RESEARCH PAPER

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Antisteroidogenic Effect of the Seed Extract of *Nelumbo nucifera* in the Testis and the Ovary of the Rat

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The petroleum ether extract of the seeds of *Nelumbo nucifera* and its fractions were administered orally to sexually immature female rats and mature male rats on alternate day for 15 days. The treatment caused a remarkable delay in sexual maturation in prepubertal female rats as evidenced from age of vaginal opening and first estrus (cornified smear) and a significant reduction in the sperm count and motility in mature male rats. In both the cases treatment resulted in accumulation of cholesterol and ascorbic acid and reduction in Δ^5-3β-hydroxysteroid dehydrogenase and glucose-6-phosphate dehydrogenase activity in the ovary and testis of female and male rat respectively. These results indicate suppression of steroidogenesis in both ovary and testis.

NELUMBO *nucifera*, family : *Nymphaeceae*, is an aquatic herb, commonly known as 'lotus' or 'padma'. Different parts of the plant are used as medicine in Ayurvedic, Unani and modern practices all over the Eastern world.[1-3] The leaves of lotus contain alkaloids roemerine and nuciferine, which are antispasmodic.[1] The plant is also used as source of perfume.[1] An antihemmorrhagic principle, quercetin, has been isolated from the dried receptacle.[4] The plant is used even today by some tribals for birth control.[1-4] Ancient Indian scholars recommended the name of a large number of plants, including *Nelumbo nucifera*, for antifertility action.[1,5] The petroleum ether extract of the seeds of *N. nucifera* on intraperitonial administration to sexually mature female mice has been reported to arrest the estrus cycle and increase the cholesterol and ascorbic acid content in the ovarian tissue.[6] In the present work, experiments were designed to determine the possible mode of action of the petroleum ether extract of the seeds of *Nelumbo nucifera* and its fractions on the gonadal organs of sexually immature female and mature male rats.

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EXPERIMENTAL

Preparation of extract

The seeds of *Nelumbo nucifera* were obtained from a local supplier (United Chemicals and Allied Products, Calcutta, India) and were identified in the Pharmacognosy laboratory of our department. The seeds were air dried, ground and extracted in soxhlet with petroleum ether (60-80). The crude seed extract of *Nelumbo nucifera* was dried under vaccuum. One part of the crude extract was saponified. From the saponified mixture the nonsaponified compounds were first extracted with chloroform, and then the fatty acids were extracted, after acidification of the mixture, with chloroform. Both the nonsaponified compound mixture and the fatty acid mixture were freed from solvent under vacuum. The fatty acid mixture was then converted into methylesters by boiling with dry methanol in presence of trace amount of con. sulfuric acid. The crude extract (CR), nonsaponified compounds (NS) and methylesters of fatty acids (FAME) were used as test compounds in the present investigation.

Table I: Effects of oral administration of Petroleum Ether (60-80) extract of seeds of *Nelumbo nucifera* on onset of puberty, body weight, weight of ovary and uterus of prepubertal female rats.

Sl. No.	Group	Vaginal opening (Mean age in Days ± SE)	First estrus (Mean age in Days ± SE)	Difference in Body weight in 9 (Mean ± SE) in 15 days	Weight in mg+ (Mean ± SE)	
					Ovary	Uterus
01.	Untreated (12)*	51.19 ± 1.22	56.9 ± 6.82	+(16.33 ± 1.32)	13.66 ± 0.99	57.77 ± 0.42
02.	Vehicle treated (12)* (10 ml/Kg Refined Groundnut Oil)	50.91 ± 1.10	52.6 ± 0.92	+(19.66 ± 3.97)	12.66 ± 1.04	58.66 ± 0.56
03.	Seed extract treated (12)* (2.5 mg/Kg b.w.)	60.31 ± 0.66#	66.1 ± 1.12#	+(3.8 ± 0.74)#	10.42 ± 0.18#	52.10 ± 0.81#
04.	Seed extract treated (12)* (5.0 mg/Kg b.w.)	62.87 ± 1.20#	66.6 ± 0.98#	+(3.8 ± 0.34)#	9.92 ± 0.69)#	50.29 ± 0.61#
05.	Seed extract treated (12)* (7.5 mg/Kg b.w.)	70.64 ± 0.92#	69.8 ± 0.86#	+(3.2 ± 0.16)	10.26 ± 0.45#	47.37 ± 0.96#

* - Figures in parenthesis indicate the number of animal used.
+ - Weights of the ovaries and uterus have been calculated as percent body weight
# : $p < 0.05$

Effect of the extracts on onset of maturity in female rats

Sixty prepubertal female Wister strain albino rats, collected from Indian Institute of Chemical Biology, Calcutta, India, were selected for the present investigation. Food pellets (Hindustan Lever Ltd., India) and tap water were provided *ad libitum*. Rats were divided into five groups, each containing twelve rats.

Group one & two received normal saline (5ml/kg body wt.) and refined groundnut oil at a dose of 10 ml/kg orally.

Crude seed extract was administered orally as a suspension in refined groundnut oil at a dose of 2.5 mg/kg, 5.0 mg/kg and 7.5 mg/kg to the rats of 3rd, 4th and 5th group respectively.

Effect of the seed extract and its fractions on gonadal organs of male rat

Fifty healthy albino male rats (Wister) were selected for present study. The animals were equally divided into five groups, each containing ten.

Group one and two received saline (5mg/kg), and refined groundnut oil at a dose of 10 ml/kg orally which serve as normal and vehicle control. Group three received 7.5 mg/kg of crude seed extract orally while Group four and five received orally 2 mg/kg of NS and FAME as a suspension in refined groundnut oil respectively.

The vehicle, crude extract and its fractions were administered orally, on alternate days for 15 days after 18 of fasting. Body weights of each group were

Table II: Effects of the oral administration of Petroleum Ether (60-80) extract of seeds of *Nelumbo nucifera* on cholesterol and ascorbic acid content and the enzymes G-6-PD and ^{5}-38-HSD activities in ovarian tissue of prepubertal female rats

Sl. No.	Group	Concentration of (mcg/mg tissue) (mean ± SE)		G-6-PD activity in Ovary (Unit change/mg protein X 10^{-2} (Mean ± SE)	Δ^5-38-HSD activity in Ovary (unit/mg protein X 10^{-3} (Mean ± SE)
		Cholestrol	Ascorbic Acid		
01.	Untreated (12)*	21.53 ± 1.40	72.65 ± 2.23	18.37 ± 0.33	88.63 ± 2.17
02.	Vehicle Treated (12)* (10 ml/Kg Refined Groundnut Oil)	23.19 ± 0.98	70.98 ± 0.76	16.47 ± 0.55	91.39 ± 1.20
03.	Seed extract treated (12)* (2.5 mg/Kg b.w.)	30.33 ± 1.89#	79.36 ± 1.12#	13.96 ± 1.18#	85.41 ± 3.62#
04.	Seed extract treated (12)* (5.0 mg/Kg b.w.)	38.41 ± 1.89#	88.93 ± 1.69#	13.32 ± 0.58#	83.05 ± 2.86#
05.	Seed extract treated (12)* (7.5 mg/Kg b.w.)	46.22 ± 0.75#	91.67 ± 0.08#	12.69 ± 0.13#	72.29 ± 1.542#

* - Figures in parenthesis indicate the number of animal used.
: $p < 0.05$

taken before and after treatment period. The female rats were inspected between 10-11 A.M. and 6-7 P.M. for vaginal opening and after vaginal introitus, a daily vaginal lavage was taken from each rat to determine the age at first estrus (cornified smear). Eight rats from each group of both female and male rats were sacrificed 24 hours after the last dose. Ovaries and the uterus of female rats and testis, cauda epididymis and adrenal glands of male rats were immediately dissected out, trimmed off from adherent fat and weighed. Sperm from cauda epididymis were released in phosphate buffer saline (PBS, pH-9). The sperm count and sperm motility were recorded, after proper dilution of (200 times) sperm suspension, in a Neubauer chamber. The cholesterol[7] and ascorbic acid[8] content and the activities of Δ^5-3B- hydroxysteroid dehydrogenase (EC1.1.1.51)[9] and glucose-6- phosphate dehydrogenase (EC 1.1.1.49)[10] in the ovary of female rats and the testis of the male rats were determined biochemically.

RESULTS

In Table - I, it is shown that the *N. nucifera* seed extract treatment in female immature rats significantly retarded the onset of sexual maturity as indicated by the age of vaginal opening and appearance of first estrus in contrast to control groups. About 38% inhibition of vaginal opening and 32% inhibition of first estrus has been observed at the highest dose of the extract. Weight of the body, ovary and uterus also decreased by 16.3%, 57.3% and 80.8% in the treated rats. From Table-II it is evident that in the ovary the cholesterol content is increased by 99% and ascorbic acid content is in-

Table III: Effects of the oral administration of Petroleum Ether (60-80) extract of seed of *Nelumbo nucifera* (CR), nonsaponified fraction (NS) and methylesters of fatty acids (Fame) on body weight, weights of testis, epididymis and adrenal glands of male rats

Sl. No.	Group	Difference in body weight in 9 (Mean ± SE) in 15 days	Weight in mg$^+$ (Mean ± SE)		
			Testis (Both side)	Epididymis	Adrenal (Both side)
01.	Untreated (10)$^{\#}$	+(14.33 ± 0.66)	1535 ± 2.86	194.3 ± 2.53	23.16 ± 0.74
02.	Vehicle treated (10)* (10 mg/Kg Refined Groundnut Oil)	+(15.66 ± 0.98)	1569 ± 4.66	189.0 ± 0.83	24.05 ± 0.59
03.	CR treated (10)* (7.5 mg/Kg b.w.)	+(12.55 ± 0.75)$^{\#}$	1439 ± 2.68$^{\#}$	158.6 ± 2.68$^{\#}$	18.7 ± 0.49$^{\#}$
04.	NS treated (10)* (2.0 mg/Kg b.w.)	+(8.66 ± 0.86)$^{\#}$	1283 ± 0.98$^{\#}$	114.3 ± 2.16$^{\#}$	16.3 ± 0.82
05.	Fame treated (10)* (2.0 mg/Kg b.w.)	+16.91 ± 0.83)	1541 ± 4.97	191.6 ± 3.14	22.7 ± 0.38

* - Figures in parenthesis indicate the number of animal used.
\+ - The weights of testis, epididymis and adrenal glands have been calculated as percent body weight
# : $p < 0.05$

creased by 29% whereas Δ^5-3β-HSD and G-6-PD activities was significantly supressed by 21% and 23% respectively in the treated rats. A marked reduction in the weight of testis, epididymis and adrenal gland and rate of body growth (weight gain) in CR and NS treated rats was noted. (Table-III). Sperm count and motility also decreased in male rats (Table-IV). Cholestrol and ascorbic acid content in the testis increased whereas Δ^5-3B-HSD and G-6-PD activities decreased significantly in CR and NS treated rats (Table-V). From Table-III, IV and V it is also clear that FAME cause no significant change in male gonadal organ.

DISCUSSION

Cholesterol as a precursor molecule[11] and important role of ascorbic acid[12] in the process of gonadal sterodogenesis are well established. An increased content of cholesterol and ascorbic acid in the hypofunctioning ovaries has been reported.[13] Accumulation of cholesterol and ascorbic acid in the ovaries of treated prepubertal rats provided additional support to the suppression of ovarian steroidogenic activity. Delayed onset of sexual maturity as well as diminished weight of uterus may be due to insufficient production of estrogen.[14-18] From this point of view the accumulation of cholesterol and ascorbic acid in the prepubertal ovaries (Table V) in treated rats gives support to the depressed ovarian steroidogenic activity. Synthesis of estrogen in the immature rat ovaries has been reported.[17] An increased steroid hormone biosynthesis from the prepubertal ovarian compartment is associated with concomitant increase in the NADPH supplying G-6-PD in ovarian sites[20]. Further, positive correlation between estrogen secretion from the prepubertal rat ovaries and ovarian G-6-PD activity in terms of pen-

Table IV: Effects of the oral administration of Petroleum Ether (60-80) extract of seeds of *Nelumbo nucifera* (CR), nonsaponified fraction (NS) and methylesters of fatty acids (FAME) on sperm count and sperm motility of male rats

Sl. No.	Group	Sperm count (millions/ML (Mean ± SE)	Sperm Motility (Per Cent) (Mean ± SE)
01.	Untreated (10)*	52.86 ± 1.39	60.50 ± 1.32
02.	Vehicle treated (10)* (10 ml/Kg Refined Groundnut Oil)	53.79 ± 2.39	62.20 ± 1.18
03.	CR treated (10)* (7.5 mg/Kg b.w.)	41.92 ± 3.45#	46.82 ± 3.92#
04.	NS treated (10)# (2.0 mg/Kg b.w.)	32.96 ± 1.98#	38.33 ± 0.99 #
05.	FAME treated (10)* (2.0 mg/Kg b.w.)	50.62 ± 4.18	64.81 ± 2.36

* - Figures in parenthesis indicate the number of animal used.
# : $p < 0.05$

tose phosphate pathway has been established by many workers.[18,21]

Knorr successfully established that Δ^5-3β- HSD is an important enzyme in the production of steroid hormone.[22] Therefore fall in G-6-PD and 3β-HSD activities after treatment with seed extract of *Nelumbo nucifera* suggests a decrease in the ovarian steroidogenesis. Accumulation of the substrate and reduction of the activities of the enzymes G-6-PD and Δ^5-3βHSD indicate the inhibition of steriodogenesis. Biosynthesis of the specific steroids (testosterone) by the sertoli cells is responsible for the spermatogenic activity and thereby maintain the activity of accessory reproductive organ in male. A marked reduction in the number of spermatozoa (sperm count) and their motility is due to dimished synthesis of androgen and thereby it reduces the weights of testes and accessory reproductive organs. The cholesterol and ascorbic acid are the principal precursor for the formation of androgens in the biogenic pathway, in the testis.[11,12]

From the sperm content and motility and the weights of the tests and accessory organs, it is evident that inhibition of steroidogenesis take place in CR and NS treated rats (Table: III, IV). But from the data it is clear that FAME fraction caused no significant change in steroidogenic activity in male reproductive organs.

Therefore, on the basis of experimental observations, it may be concluded that the delay of onset of puberty in female rats following treatment with the seed extract of the *nucifera* is possibly due to the reduction in ovarian steroidogenesis. It may also be concluded that the hypogonodal activity in male rats is due to inhibition of testicular steroidogenesis. Probably the site of action of the seed extract either directly on the gonad or *via* gonadotropin secretion is subject to further clarification.

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Table V: Effects of the oral administration of crude Petroleum Ether (60-80) extract of seeds of *Nelumbo nucifera* (CR), non saponified fraction (NS) and methylesters of fatty acids (FAME) on cholesterol and ascorbic acid content and enzymes G-6-PD and Δ^5-3β-HSD activities in the testis of male rats

Sl. No.	Group	Concentration of (mcg/mg tissue) (mean ± SE)		G-6-PD activity in Ovary (Unit change/mg protein X 10^{-2} (Mean ± SE)	Δ^5-3β-HSD activity in Ovary (unit/mg protein X 10^{-4} (Mean ± SE)
		Cholestrol	Ascorbic Acid		
01.	Untreated (10)*	51.96 ± 0.86	83.46 ± 1.74	3.11 ± 0.13	64.39 ± 0.06
02.	Vehicle treated (10))* (10 ml/Kg Refined Groundnut Oil)	54.31 ± 2.62	81.23 ± 0.98	3.71 ± 0.88	61.02 ± 0.03
03.	CR treated (10)* (7.5 mg/Kg b.w.)	74.71 ± 0.68#	92.76 ± 1.31#	2.38 ± 0.47#	50.23 ± 0.08#
04.	NS treated (10)* (2.0 mg/Kg b.w.)	89.17 ± 1.32#	102.61 ± 2.76#	1.97 ± 0.92	39.78 ± 0.04#
05.	FAME treated (10)* (2.0 mg/Kg b.w.)	52.66 ± 2.13	80.62 ± 3.61	3.16 ± 0.88	59.13 ± 0.082

* - Figures in parenthesis indicate the number of animal used.
# : $p < 0.05$

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