Antispermatogenic effects of *Nyctanthes arbortristis* in male albino rats

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Abstract

The present is an attempt to evaluate the antispermatogenic activity of methanol extract of *Nyctanthes arbortristis* stem bark in male albino rats. Methanol extract of *N. arbortristis* stem bark was administered orally to adult male albino rats at the dose level of 100 mg/kg b.wt. for 60 days. On day 61st the rats were autopsied, the testes and accessory reproductive organs were removed and weighed. The organs were processed for biochemical estimations and histopathological observations. Sperm motility and sperm density in cauda epididymis were also assessed. The results revealed that the *Nyctanthes* stem bark extract treatment caused a significant decrease in weight of testes and accessory reproductive organs whereas body weight did not showed any significant change when compared to control. Sperm motility as well as sperm density reduced significantly, which resulted in total suppression of fertility. Significant reduction was also observed in biochemical estimation of testicular protein, sialic acid, glycogen and seminal vesicular fructose whereas cholesterol content of testes was increased. A marked diminution in the germ cell population was noticed. Production of spermatids declined by 64.29 %. Seminiferous tubular diameter and Leydig cell nuclear area showed notable reduction. The Sertoli cells count and its cross-sectional surface area was also significantly reduced. There was no significant alteration in the blood and serum parameters throughout the course of investigation. In conclusion *Nyctanthes arbortristis* methanol stem bark extract causes impairment of testicular function and affects spermatogenesis in male rats.

Key words: *Nyctanthes arbortristis*, Testicular cell population, Sperm motility, Leydig cells

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Introduction

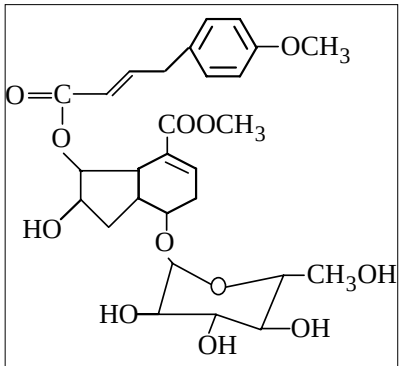
Nyctanthes arbortristis Linn. commonly known as Harsinghar (Hindi) and Sephalika (Sanskrit) belonging to family Oleaceae. It is distributed in E. Asia and in India it is found wild in the forest of Madhya Pradesh, Rajasthan, Uttarpradesh and in Sub-Himalayan tracts. Leaves of *Nyctanthes* being used in the treatment of sciatica and arthritis are also advocated for various kinds of fevers and painful conditions by the Ayurvedic physicians. Transquilizing, antihistaminic and purgative activities was also exhibited by the leaves extract [1]. Roots of it are used for emaciation. Stem bark of this plant is taken to cure dysentry, ulcer of palate and internal injuries [2]. It has been reported that *Nyctanthes* possesses analgesic, antipyretic, ulcerogenic, anti-inflammatory [3] antileishmanial, antiviral, antifungal [4] and antibacterial properties [5]. However no work have done its effect on male reproductive system therefore the present work is an attempt has been made to find if the plant could be used as a male fertility regulating agents.

Materials and methods

Extraction and isolation of compounds

Stem bark of *Nyctanthes arbortristis* were collected around Jaipur city. It was identified and authenticated by Dr. N.J. Sarana, Associate Professor, Department of Botany, University of Rajasthan. Shade dried stem bark (3 Kg) were crushed and coarsely powdered and then extracted in a soxhlate apparatus with 70% methanol for 40 to 45 hrs. The extract was filtered and methanol was removed under reduce pressure to obtain dark viscous brown mass. The concentrated extract (40g) was treated with acetonitrile (CH_3CN) to remove fats. The non-fatty portion was then treated with charcoal to remove colored impurities.

The filtrate was concentrated (9g). A part of the extract was fed to rats at 100 mg/kg and some portion of this extract was subjected to chromatograph over silica gel coloumn eluted with chloroform and methanol in ratio of 9:1. It is reported that this extract contain following compounds: β-amyrin, arbortristoside-A[1], oleanolic acid, nyctoside-A[2], nyctanthic acid, 6-β-hydroxyloganin[3]. Among these arbortristoside-A was the major compound, which is recrystallized by methanol to obtain pure compound arbortristoside-A. Therefore looking to medicinal activities of this plant, arbortristoside-A was subjected to antifertility activity in male rats. The structure was established on the basis of spectroscopic methods [6].



Arbortristoside-A

Animals

Healthy, adult (4-5 months old) male albino rats of Wistar strain weighing 150-180g, which were obtained from Jamia Hamdard, Hamdard University, New Delhi (India). The rats were housed in plastic cages under standardized conditions (12 h light / 12 h dark; 25°C±3°C and 35-60% humidity) and were maintained on standard rat pellet diet (Hindustan Lever) and tap water ad libitum.

Study protocol

Male rats of proven fertility were divided into 2 groups of 10 rats each.

Group – I: Rats received distilled water 0.5 ml for 60 days.

Group – II: Rats treated with *Nyctanthes arbortristis* stem bark extract dissolved in 0.5ml distilled water (100 mg/rat/day) for the period of 60 days.

Fertility test

Fertility testing in both groups was investigated from day 55 to 60. Males from control and treated groups were caged overnight with normal proestrous females in the ratio of 1:2 for normal mating. Presence of sperms in the vaginal smear confirmed the positive mating and the day was taken as an index of Day-I of gestation. The implantation sites of mated females were checked on day 16^{th} by laparotomy.

Autopsy schedule

The male rats were weighed and sacrificed under ether anesthesia after 24 hours of the last drug administration of 60 days duration. Blood was collected by cardiac puncture and serum was separated and reproductive organs i.e. testes, epididymis, seminal vesicle and ventral prostate were removed, cleared off fats, connective tissues and weighed on an electronic balance and kept at -20°C for biochemical estimation.

Sperm analysis

The motility of cauda epididymal sperm was determined with haemocytometer and sperm density was assessed in testes and cauda epididymis in Neubauer's counting chamber by the method of Prasad *et al*. [7].

Biochemical analysis

The estimation of protein and sialic acid were performed in testis and epididymis. Glycogen, cholesterol in testes and fructose in seminal vesicle were also estimated. Blood was analyzed for R.B.C., W.B.C. count, hemoglobin, haematocrit and blood sugar. Serum was analyzed to estimate total cholesterol, total protein, phospholipids and tryglycerides.

Histological preparation

For examination of pathological changes, tissues were fixed in Bouin's fluid, dehydrated in graded ethanol, cleared in xylene and embedded in paraffin wax, sections cut at 5 µm stained with hematoxylin and eosin for the discrimination of the stages of spermatogenesis.

Quantitative analysis

Quantitative evaluation of cell population was based on the calculation made for each cell type per cross tubular section, only round sections of spermatogonia, spermatocytes and Sertoli cells were counted using X800. At least 20 round tubular cross sections were counted for each stage of spermatogenesis. These crude counts were corrected by using Abercrombie's correcting factor. Interstitial cell types such as fibroblast, mature and degenerating Leydig cells were estimated applying a differential count which were statistically verified by the binomial distribution. Mean seminiferous tubular diameters were determined by measuring and tracing an average of 100 selected seminferous tubules. The Leydig cell nuclei area and Sertoli cell area were measured at X800.

Statistical analysis

Mean and standard error of mean [SEM] were calculated and the significance of difference was analyzed by applying student's 't' test.

Results

Body and organ weight

Table 1: Effect of *Nyctanthes arbortrstis* extract on the body weight and the organs weight

Treatment	Body weight (g)	Organ weight (mg/ 100g b.wt)			
		Testes	Epididymis	Seminal vesicle	Ventral prostate
Group-I Control	230 ±6.50	1407 ±32.00	614.15 ±11.20	562.00 ±22.50	308.50 ±2.02
Group-II *N. arbortristis* 100 mg $kg^{-1}day^{-1}$	210 ±10.00[a]	1200 ±15.00[c]	508.50 ±5.00[c]	430.75 ±25.18[b]	229.99 ±1.80[c]

Values are mean ± SEM (n=10); ns non significant, [a] $P > 0.05$ non significant; [b] $P < 0.05$ significant; [c] $P < 0.01$ highly significant v/s control.

Body weights of rats, after the treatment did not alter significantly, however there is significant reduction in the weight of reproductive organs i.e. testis, epididymis, seminal vesicle and ventral prostate (Table-1).

Sperm dynamics and fetility

Table 2: Effect of *Nyctanthes arbortristis* on sperm dynamics and fertility

Treatment	Sperm motility % (Cauda epididymis)	Sperm density (million/ml)		Fertility (%)
		Cauda Epididymis)	Testes	
Group-I Control	72.00 ±2.50	46.57 ±1.42	4.46 ±0.44	100 (+) ve
Group-II *N. arbortristis* 100 mg $kg^{-1}rat^{-1}$	30.04 ±1.25[c]	11.20 ±0.70[c]	1.91 ±0.11[c]	100 (-) ve

Values are mean ± SEM (n=10); [c] $P < 0.01$ vs control

Sperm motility of the cauda epididymis declined by 62.75 % and sperm concentration of testis and cauda epididymis reduced significantly ($P \leq 0.01$) which resulted in the suppression of male fertility by 100% (Table-2).

Cell population dynamics

Table 3: Effect of *Nyctanthes arbortristis* on testicular cell population dynamics

Treatment	Testicular cell counts (number/10 cross section)					
	Sertoli cell	Spermatogonia	Preleptotene	Pachytene	Secondary spermatocyte	Round Spermatid
Group-I Control	2.81 ±0.02	6.87 ±1.20	20.25 ±1.78	29.29 ±1.09	46.50 ±1.00	36.55 ±3.25
Group-II *N. arbortritis* 100 mg $kg^{-1}rat^{-1}$	2.06 ±0.04[c]	5.40 ±0.52[a]	8.40 ±0.98[c]	13.41 ±0.78[c]	28.50 ±2.30[c]	13.05 ±2.25[c]
	(-) 26.65		(-) 58.51	(-) 54.21	(-) 38.70	(-) 64.29

Values are mean ± SEM (n=10); [b] $P < 0.05$; [c] $P < 0.01$ vs control; Percent variations vs control in parentheses

Administration of *Nyctanthes* stem bark extract caused an effective inhibition of spermatogenesis. There was a significant reduction in most of the cell types of seminiferous tubules. Population of preleptotene, pachytene, secondary spermatocytes, rounded spermatids were reduced by 58.51 %, 54.21 %, 38.70 % and 64.29 % respectively. Spermatogonial population did not show any significant alteration when compared to control (Table-3).

Table 4: Effect of *Nyctanthes arbortristis* stem bark extract on Leydig Cell Differential Counts and Testicular histometery

Treatment	Leydig Cell Differential Counts			Testicular histometery		
	Fibroblast	Mature	Degenerated	Seminiferous Tubular Diameter (µm)	Leydig Cell Nuclear Area (µm)	Sertoli Cell Area (µm)
Group-I Control	54.00 ± 3.60	99.70 ± 0.26	57.00 ± 2.80	268 ±9.00	11.10 ± 0.05	48.90 ± 3.20
Group-II *N. arbortritis* 100 mg kg^{-1}rat^{-1}	55.30 ±2.90[b]	63.55 ±3.65[c]	81.15 ±2.60[c]	189 ±7.50[c]	6.80 ±0.12[c]	15.50 ±2.00[c]

Values are mean ± SEM (n=10); [b] P < 0.05 significant; [c] P < 0.01 highly significant v/s control

Cross sectional surface area of Sertoli cells showed a notable depletion. Number of mature Leydig cells was decreased significantly (P≤0.01) where as degenerating cell number was increased as compared to control animals. Significant (P≤0.01) reduction in the seminiferous tubular diameter and Leydig cell nuclear area also have been observed (Table-4).

Biochemical findings

Table 5: Effect of *Nyctanthes arbortristis* stem bark extract on tissue biochemistry

Treatment	Protein (mg/g)		Sialic acid (mg/g)		Glycogen (mg/g)	Cholesterol (mg/g)	Fructose (mg/g)
	Testes	Cauda epididymis	Testes	Cauda epididymis	Testes	Testes	Seminal vesicle
Group-I Control	204.42 ±4.44	255.25 ±3.25	4.62 ±0.16	5.30 ±0.15	3.88 ±0.10	7.80 ±0 .30	4.20 ±0.19
Group-II *N. arbortritis* 100 mg kg^{-1}day^{-1}	165.00 ±2.22[c]	203.00 ±5.45[c]	3.75 ±0.03[c]	3.42 ±0.10[c]	1.81 ±0.07[c]	12.10 ±0.50[c]	3.20 ±0.12[b]

Values are mean ± SEM (n = 10); [b] P < 0.05; [c] P < 0.01 vs control

Biochemical parameters showed significant alteration after treatment. Protein content of testes and epididymis were reduced significantly (P≤0.01) after stem bark extract treatment. Content of sialic acid depleted significantly in testes and epididymis (P≤0.01). Testicular glycogen was decreased significantly (P≤0.01) in treated groups whereas level of

cholesterol in testes was elevated (P≤0.01). Fructose content of seminal vesicle also showed significant (P≤0.05) reduction (Table-5).

Blood and serum analysis

Table 6: Effect of *Nyctanthes arbortristis* stem bark extract on blood

Treatment	R.B.C. (Million/mm^3)	W.B.C. (Million/mm^3)	Haemoglobin g%	Haematocrit (%)	Blood sugar (mg/dl)
Group-I Control	5.10 ±0.16	8540 ±50.00	14.09 ±0.40	44.60 ±4.40	90.85 ±1.45
Group-II *N. arbortristis* 100 mg kg^{-1} rat^{-1}	5.02 ±0.12^a	8300.00 ±100.00^a	13.20 ±0.50^a	43.60 ±0.80^a	85.35 ±3.00^a

Values are mean ± SEM (n = 10); ^{a} P > 0.05 (^{a} = non significant)

Blood estimation i.e. R.B.C., W.B.C., haemoglobin, haemotocrit, blood sugar was almost in the range like that of control animals (Table-6).

Table 7: Effect of *Nyctanthes arbortristis* stem bark extract in serum

Treatment	Total Protein	Total Cholesterol	Phospholipid	Tryglyceride
	(mg/dl)			
Group-I Control	15666.64 ±333.10	104.50 ±4.30	102.05 ±3.05	110.30 ±3.70
Group-II *N. arbortristis* 100 mg rat^{-1} day^{-1}	15111.09 ±550.40^a	98.05 ±3.05^a	92.75 ±4.75^a	102.25 ±4.75^a

Values are mean ± SEM (n = 10); ^{a} P > 0.05 (^{a} = non significant)

Protein, cholesterol, phospholipid and tryglyceride contents of serum did not change significantly (Table-7).

Discussion

Animal treated with *Nyctanthes arbortristis* stem bark extract showed a notable depression of spermatogenesis. There is significant reduction in testes weight, which can be attributed for the loss of germ cell [8].

Decrease seminiferous tubule diameter reflects tubular shrinkage, which may occur due to cell death or sloughing of epithelial cells [9]. Reduction in the weight of accessory reproductive organs directly supports the reduced availability of androgens [10]. Since male accessory organs are essential under the control of androgens through their specific receptors and androgenic deprivation in the rat prostate up regulates the concentration of transcripts for the androgen receptors [11].

Marked inhibition of sperm motility may be due to low level of ATP content [12]. Slight reduction due to alteration in the metabolism of the testes has serious repercussion on sperm motility and fertility rate, since normal internal milieu of epididymis is necessary for proper maturation of sperm [13].

Spermatogenesis requires functional integrity and cooperation of the Sertoli cells as they occupy the full thickness of the seminiferous tubules and are in close contact with germinal cells. Secretory activity of Sertoli cells i.e. A B P (Androgen Binding Protein) production is modulated by germinal cells particularly by pachytene and early spermatids [14]. Alteration of Sertoli cells affects the production of ABP, which in turn leads to the arrest of spermatogenesis. There is also evidence that the disturbance of Sertoli functions results in the damage of spermatogenesis [15]. Results showed low counts of Sertoli cells and some structural changes in Sertoli cell after administration of extract of *Nyctanthes arbortristis* stem bark. Similar results have been observed with *Albizzia lebbeck* pod extract [16].

In the interstitial compartment of treated rats there appeared to be severe depletion of the number of mature Leydig cells. Leydig cells maintain concentration of testicular testosterone in testicular fluid surrounding the seminiferous tubules. Degenerative changes in Leydig cells effect on functional ability of these cells to synthesize testosterone, which is required for maintenance of spermatogenesis [17].

It is evident that testicular function would be altered by reduced protein content [18]. Results revealed that protein and sialic acid content of epididymis reduced significantly. The principle cells of the epididymis are responsible for the synthesis of proteins and sialic acid, which are directly poured into the epididymal lumen [19]. Reduction in testicular sialic acid either by due to absence of spermatozoa or reduced androgen production could affect metamorphosis and maturational stages of spermatid [20]. According to Du Toit [21] it is also postulated that bounded sialic acid and sperm ATP concentration negatively correlated with sperm motility. The glycogen content in the cell indicates energy storage. Sertoli cells and spermatogonia often contain glycogen and secrete substrate from the blood and provide source of reserve carbohydrates for seminiferous tubular cells and the glycogen level is found to be directly proportional to the steroid hormones [22]. The decrease glycogen content of the testes after the administration of *Nyctanthes arbortristis* stem bark extract may reduce the energy source for spermatogenic activity which might have resulted in spermatogenic arrest. Similar results have been revealed by Seetharam *et al* [23]. High accumulation of cholesterol in the testes of the extract treated rats may be due to decreased steroidogenesis [24]. Curry and Atherton [25] suggested that the reduction in seminal fructose might be due to reduced synthesis and secretion of circulating androgens. It has been observed that blood and serum parameter were within normal range, indicating non-toxic nature of plant material administered. In conclusion: The methanol extract of *Nyctanthes-arbortristis*, stem bark is effective in suppressing the spermatogenesis with the changes in structural activity of Sertoli cell and Leydig cell.

Acknowledgement

The authors are thankful to the Head, Department of Zoology, Prof. N. K. Lohiya, Coordinator CAS, Department of Zoology, University of Rajasthan, Jaipur for providing necessary facilities, as well as U.G.C., Regional Office, Bhopal, M.P. (INDIA) for financial support.

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