WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 15, 1087-1096. Research Article ISSN 2277– 7105

ROLE OF *CATHARANTHUS ROSEUS* LINN. (NAYANTARA) ON SPERM COUNT AND SERUM TESTOSTERONE LEVEL OF ADULT MALE ALBINO RAT

[1]Kartik Shaw, [2]Krishna Nandi, [3]Ramjyoti Ganguly and [4]Chandan Roy*

[1]M.Sc, Dept. of Physiology, Krishnath College, University of Kalyani.

[2]Associate Professor and H.O.D., Undergraduate Section, Dept. of Physiology, Krishnath College, University of Kalyani.

[3]Ex – H.O.D. and Associate Professor, Postgraduate Section, Dept. of Physiology, Krishnath College, University of Kalyani.

[4]Guest Lecturer, Dept. of Physiology, Berhampore Girls' College, University of Kalyani.

Article Received on
28 Sep. 2017,
Revised on 19 Oct. 2017,
Accepted on 10 Nov. 2017
DOI: 10.20959/wjpr201715-10171

***Corresponding Author**
Chandan Roy
Guest Lecturer, Dept. of Physiology, Berhampore Girls' College, University of Kalyani.

ABSTRACT

Male reproductive system is a multifaceted process that involves the testes, epididymis, accessory glands and associated hormones. Testes perform two highly organized and indicative events, called spermatogenesis and steroidogenesis, which are vital for the perpetuation of life. On the other hand, *Catharanthus roseus* (CR) Linn. (Nayantara), Apocynaceae family, has an effect on blood sugar. It reduces blood sugar, in rural areas people often take some leaves of this ornamental plant and chew it. This project shows that long term use of the leaves of Nayantara leaf extract causes reduction in epididymal sperm count and lowering of serum testosterone. There were two set of animals having 3 subsets. Main two sets were before treatment group and other was after treatment group. The subsets were normal group, low dose group and high dose group. Leaves were collected and water extract was made and then administered orally to the animals daily for 30 days. After 30 days of treatment with extract, animals were sacrificed and the parameters were checked. In this study, it has been seen that treatment with nayantara leaf extract significantly decreased the epididymal sperm count and serum testosterone level respectively.

KEYWORDS: *Catharanthus roseus* Linn., Nayantara, Apocynaceae, testosterone, epididymal sperm count.

INTRODUCTION

Male reproduction is a multifaceted process that involves the testes, epididymis, accessory sex glands and associated hormones. Testes perform two highly organized and intricate events, called spermatogenesis and steroidogenesis, which are vital for the perpetuation of life. Spermatogenesis, a highly dynamic and synchronized process, takes place within the seminiferous tubules of the testis with the support of somatic Sertoli cells, leading to the formation of mature spermatozoa from undifferentiated stem cells.[1] The interstitial compartment, which comprises Leydig cells, are the site of steroidogenesis.[2] Subsequent to the process of spermatogenesis, spermatozoa transits from the testis to the ejaculatory ducts, undergoing a sequence of modifications that results in the accomplishment of its ability to move, capacitate and to interact with zona pellucida of the female ovum.

The testis is one of the organs that are very vulnerable to assault, which is reflected by the adverse trend in male reproductive health during the past several years. The male reproductive system is extremely sensitive to various environmental factors such as life style, drugs, radiation, pollution and toxicants, the result of which could be congenital abnormalities in infants and functional alterations in adults.[3] Several natural and synthetic products are reported to target the testes at the hormonal level or spermatogenesis or both.

In this review, we discuss on the effect of Nayantara extract that hampers the functionality of the testis, thereby leading to infertility. The sacred knowledge about the healing powers of plants was initially passed down orally through generations and as civilizations grew written records were prepared for the benefit of the population.[4] *Catharanthus roseus*(Nyantara), *Azadirachtaindica*(Neem), *Allium sativum*(Garlic) are medicinal plants, used inAyurveda for treating various diseases, one of which is diabetes mellitus. In the present study of 12 months period fromJanuary to December 2007, aqueous extract of this plants were prepared and blood glucose lowering effect and improvementof body weight gain in Streptozotocin (50 mg/kg bwti.p.) induced diabetic rats were measured and compared with that of apatent drug glimepride in the Department of Pharmacology, Bangladesh Agricultural University, Mymensingh. Rats wereadministered *Catharanthusroseus, Azadirachtaindica, Allium sativum*extracts at the dose rate of 1g/kg, 500 mg/kg and1g/kg bwt orally for 14 days, respectively. Blood glucose level and body weight was measured by Glucotrend kit andElectronic balance and that compared with a patent drug Glimepride at a dose rate of 100 mg/kg bwt. The data werecompared statistically by using student's unpaired *t*-test. The herbal

preparations of these plants significantly increased bodyweight gain and decreased blood glucose as compared with the patent drug. The present study clearly indicated the significantantidiabetic activity of *Catharanthus Roseus, Azadirachtaindica* and *Allium sativum*and supports the traditional usage of theherbal preparations by Ayurvedic physicians for the therapy of diabetes.[5]

It contains tannins, steroids, saponin glycosides, cardiac glycosides, anthraquinone glycosides and flavonoids.[6] It produced pathological changes in the principle and apical cells of caput and nuclear cells of cauda causing impairment of epididymal functions.[7,8] It affected spermatogenic cell lines other than spermatogonia.[9]

Oral administration of Catharanthus roseus Linn. leaf extract caused widespread testicular necrosis, hyalinization of tubules and scrotal –cell-only syndrome. Biochemical studies revealed notable reduction in glycogen and fructose levels in reproductive tissues.[10]

MATERIALS AND METHODS

Collection of C. Roseus leaves: Matured leaves of Catharanthus Roseus were collected from Krishnath College's compound and some of my friend's garden. Collected leaves were green and matured with a size of 5-6 cm long and 2-3 cm flattened.

Extraction of C. Roseus leaves

Collected leaves were washed thoroughly to wash out the unwanted dust and soil. Then the leaves were left for about 30 minutes to remove the water droplets due to washing. Water extraction of the leaves was done by weighing leaves and water. The ratio of leave and water was 1:2; it means 10 gm of leave paste was mixed with 20 gm of water. First of all water and leaves were weighed accurately in digital weighing machine provided by the college. Then in a motor-pestle leaves were crushed as much as possible and a paste was made. Then added the water in the paste and mixed thoroughly by crushing again and again. After that the mixture was filtered using filter paper and collected in a glass tube.

Doses: Two varieties of doses were prepared. One of them was high dose containing the crude extract and the second was low dose diluting the crude extract 10 times. 0.5 ml/ 100 gm of body weight of the prepared drug was given orally to the rats. It was continued for 4 weeks regularly. After completion of the duration of 4 weeks, several tests were done to measure the effect of the drug induced.

Epididymal Sperm Count

Phosphate Buffer Solution

The most common composition of PBS	
Salt	**Concentration (g/L)**
NaCl	8.0
KCl	0.2
Na_2HPO_4	1.42
KH_2PO_4	0.24

Started with 800 ml of distilled water to dissolve all salts. Ph adjusted to 7.4 with HCl. Volume made upto 1L. The resultant 1xPBS should have a final concentration of 10 mM PO_4^{3-}, 137 mM NaCl, and 2.7 mM KCl.

Counting of Sperm

1. Epididymis taken out from the Rat.
2. 1 ml saline taken into a clean Petri Dish, teased the epididymis until the solution become becomes cloudy.
3. In a WBC pipette, solution was drawn upto 0.5 mark, and diluted with buffer upto the mark.
4. Counted in Neubaur's chamber and calculated.

Step 1 – Averaging: If one did not count all of the cells in a large square (1mmx1mm) then it is necessary to average the results first before proceeding. For the purpose of this example, I use an average cell count of Y cells.

Step 2 – Computing the volume: It is necessary to determine the volume represented by the square. The width and height of the square (e.g. 0.25mm x 0.25mm) must be multiplied by the height of the sample (often printed on the hemocytometer, in this example it is 0.1mm): v = 0.25mm x 0.25mm x 0.1mm = 0.00625mm^3 = 0.00625ul (where ul is microliters).

Step 3 – Calculating the number of cells in 1 ml: If there are Y cells in 0.00625ul, then how many cells are there in 1ml (=1000ul)? We do simple direct proportion, Y cells/0.00625ul = X/1000ul (Ycells*1000ul)/0.00625ul = X (the ul cancel out).

Step 4 – Correcting for dilution: If the sample was diluted before counting, then this must be taking into consideration as well. We assume that the sample was diluted 1:10. The final result is therefore (Ycells*1000ul)/0.00625ul cells x 10 = 197 529 600 cells in 1 ml.

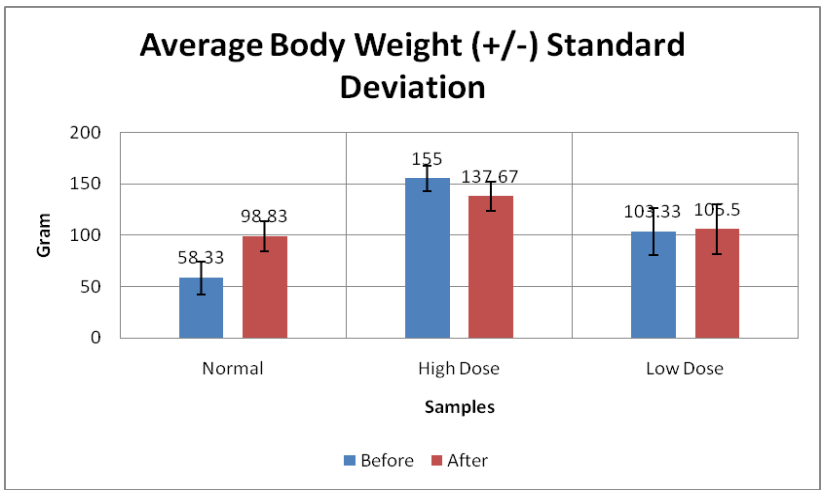
Histological studies

1. Tissue sections were taken and fixed in 4% Formol solution.
2. Dehydrated, Embedded and blocks were made.
3. Sliced with the help of Microtome and stained with Eosin - Haematoxylin staining procedure.
4. Observed under microscope.

RESULTS

Table. 1: Effect of leaf extract of Catharanthus roseus on body weight.

Body weight in gm						
	Before treatment			After treatment		
	Normal dose	High dose	Low dose	Normal dose	High dose	Low dose
	30	160	140	70	155	150
	60	150	130	97	135	115
	80	150	90	110	125	90
	60	140	80	95	125	95
	60	150	90	90	130	98
	60	180	90	107	156	85
Mean	58.33333	155	103.3333	94.83333	137.6667	105.5
SD	16.02082	12.58306	22.85218	14.30268	14.30618	24.072806



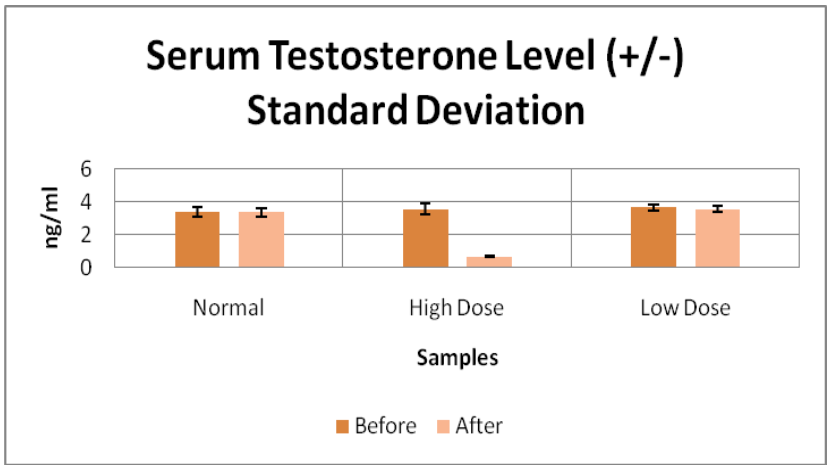
The computed t is found to be higher than the critical $t_{0.01}$, in case of High dose group of animals. So, the probability P of getting the observed differences between the means by chance due to random sampling amounts to less than 0.01, which may be considered very

low. Hence, H_0 may be rejected. So the Body Weight of animals differ significantly after treatment ($P<0.01$).

In case of Normal group and Low dose group, $P>0.01$. So, Body weight in these groups does not differ significantly after the treatment.

Table. 2: Effect of leaf extract of Catharanthus roseus on serum testosterone level.

Serum Testosterone (ng/ml)						
	Before treatment			After treatment		
	Normal dose	High dose	Low dose	Normal dose	High dose	Low dose
	3.81	2.98	3.66	3.56	0.8	3.65
	3.12	3.68	3.89	3.01	0.6	3.72
	3.56	3.92	3.46	3.61	0.71	3.31
	2.91	3.79	3.35	3.01	0.69	3.21
	3.14	3.08	3.63	3.18	0.58	3.71
	3.72	3.74	3.81	3.68	0.7	3.67
Mean	3.376667	3.531667	3.633333	3.341667	0.68	3.545
SD	0.32	0.334444	0.153333	0.275	0.06	0.19

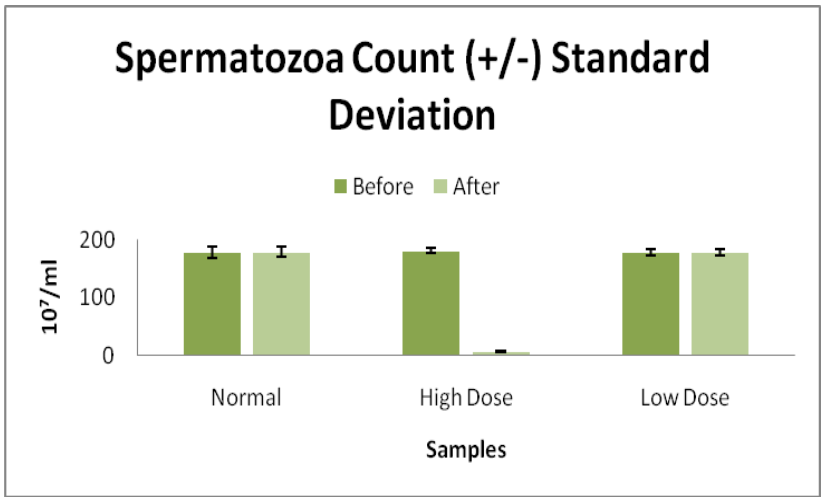


The computed t is found to be higher than the critical $t_{0.01}$, in case of High dose group of animals. So, the probability P of getting the observed differences between the means by chance due to random sampling amounts to less than 0.01, which may be considered very low. Hence, H_0 may be rejected. So the Serum Testosterone level of animals differ significantly after treatment ($P<0.01$).

In case of Normal group and Low dose group, $P>0.01$. So, Serum Testosterone level in these groups do not differ significantly after the treatment.

Table. 3: Effect of leaf extract of Catharanthus roseus on spermatozoal count.

Spermatozoa Count 10^7per ml						
	Before treatment			After treatment		
	Normal dose	High dose	Low dose	Normal dose	High dose	Low dose
	187.7	180.31	178.9	186.98	2.28	177.76
	160.8	182.7	189.81	163.76	7.65	186.54
	167.9	171.97	168.48	166.82	6.31	169.98
	183.4	176.88	173.63	183.9	7.83	171.42
	184	185.65	182.24	185.78	8.75	182.79
	186.98	187.87	179.61	185.42	7.41	180.6
Mean	178.4633	180.8967	178.7783	178.7767	6.705	178.1817
SD	9.408889	4.51	5.148889	8.991111	1.606667	5.128333



Normal value is close to that reported for Sprague-Dawley albino rats by Turner, Hartmann and Howards (1977), who detected 1· 84 (± 0·10 SEM) x 10^9 spermatozoalml in the cauda epididymis of adult animals, using a micropuncture technique.[11]

The computed t is found to be higher than the critical $t_{0.01}$, in case of High dose group of animals. So, the probability P of getting the observed differences between the means by chance due to random sampling amounts to less than 0.01, which may be considered very low. Hence, H_0 may be rejected. So the Body Weight of animals differ significantly after treatment ($P<0.01$).

Histological changes

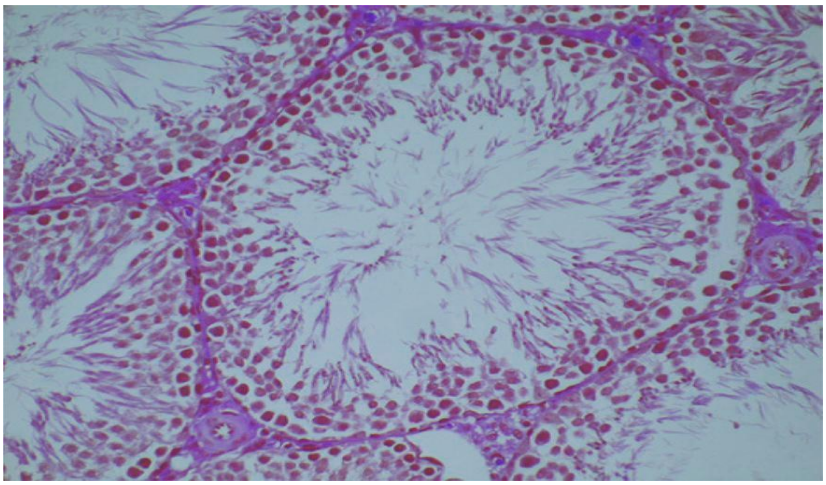


Fig. 1: Histology of normal testes.

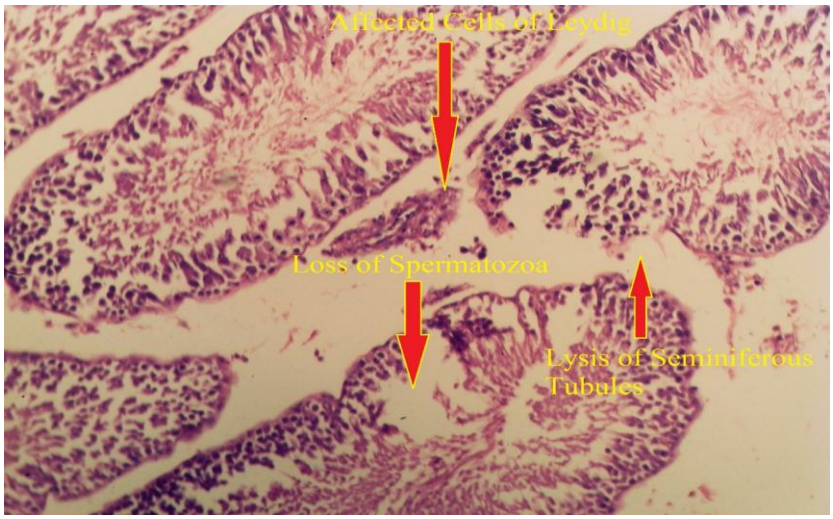


Fig. 2: Lysis of seminiferous tubule and loss of spermatozoa after treatment with CR leaf extract (high dose).

Lysis of Seminiferous tubules and loss of spermatozoa, leydig cells destruction are has been seen maximally in high dose group. But in case of normal and low dose group, there are minute changes.

DISCUSSION

The leaves and stems are the sources of dimeric alkaloids, vinacristine and vinblastine that are indispensable cancer drugs, while roots have antihypertensive, ajmalicine and serpentine.[12] The leaves are used traditionally in various regions of the world including India, West Indies as well as Nigeria to control diabetes.[13] In our experiment we have seen a slight decrease in body weight, which is not significant. It has been seen that two weeks of

daily treatment of various extract of Vinca rosea lead to a dose-dependent fall in blood sugar levels by 25%–50%. Effect was maximum till 14 days of treatment. Vehicle control animals were found to be slightly increased in their body weight but diabetic rats showed significant reduction in body weight during 14 days. Decrease in body weight was may be due to lesser food availability.

In rural areas, people often tear 3-4 leaves and eat each day to reduce their blood sugar as it's popularly known as antidiabetic. But it has some side effects in our reproductive system, very few people know about this fact. From this experiment and the results obtained one can conclude that CR leaf extract has a high range of side effect in our reproductive system. This can lower the blood cells as it is used as anticancer drug preparation. It contains Vincristine, Vinblastine, etc. Any of these elements may cause the changes in reproductive system. It has been observed that, vincristine is majorly responsible for the changes.

It is our kind duty to make people aware of the side effects of this CR leaf extract. It reduces the sperm count and serum testosterone level, which may cause infertility and death to the peoples using it regularly.

ACKNOWLEDGEMENTS

We are expressing our sincere thanks to Mr. Subrata Kumar Das, Laboratory attendant, Post Graduate Dept. of Physiology, Krishnath College (K.U.) for his co-operation.

REFERENCES

1. Spermatogenesis and cycle of the seminiferous epithelium. Hess RA, Renato de Franca L. s.l. : Adv Exp Med Biol, 2008; 636: 1–15.
2. The role of the Leydig cell in spermatogenic function. Ge R, Chen G, Hardy MP. Adv Exp Med Biol, 2008; 636: 255–69.
3. Effect of environmental contaminants on male reproduction. Saradha B, Mathur PP. Environ Toxicol Pharmacol, 2006; 21: 34–41.
4. Indian Medicinal Plants. Kritiker KR, Basu BD. 1975; 8: 785–8.
5. Antidiabetic effects of Catharanthus roseus in experimentally diabetic induced rat. M. Mostofa, M. E. Choudhury, M. A. Hossain, M. Z. Islam, M. S. Islam and M. H. Sumon, Bangl. J. Vet. Med., 2007; 5: 99–102.

6. Yadav PD, Bharadwaj NSP, Yedukondalu M, Methushala CH and Ravi Kumar A. Phytochemical evaluation of Nyctanthes arbortristis, Nerium oleander and Cantharanthus roseus. Indian Journal of Research in Pharmacy and Biotechnology, 2013; 1: 333-338.
7. Averal HI, Stanley A, Murugaian P, Polanisamy A and Akborsha MA: Specific effect of vincristine on epididymis. Indian Journal of Experimental Biology, 1996; 34: 53-56.
8. Murugavel T, Ruknudin A, Thangauelu S and Akbarsha MA: Antifertility effect of Vinca rosea (linn.) leaf extract on male albino mice. A sperm parametric study. Current Science, 1989; 58: 1102-1103.
9. Murugavel T and Akbarsha MA. Antispermatogenic effect of Vinca rosea Indian Journal of Experimental Biology, 1991; 29: 810-812.
10. Mathur R and Chaudan S. Antifertility efficacy of Catharanthus roseus Linn: a biochemical and histological study. Acta Eur Fertil., 1985; 16: 203-205.
11. In vivo sodium, potassium and sperm concentrations in the rat epididymis. Turner TT, Hartmann PK & Howards SS. Fertility and Sterility., 1977; 28: 191-194.
12. R. N. Kulkarni, K. Baskaran, R. S. Chandrashekara and S. Kumar, "Inheritance of morphological traits of periwinkle mutants with modified contents and yields of leaf and root alkaloids," Plant Breeding, 1999; 118(1): 71-74.
13. R. C. Cowley and F. C. Bennett, "Vinca rosea," Australian Journal of Pharmacy, 1928; 9: 61.