Pharmacological Evaluation For The Antifertility Effect of the Ethanolic Seed Extract of *Nelumbo Nucifera* (Sacred Lotus)

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Summary

To evaluate the effect of *Nelumbo nucifera* Gaertn. (Nymphaeaceae) on male reproductive function and fertility, 50% ethanolic extract of its seeds (NnSEt) was administered orally to male rats at the dose levels of 50, 100 and 200 mg/rat per day for 60 days. A dose-dependent response was found after this treatment. The body weights were not affected, whereas the weights of reproductive organs decreased significantly after this treatment. Significant suppression of cauda epididymal sperm count and motility was observed. Fertility was decreased in this treatment by 100% in *Nelumbo nucifera*-treated rats. Oral administration of this drug at all the dose levels did not alter the blood and serum profiles, whereas testosterone level of serum was declined significantly. The concentration of testicular cholesterol was significantly elevated, whereas protein, sialic acid, glycogen and fructose content were reduced significantly. It is concluded that *Nelumbo nucifera* treatment has an antispermatogenic effect in male rats.

Keywords: *Nelumbo* nucifera, sperm motility, sperm density, Testicular cell population dynamics, serum testosterone.

1. Introduction:

Nelumbo nucifera Gaertn. (Nymphaeacae) also known as sacred lotus is a large aquatic herb with stout, creeping rhizome found throughout India. *Nelumbo nucifera* is a native of China, Japan and possibly India. Almost all parts of the plant are eaten as vegetable and also used in indigenous system of medicine (1). *Nelumbo nucifera* is reported to possess' antidiarrhoeal(2),psychopharmacological (2), diuretic (3), antipyretic (4), antimicrobial (3,5,6), hypoglycaemic (7). Antioxidant activity of various parts of *Nelumbo nucifera* is well established, e.g. leaf (8), stamens (9) and rhizomes (10, 11).

Lotus seeds are sold in the Indian market in the name of kamal gatta, as a vegetable (1). *Nelumbo nucifera* seeds are commonly used as folk remedy in the treatment of tissue inflammation, cancer, antiemetic, given to children as diuretic and refrigerant (12). It is also used as cooling medicine for skin diseases, leprosy and considered as antidote to poison (13). The seeds are reported to possess hepatoprotective and free radical scavenging activity (14), antifertility activity (15) and also suppress cell cycle progression, cytokine genes expression, and cell proliferation in human peripheral blood mononuclear cells (12). The major phytochemical constituents present in the seeds are alkaloids like dauricine, lotusine, nuciferine, pronuciferine, liensinine, isoliensinine, roemerine, nelumbine and neferine (16, 1, 17). Procynadines were isolated from the seedpods of *Nelumbo nucifera* by Ling and co-workers (18), they also reported the antioxidant activity of procyanidines. *Nelumbo*

nucifera seeds also contain saponins, phenolics and carbohydrates (1).

Above cited literature prompted us to carry out work on the antifertility activity of *Nelumbo nucifera* seeds in male albino rats. So, in this article, we wish to report the antifertility effect of the *Nelumbo nucifera* 50% ethanolic extract of seeds (NnSEt) in male albino rats with special reference to testicular cell population dynamics.

2. Materials and Methods:

2.1 Plant Material and Extract Used

Standarized 50% ethanolic extract of *Nelumbo nucifera* seeds (NnSEt) was procured from Tulsi Amrit Pvt. Ltd., Indore, India.

2.2 Experimental Model used

Fourty adult male Wistar rats weighing 180-190 gm were used for the present investigation. Animals were housed in standard rat cages and maintained under standard condition (12-h light/dark cycle: 25±3°C; 35-60 relative humidity), water and food (commercial diet) were available *ad libitum*. Drug and/or vehicle was administered to all animals by oral intubations. The study was approved be ethical committee of the University, Department of Zoology, Jaipur, India. Indian National Science Academy, New Delhi (19) guidelines were followed for maintenance and use of the experimental animals.

Before starting the experiments, the male rats were kept for fertility test, the number and size of litters were recorded.

2.3 Fertility Test

The fertility test of individual males was done before the experiment and from days 55 to 60 in both control as well as in the treated group animals. The male rats were cohabited with proestrous females in ratio of 1:2. Vaginal smears were checked for positive mating. The inseminated females were separated and the numbers of litters delivered were recorded (20).

2.4 Experiment Design

The rats were divided in to 4 Groups containing 10 animals in each.

Group I : Control Group receiving Vehicle only (distilled water 0.5 ml/day/rat)

Group II : Treatment Group receiving 50 mg/kg b. wt./day/rat of NnSEt for 60 days.

Group III : Treatment Group receiving 100 mg/kg b. wt./day/rat of NnSEt for 60 days.

Group IV : Treatment Group receiving 200 mg/kg b. wt./day/rat of NnSEt for 60 days.

The animals were weighed and autopsied under light ether anesthesia 24 h after the last dose of the treatment.

Blood samples were collected by cardiac puncture in the heparinized tubes for hematological studies.

2.5 Body and Organ Weight Measurements

Initial and final body weights of the animals were recorded. At autopsy, the reproductive and accessory sex organs (testes, epididymis, seminal vesicle, ventral prostate and vas deferens) along with liver were dissected out, freed from adherent tissues and weighed, slices of tissues were weighed on an electronic top pan balance to the nearest mg.

2.6 Sperm Motility and Sperm Density

After anesthetizing the rat, the epididymis was exposed by scrotal incision, and spermatozoa were expressed out by cutting the distal end of the cauda epididymal tubule.

The motility of cauda epididymal spermatozoa was recorded and the percentage of motile sperms was calculated per unit area (21). Cauda epididymal sperm density was assessed using a Neubauer's haemocytometer (22).

2.7 Assay of Serum testosterone concentration

Serum concentration of testosterone was measured following the immunoenzymatic method in ELISA reader (Merck, Japan) and according to the standard protocol given by National Institute of Health and Family Welfare (23).

2.8 Tissue Biochemistry:

On autopsy the testes, epididymis, seminal vesicles, ventral prostate and liver were removed, cleared of fat and connective tissue, weighed and kept at -20°C until assayed for sialic acid (24), total protein (25), cholesterol (26), glycogen (27) and fructose (28).

2.9 Blood and Serum Analysis

Blood was collected and the values of R.B.C. and W. B.C. counts, haematocrit, hemoglobin% and Blood Sugar were estimated. The serum was analyzed to estimate the total protein, cholesterol, HDL-Cholesterol, triglycerides and phospholipids according to the standard methods.

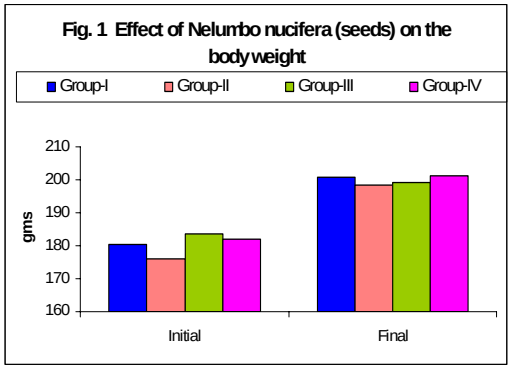
2.10 Statistical Analysis

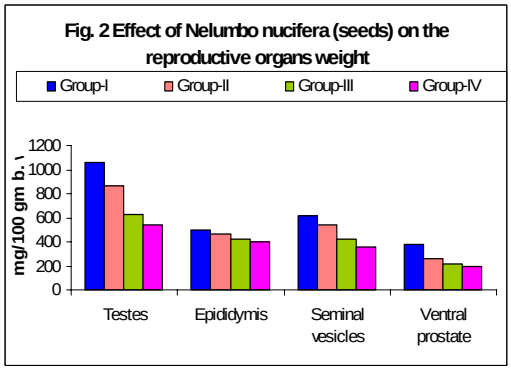
All of the recorded values of Body/Organ weight, sperm dynamics, hematological parameters and testicular cell dynamics were expressed in terms of mean ± SEM. The treated groups were compared to control using the Student's *t*-test (29).

3. Results:

3.1 Changes in Body and reproductive organs weight

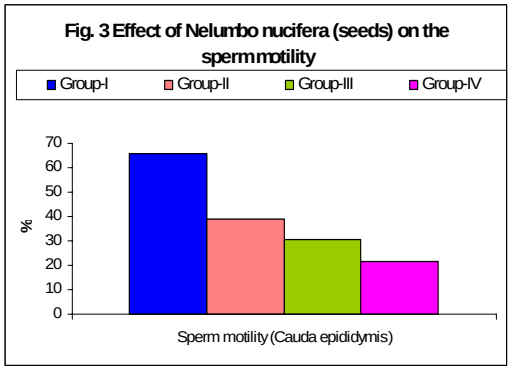
Oral administration of *Nelumbo nucifera* at all the dose levels did not cause any significant change in the body weight of treated rats. However, the weights of testes, epididymis, seminal vesicle and ventral prostate were decreased significantly ($p \leq 0.001$) (Fig.1 and 2) in a dose dependent manner.

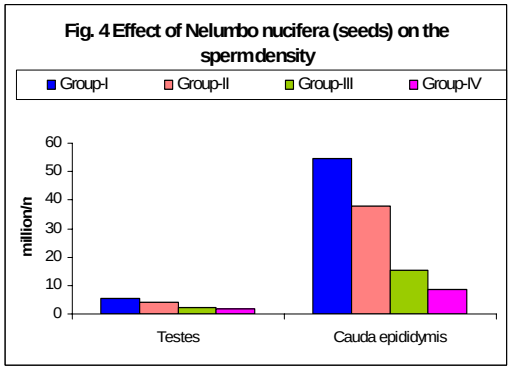


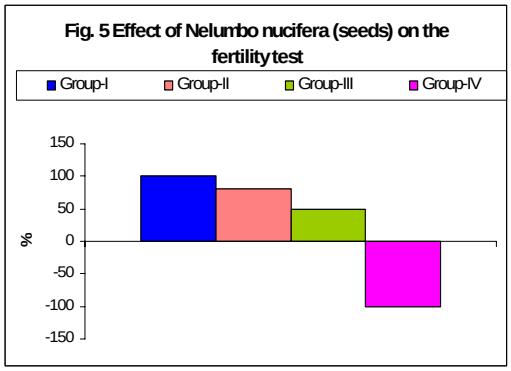


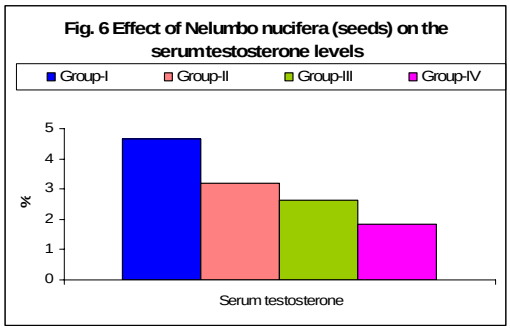
3.2 Changes in Sperm dynamics, fertility index and serum testosterone concentration

The sperm motility of cauda epididymis was significantly reduced ($p \leq 0.001$) at all the dose levels tested. Concentration of testicular and cauda epididymal spermatozoa declined by 25.04% and 30.70% at 50 mg dose level; 56.44% and 71.68% at 100 mg dose level and 63.55% and 84.14% at 200 mg dose level. The fertility of male rats reduced up to 100% after the treatment with *Nelumbo nucifera* (seeds). Serum testosterone level was declined significantly ($p \leq 0.001$) in a dose dependent manner (Fig. 3-6).



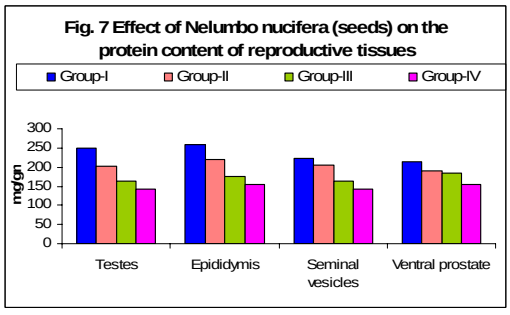


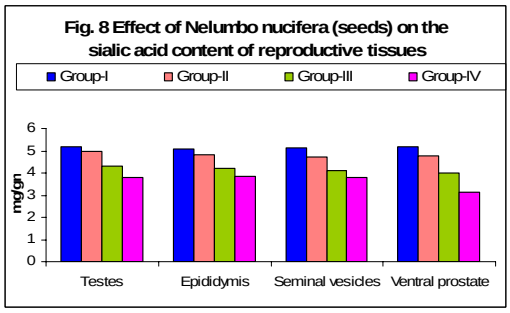


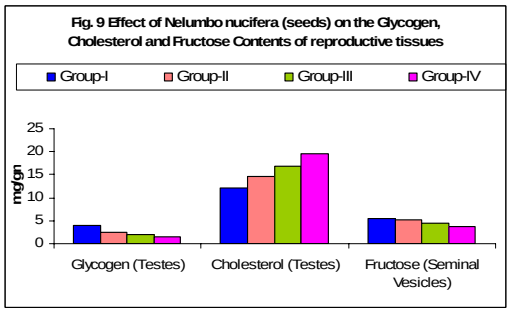


3.3 Biochemical observations

As shown in Fig. 7-9, the protein and sialic acid contents of testes, epididymis, seminal vesicles and ventral prostate were reduced significantly ($p \leq 0.001$). Also, a significant depletion ($p \leq 0.001$) was noticed in testicular glycogen and fructose contents, whereas a significant elevation ($p \leq 0.001$) was observed in testicular cholesterol content.







3.5 Changes in the Hematological and Serological profiles

The hematological and serological parameters (R.B.C. and W.B.C. counts, hemoglobin, haematocrit, Blood sugar, Serum protein, serum cholesterol, serum triglycerides, serum phospholipids and HDL-cholesterol) remained unaffected in all the treatment groups.

4. Discussion:

The oral treatment of *Nelumbo nucifera* to male rats showed a significant reduction in testis, epididymides, seminal vesicles, ventral prostate and vas deferens weights. It may be due to low plasma level of testosterone, as testicular size is the best primary assessment of spermatogenesis, since the tubules and germinal elements account for approximately 88% of the testicular mass (30). Reduction in the weight of accessory reproductive organs directly supports the reduced availability of androgens (31).

The reduction in sperm motility in cauda epididymis is of importance with regard to fertilization, it may be due to androgen deficiency (32). The 100% negative fertility test may be attributed to decreased spermatozoa density and motility of cauda epididymis. Moreover, the spermatozoa were sluggishly motile and therefore, unable to fertilize the normal cycling females. Reduction in the progressive epididymal sperm motility

of the treated rats may be responsible for the severe decrease in fertility of male rats leading to the presence of sperm toxic agent in the extract (33).

A significant decline in serum testosterone might be due to adverse effect of treatment on hormonal milieu of the testes. The reduced weights of seminal vesicle and ventral prostate further support the suppressed concentration of testosterone in circulation (34).

Sialic acids are concerned with changing the membrane surface of maturating spermatozoa, coating of spermatozoa with certain antigens and in the development of their fertilizing capacity (35). Low levels of sialic acid may suppress the fertilizing capacity of spermatozoa. Sperm immotility, in all the treated groups, suggests an undersupply of androgens to epididymis, resulting in impairment of epididymal functions and its weight. Motility of spermatozoa is due to the flagellar beat, which is dependent on the microtubular apparatus of the flagellum and adenosine triphosphate (ATP) (36). Reduced protein and glycogen content could be correlated with low sperm density (37). Low levels of fructose could inhibit the sperm motility by deficient generation of ATP (38).

No change in the values of hematological and serological parameters in treated groups showed that the drug did not effect the vital functioning of the rats.

The results, suggested that oral administration of *Nelumbo nucifera* could lead to antifertile state in male rats due to the interference of the drug in the testicular androgen levels.

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