conducted in female rats weighing (200±10g). The animals were divided into 3 groups.

The extract was administered orally starting from 500 mg/kg b.wt for ten days (0% mortality), 1gm/kg b.wt. for ten days (50% mortality) and 2gm/kg b.wt for ten days (100% mortality) doses in 1 ml of vehicle. Records of mortality and manifestation of toxicity were made during 24 hr. Based on the mortality rate, probit values; the oral LD_{50} was determined by McLeod method (8).

Estrous cycle

Vaginal smear of each animal were evaluated as described by Vogel (9).The duration of the estrous cycle together with that of various phases was determined as described by Makonnen et al (10).

Biochemical estimation

Protein and glycogen level were estimated according to the method of Lowry et al (11) and Montgomery (12) respectively. The cholesterol content was estimated according to Libermann and Burchard method (13).

Total protein

Total protein was assayed by the method of Lowry et al (11). Briefly, the assay mixture contained; 1 N NaOH, reagent D (2%) Sodium tartarate, Folin's reagent, and 10% T.C.A. Protein reacts with Folin's reagent to give colour complexes. The intensity of this colour was measured at green filter against blank on colorimeter.

Total cholesterol

Total cholesterol was determined by the method of Oser (13). Briefly it contains $FeCl_3$ solution, conc.

H_2SO_4 and glacial acetic acid. The phenathrene ring of cholesterol reacts with $FeCl_3$.$7H_2O$ and gives pinkish to brown colour depending upon the concentration of cholesterol.

Glycogen

Glycogen was assayed by the method of Montogomery (12). Briefly the assay mixture contained 30% KOH, H_2SO_4, 80% phenol and absolute alcohol.

Polysaccharides are treated with conc. H_2SO_4 and phenol due to which they undergo degradation and form a complex which is pink in colour. The intensity of this colour indicates intensity of glycogen in the tissue

Blood and serum analysis

Total erythrocyte count and Haematocrit were measured by Microhaematocrit method (14). Total leucocyte count (15) was estimated. The haemoglobin level was estimated by cynomethanoglobin method (16) and blood sugar by Astoor and King method (17).

Statistical analysis

Results are expressed as mean±SEM. Statistical analysis was done by student's t-test (18).

Results

Effects on body and organs weight

There was a non significant change in the body weight of the experimental animals but there was a significant decrease ($p<0.01$) in the weight of the reproductive organs as compared to the control as follows: Ovary; Control (43±4.75), Nelumbo extract treated (25±3.86), Uterus; Control (236±0.004), Nelumbo extract treated (214 ±0.007) and Vagina; Control (221±0.002), Nelumbo extract treated (178±0.003) mg (Table I).

Effect on tissue biochemistry

The glycogen decreased significantly ($p<0.001$) in the: Ovary; Control (8.03±0.32), Nelumbo treated (4.78±0.62), Uterus; Control (18.31±0.50), Nelumbo treated (11.66±0.81), Vagina; Control (8.71±0.48), Nelumbo treated (4.18±0.57), and protein: Ovary; Control (184±4.49), Nelumbo treated (134±3.94), Uterus; Control (181.13±6.8), Nelumbo treated (140±5.49), Vagina; Control (169.37±7.84), Nelumbo treated (119±4.66). Where as there was a significant increase in the level of cholesterol of the experimental animals as compared to the control: Ovary; Control (8.84±0.55), Nelumbo treated (12.08±0.44), Uterus; Control (4.15±0.18), Nelumbo treated (8.23±0.79), Vagina; Control (5.17±0.14), Nelumbo treated (8.41±0.65) (Figures 1-3).

Effect on haematological studies

There was a non significant change in haematological parameters. Total erythrocyte count, total leucocyte counts, haemoglobin, blood sugar and haematocrit values were found to be within normal range when compared to control (Table III).

Effect on estrous cycle of rats

Ethanolic extracts of seeds of Nelumbo nucifera prolonged the estrous cycle significantly ($p<0.05$). There had been a significant increase ($p<0.01$) in the length of the diestrous phase (Control; 1.81± 0.21 days, Nelumbo treated; 3.62± 0.42 days) (Table II).

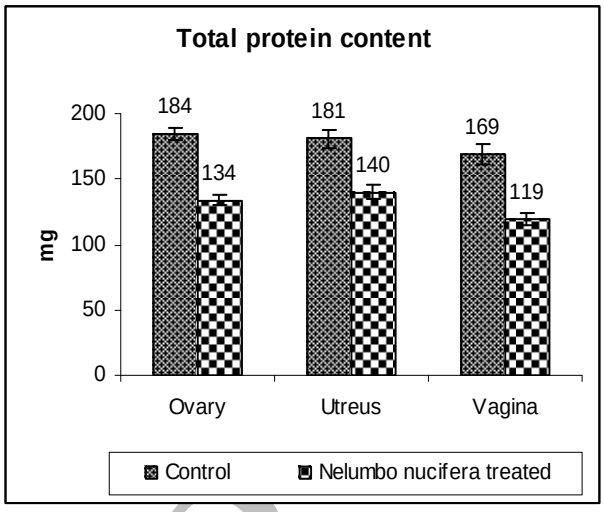

Figure 1. Effect of Nelumbo nucifera 50% ethanolic extract treatment on the protein content of ovary, uterus and vagina of female albino rats.

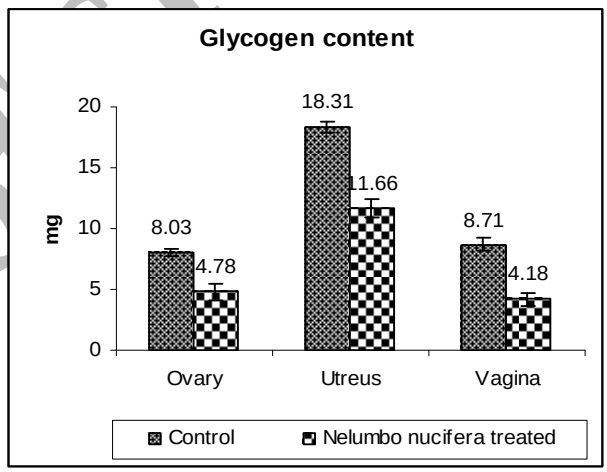

Figure 2. Effect of Nelumbo nucifera 50% ethanolic extract treatment on the glycogen content of ovary, uterus and vagina of female albino rats.

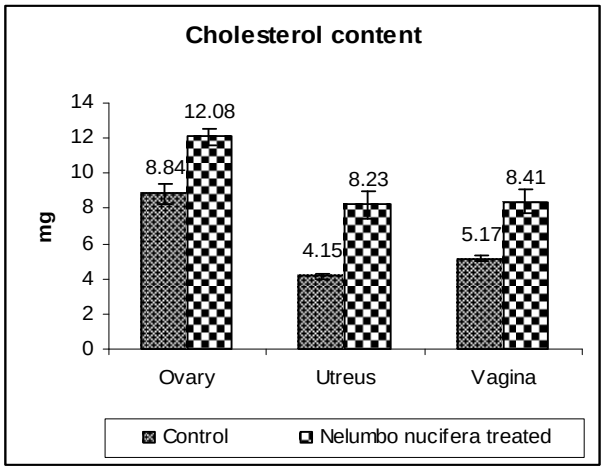

Figure 3. Effect of Nelumbo nucifera 50% ethanolic extract treatment on the cholesterol content of ovary, uterus and vagina of female albino rats.

Table I. Body and organ weight changes in female albino rats after the administration of 50 % ethanolic extract of Nelumbo nucifera.

Groups	Body weight (g)		Reproductive organs weight (mg)		
	Initial	Final	Ovary	Uterus	Vagina
Group 1: Control	200.20±2.85	209.80±2.57 ns	43±4.75	236±0.004	221±0.002
Group 2: Nelumbo nucifera treated	205.00±1.41	214.70±2.71 ns	25±3.86*	214±0.007*	178±0.003*

(Mean ±SEM of 10 animals)
Group 2 compared with group 1: * = significant (p≤0.01), ns = non-significant

Table II. Effects on estrous cycle of female albino rats after the administration of 50 % ethanolic extract of Nelumbo nucifera.

Phases	Estrous cycle	Proestrous phase	Estrous phase	Metaestrous phase	Di estrous phase
Group 1: Control	4.28±0.12	0.82±0.09	0.83±0.18	0.82±016	1.81±0.21
Group 2: Nelumbo nucifera treated	5.46±0.51*	0.45±0.12*	0.89±0.19*	0.48 ±0.21*	3.62±0.42**

(Mean ±SEM of 10 animals)
Group 2 compared with group 1: **=highly significant (p≤0.001), * = significant (p≤0.01) ns = non-significant

Table III. Haematological changes in female albino rats after the administration of 50 % ethanolic extract of Nelumbo nucifera.

Groups	RBC Count million/mm^3	WBC Count per. cu. mm.	Haemoglobin gm%	Haematocrit %	Blood Sugar mg/100 ml
Group 1: Control	3.52±0.45	6.4±0.44	13.49±0.56	49.38±1.39	81.32±0.66
Group 2: Nelumbo nucifera treated	3.80±0.06 ns	6.7±0.30 ns	13.17±0.42 ns	43.19±0.99 ns	81.41±0.81 ns

(Mean ±SEM of 10 animals)
Group 2 compared with group 1: ns = non-significant

Discussion

Plants have the property to inhibit the estrogen surge for implantation (19). In mice and humans, estrogens play a pivotal role in the implantation since they participate in the estrogen, progesterone balance and thereby in the uterine receptivity to the embryo (20).

Administration of Nelumbo nucifera to female rats caused estrogen inhibition due to its antiestrogenic nature. The decrease in the weight of ovary and uterus shows antiestrogenic nature of Nelumbo nucifera since antiestrogenic substance decreases the wet weight of the uterus (21).

The prolonged estrous cycle and diestrous phase observed with the extract suggests the antifertility effect of Nelumbo nucifera seeds .The prolongation of diestrous phase may explain the remote chances of the rats to get pregnant. The results obtained in the present study on the prolonged estrous cycle and on its diestrous phase methanolic root extract of Rumex steudelii (22).

Cholesterol derived from the different sources is the precursor for the steroidogenesis of ovarian endocrine tissue (23).

The significant increase in the cholesterol level of the group receiving extract indicates that cholesterol was not used for steroidogenesis hence accumulated in the ovary (24).

The decrease in the glycogen content of Nelumbo nucifera treated uterus confirms the antiestrogenic nature of the drug (25). Reduction in protein content of the female genital tract of Nelumbo nucifera treated rats suggests an inhibition of estrogen production (26).

Blood parameters have been found to be within normal range indicating non toxic action of Nelumbo extract on general body metabolism. Further work on the mechanism of antifertility action and histological changes of the genital organs are in progress.

Conclusion

Over all, it may be concluded that the ethanolic extract of Nelumbo nucifera seeds has anti-estrogenic effect in female rats.

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