

Evaluation of the spermatogenetic effect of the root of *Ricinus communis* Linn. in male Wistar rats.

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Abstract

As infertility becoming the burning issue day by day and Male infertility factors contribute to approximately 30% of all infertility cases and some of the treatments are expensive that every infertile couple cannot take the benefit of it. It is important to introduce a traditional unique and inexpensive treatment for such a society. Ricinus communis is a widely used drug in Ayurvedic medicine for different diseases. Acharya Charaka mentioned the root of Ricinus communis in Agryasangraha which acts as an aphrodisiac i.e. improves the quality, quantity, and abnormality of sperm. Therefore spermatogenetic effects of the root of Ricinus communis were studied in male Wistar rats. The effect of decoction of the root of Ricinus communis on the serum testosterone, sperm count, testis, and on the weight of testis and body weight was studied for the assessment of spermatogenetic activity.

The root decoction elevated the level of serum testosterone, sperm count, and body weight significantly. It may be concluded that due to its medicinal properties Root of R. communis is effective against male infertility and shows a gain in weight, testosterone, sperm count and acts as good aphrodisiac, hence increases the spermatogenesis.

Keywords – Aphrodisiac drug, Ayurved system, Ricinus communis, Spermatogenesis.

Introduction

In Indian culture, parenthood has always been most important from the very beginning and still, nowadays it occupies a primary place in our civilization. But at present day's male infertility is unexpectedly rising & putting up a bit worried for both patients as well as physicians. Infertility affects around 1 in 7 couples of reproductive age, often causing considerable mental distress. The 2017 amendment report estimates that the fertility rate of Indians (calculated as the number of children born to a woman), has plummeted by more than 50 percent, from 4.97 for the duration of the 1975-1980 phase to 2.3 for the current stage of 2015-2020. By 2025-2030, the report projects, the rate will drop further to 2.1, touching 1.86 from 2045-2050 and 1.78 from 2095-2100¹. In men, infertility may consequence from impaired sperm quality, quantity, and abnormality. Azoospermia or Oligospermia is usually idiopathic but may be a consequence of hypogonadism². One more problem which is difficult for the present generation is male sexual dysfunctions i.e. impotence. India, with its crowded millions, it is contradictory to face Infertility as trouble, though to an infertile couple who are hoping for a child, it is a stressful aspect. From the beginning, however, the inconsistencies in the attitudes of the male towards marital infertility have been mysterious, and no attempt was made to study the part that he might play in infertility. Thus, for centuries the woman was to blame for any failure to reproduce, and the man has remained curiously separate from any blame. Even though there are advents of so many modern techniques, such as Assisted Reproductive Technologies (ART), such as IVF (in vitro fertilization). ART does not cure or treat the cause of infertility but they can help couples attain a pregnancy, even if the man's sperm count is very low. All these treatments may be expensive, prolonged which cannot possible for every couple and there is no reassurance about the fertility after these treatments. It ultimately results that some men have to deals with the reality that nothing can be done about their infertility. Traditional systems of medicine carry on being broadly adept on many accounts. Now, the entire world is considering the *Ayurvedic* herbal drug to find relief from not curable ailment because of their acceptable result and comparative safety compared to modern drugs. Among ancient civilizations, India has been well-known to be rich depository of medicinal plants. About 8,000 herbal remedies have been codified in AYUSH systems in INDIA. Recently, World Health Organization estimated that 80 percent of people worldwide rely on herbal medicines for some aspect of their primary health care needs. According to WHO, around 21,000 plant species have the potential for being used as medicinal plants.³ Ayurveda delineate the uses of *R. communis* for the management of different disease conditions in Classical texts. In *Charak samhita*, Acharya Charaka mentioned about root of *R. communis* (*Erandamoola*) in *Agryasangraha* which acts as a aphrodisiac (*Vrushya*) i.e. improves the quality, quantity and abnormality of sperm “एरण्डमूलं वृष्यवातहराणं”⁴. Here, the term *Vrushya Karma* is projected to deal with both the problems of male infertility (*Vandyatva*) and male sexual dysfunctions (*Klaibya*). In this article, an attempt has been made to assess the root of *R. communis* as an aphrodisiac on scientific base which may be ray of hope in case of male infertility.

Material and Method

Collection, authentication, and preparation of decoction of the root of *R. communis*-
The field sample of *R. communis* root was collected in the month of February from the field of Takali village, Wardha, and further authenticated from FRLHT (The Foundation for Revitalisation of Local Health Traditions), Bangalore. The drug was powdered and coarsely powdered root of *R. communis* Linn. was taken in wide-mouth steel vessel containing distilled water in the ratio of 1:8 (*R. communis* Linn: distilled water). This mixture was heated on low flame until a total content reduces up to the 1/4th quantity. After a reduction of 1/4th quantity, the kwath was used for further study.

*Note: Daily fresh decoction was prepared and used.

Housing and handling of an animal-

All the animals were out in the open to 12 hours light and 12 hours dark cycle with the comparative humidity of 50 to 70% and the ambient temperature was $22 \pm 03^{\circ}\text{C}$. All animals were kept on the similar ecological surroundings.

Spermatogenetic activity-

18 Male Wistar rats weighted from 300gm to 450gm were used for the experimentation. The rats were obtained from Animal house DMIMS (DU), Wardha. The experimental protocols were approved by the Institutional Animal Ethics Committee (DMIMS(DU)/IAEC/2016-17/12, dated 11/12/2016) in accordance with the guideline formulated by CPCSE, India. These animals were fed by Amrut brand rat pellet feed supplied by Pranav Agro Ltd. throughout the study period. Drinking water was given in polypropylene bottles with a stainless steel sipper tube. The animals fasted overnight before experimentation.

The animals were divided into 3 groups of 6 animals each. Animal of the group I, II, and III were administered orally with (1 ml/day) normal distilled water (control), a minimum dose of decoction(20gm/bodyweight/day)and with a maximum dose of decoction(30gm/bodyweight/day) respectively for 45 days. The dose of the drug was calculated by extrapolating the human therapeutic dose to rat on the basis of body surface area ratio (conversion factor 0.018 for rat) by referring to the table of Paget and Barnes (1964)⁵.

Route of administration: Oral route.

Dose Duration: Once a day (In the morning 10 am to 10.30 am)

Study Duration: 45 days.

Blood collection for serum testosterone was done on 0 day and 47th day of treatment i.e. before and after treatment through orbital region as shown in plate V. The Blood was collected in the plane bulb (plate V: fig. 1 to 3) and send to the central research laboratory of DMIMS. Collected blood was kept for 45 min at room temperature and serum was obtained after centrifugation for 15 min in serum separator tubes (Vacutainer; Becton Dickinson, Rutherford, NJ). Serum was prepared (plate VI) testosterone was assayed using a testosterone ELISA kit (Diagnostic Products Corp., U.S.A). Enumeration of Sperm count was done through Cauda Epididymis by the PESA method. Then the sample was sent to the pathology laboratory of DMIMS. On 47th day rats from each group were anesthetized with halothane and ketamine injection for Histopathology investigations. Part preparation of testis was done as shown in plate II fig. 1 to 3. Then the left testis and epididymis were isolated, preserved processed for light microscopy. The suturing was done by 2-0 catgut suture (round body) and post-operative care was taken.

Statistical analysis -

The results were analyzed by using the Unpaired t-test and the p-value of <0.05 was considered as significant.

Results

The results are presented in tables 1, 2, 3, and 4. The results indicate that the serum testosterone level, sperm count, and weight of rats were improved significantly in the animal

treated with 20gm/kg/day decoction. However, there were no significant changes in histopathological investigations.

Discussion –

Infertility is rising as one of the upsetting problems in the current world. It is common for 10% of women age 15 to 44. The cause can be due to the woman (33%), the man (33%) and by both sexes or due to unknown etiology (33%), approximately. A number of studies reflect on these aspects as per GBD study⁶⁻¹⁰. In India, the prevalence of infertility is around 23%. In a preliminary study by the World Health Organization's multi-center study, 45% of infertile men were found to be affected by oligozoospermia. Few of the related studies from this region have been reported¹¹⁻¹³. A number of studies of Ayurvedic preparations in different conditions have been reported¹⁴⁻¹⁸. The main causes of male infertility include varicocele, low or absent sperm count, sperm damage, or certain diseases. Risk factors for male infertility are toxins, alcohol, smoking, age, health problems and drug use, chemotherapy, radiation, and medicines, etc; sterility may be treated with conservative treatments, surgery, artificial insemination, or assisted reproductive technology, based on the couples test results and other factors but all these treatments may be highly expensive, time-consuming. Ayurveda describes numerous single drugs having an incremental effect on the libido as well as on the level of seminal parameters. This study aimed at enlightening and updating the available scientific information on the spermatogenetic activity of the root of *R. communis*. For this purpose animal, an experimental study was conducted in 18 Male Wistar rats. In this study, the efficacy of dose given to group II was seen more than that of the third group in all three aspects i.e. in sperm count, serum testosterone levels, and weight gain. At the last day, the efficacy of dose given to the group II (20gm/kg) was seen more than that of group III in all three aspects i.e sperm count, serum testosterone level, the effect on body weight, also the histopathological investigations show no significant changes in cells of the testis, epididymis, and tubular. It indicates that the drug is absolutely safe and does not create any adverse effects on organ on prolonged administration.

Conclusion

Spermatogenesis and male infertility are dependent upon the serum testosterone and sperm count. As a result, the root of *R. communis* improves the serum testosterone in rats at the dose of 20 gm/kg and multiple majors of sexual behavior (excitement). Therefore study suggests that the root of *R. communis* improves serum testosterone to treat sexual dysfunctions caused by low serum testosterone levels and low sperm count.

Table 1. Spermatogenetic effect of root of *Ricinus communis* in group I and II.

	Group	N	Mean	SD	SE	t-Value	P-Value
Sperm Count	Group I	6	27.83	5.00	2.04	-6.373	0.000
	Group II	6	43.17	3.13	1.28		
Testosterone	Group I	6	0.16	0.42	0.17	-2.432	0.035
	Group II	6	0.68	0.32	0.13		
Change in Weight	Group I	6	37.17	31.64	12.92	-1.686	0.123
	Group II	6	68.50	32.71	13.35		

Table 2 - Spermatogenetic effect of root of *Ricinus communis* in group II and III.

	Group	N	Mean	SD	SE	t-Value	P-Value
Sperm Count	Group II	6	43.17	3.13	1.28	2.395	0.038
	Group III	6	37.00	5.48	2.24		
Testosterone	Group II	6	0.68	0.32	0.13	1.041	0.322
	Group III	6	0.30	0.85	0.35		
Change in Weight	Group II	6	68.50	32.71	13.35	2.624	0.025
	Group III	6	29.83	15.25	6.22		

Table 3. Spermatogenetic effect of root of *Ricinus communis* in group I and III.

	Group	N	Mean	SD	SE	t-Value	P-Value
Sperm Count	Group I	6	27.83	5.00	2.04	-3.029	0.013
	Group III	6	37.00	5.48	2.24		
Testosterone	Group I	6	0.16	0.42	0.17	-0.364	0.724
	Group III	6	0.30	0.85	0.35		
Change in Weight	Group I	6	37.17	31.64	12.92	0.511	0.620
	Group III	6	29.83	15.25	6.22		

Table 4. Histopathological findings of testis and epididymis tissue

Groups	No. of animals	Histopathology result
Group I (Distilled water)	Rat 1	Testes – normal, no changes. Epididymis – congested.
	Rat 2	Testes – normal, congested on peripheral portion. Epididymis – some changes are seen.
	Rat 3	Testes – normal, no changes. Epididymis – congested
	Rat 4	Testes – normal, congested at peripheral site. Epididymis – congested
	Rat 5	Testes – normal, no changes. Epididymis – fibro fatty tissue are observed.
	Rat 6	Testes – normal, no changes. Epididymis – congested
Group II (20gm/bodyweight/day)	Rat 1	Testes – normal, congestion at peripheral portion. Epididymis – fatty tissue shows congestion.

	Rat 2	Testes – normal, mild congestion. Testis and Epididymis – Normal.
	Rat 3	Testes – normal, no changes. Epididymis – congested
	Rat 4	Testes – Normal, no changes. Testis and Epididymis – Normal with congested epididymis.
	Rat 5	Testes – normal, no changes. Fatty tissues with congestion.
	Rat 6	Testes – normal, no changes. Epididymis – congested
	Group III (30gm/bodyweight/day)	Rat 1
Rat 2		Testes – Normal. Epididymis – Normal.
Rat 3		Testes – normal, no changes. Epididymis – congested
Rat 4		Testes – Normal, no changes. Epididymis – Normal.
Rat 5		Testes – Normal, no changes. Epididymis – Normal.
Rat 6		Testes – Normal, no changes. Epididymis – Normal.

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