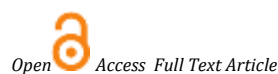


Available online on 15.06.2023 at <http://jddtonline.info>

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

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Research Article

Comparative Spermatogenesis Activity of Eranda Moola (*Ricinus Communis*) Collected In Different Seasons as per Dravya Samgrahana Kaala: An Experimental Study

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Article Info:

Abstract



Article History:

Received 11 April 2023
Reviewed 16 May 2023
Accepted 29 May 2023
Published 15 June 2023

Cite this article as:

Bingi A, Mallya SV, Comparative Spermatogenesis Activity of Eranda Moola (*Ricinus Communis*) Collected In Different Seasons as per Dravya Samgrahana Kaala: An Experimental Study, Journal of Drug Delivery and Therapeutics. 2023; 13(6):125-130

DOI: <http://dx.doi.org/10.22270/jddt.v13i6.5881>

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Introduction: Dravya sangraha is one of the criteria where different seasons are indicated for collecting different parts of the plants. As per ancient drug collection practice roots are to be collected in *Hemanth-Shishira* (winter) or *Greeshma rutu* (summer). *Eranda (Ricinus communis)* is an important medicinal plant roots of which are particularly indicated as *Vrishya* (spermatogenic). Hence study has been planned to conduct a comparative spermatogenic activity of *Eradamoola* collected in *Greeshma rutu* and *Pravarat rutu* and *Shishira ritu*. **Materials and methods:** Roots of *Eranda (Ricinus communis)* collected during *Pravrut Ritu* (EMP), *Greeshma Ritu* (EMG) and *Shishira Ritu* (EMS) shade dried separately, powdered. Spermatogenic activity study conducted on healthy albino rats divided among five groups. Testosterone was used as standard drug, whereas other three groups received sample collected in three different seasons for a period of 60 days. Next sperm analysis and histopathological study of prostate and seminal vesicle was conducted. **Discussion and conclusion:** *Eradamoola* (roots of *Ricinus communis*) has shown best spermatogenesis activity in experimental animals proving our ancient quotation as *Vrishya*. Also roots collected in different seasons have shown their difference among activity. Histopathological study of genital organs also shown positive result among spermatogenic activity.

Key words: *Dravya sangraha*, *Eranda (Ricinus communis)*, *Vrishya*, spermatogenesis activity

INTRODUCTION:

Ayurveda states the importance of Good Collection practice to achieve maximum efficacy of medicament¹. *Dravya sangraha* is one of the criteria where different seasons are indicated for collecting different parts of the plants². Plants show variation among physical and chemical properties as it grows in different seasons. Hence it is essential to collect the plant or part of the plant as medicine when it is rich in its phytoconstituents³. As per ancient drug collection practice roots are to be collected in *Hemanth-Shishira* (winter) or *Greeshma rutu* (summer)⁴.

Eranda (Ricinus communis) is an important medicinal plant where all parts of this drug are used in different pathological condition. Castor seeds are purgative, leaves used as analgesic, anti-inflammatory⁵. The roots are particularly indicated as *Vrishya* and *Vathara* ie analgesic and aphrodisiac⁶. *Vajikarana* is one among *Ashtanga Ayurveda*. It is mainly related to getting a healthy progeny⁷. *Vrishya dravyas* are those, which increase sexual vigor, improve seminal quality and enhance spermatogenesis⁸. Due to the presence of aphrodisiac activity and spermatogenic effect a *Vrishya dravya* is thought to be effective either in the treatment of Sexual dysfunction or Infertility or both in the male⁹. Though many herbs with spermatogenic property are mentioned in Ayurveda, few are

spermatogenic, few towards amending sperm production related matters.

Hence with all this background a study has been planned to conduct a comparative spermatogenic activity of *Eradamoola* collected in *Greeshma rutu* and *Pravarat rutu* and *Shishira ritu*.

MATERIALS AND METHODS:

Plant material¹⁰

The root of *Eradamoola (Ricinus cumunis)* belonging to the family *Euphorbiaceae* were collected from Natural habitat during the *Greeshma* (April-May), *Pravrut ritu* (May-June) and *Shishira ritu* (December- January) authenticated using floras and botanists opinion, sample deposited at SDM Centre for research in Ayurveda and allied sciences, Udupi.

Extraction and preparation of test sample

The root sample collected during *Pravrut Ritu* (EMP), *Greeshma Ritu* (EMG) and *Shishira Ritu* (EMS) shade dried separately, powdered and kept labelled and used for further study.

Methodology

Animal selection¹¹:

Healthy Albino rats were taken from animal house attached to SDM center for research and Allied sciences, Udupi. The experimental protocol was approved by IAEC with approval no SDMCRA/IAEC/ 07/01/2019. The animals were fed with normal diet, water and libitum throughout the study. The housing provided has the controlled lighting of 12:12 hour light and dark cycle. 25°C temperature and approximately 50% of relative humidity. They were kept in clean dry cages week before the beginning of the experiment to acclimatize with the experimental conditions. The animals were fed with standard pelleted diet (Lipton India Ltd, Mumbai) and distilled water ad libitum was maintained at 21°C-23°C under a constant 12hrs light and dark cycle. The animal care and experimental protocols were in accordance with CPCSEA/IA

Spermatogenetic Activity:

Preparation of animals¹²:

Healthy male Albino rats were housed in groups of six in each clean cage, the bedding material of the cages were removed and replaced thrice a week with fresh materials as often as necessary to keep the animals clean and dry. The animals were selected in such a way that they were free from illness, injury, disease and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. Only those animals which are healthy having weights 150-200 grams were selected and maintained at standard laboratory conditions.

Preparation and administration of doses¹³:

All the doses were prepared in distilled water using 5% Tween 80 solution as suspending agent and administered orally. In all cases, the concentrations were prepared in 1 ml/100g of body weight. The test substances were administered in a single dose using a gastric intubation tube

Test drug dose:

This will be calculated using following formula;

Human dose x body surface area constant of rats

$$= 20 \times 0.018 \times 5 / \text{kg body wt}$$

$$= 1.81 \text{ gm/kg}$$

Standard drug testosterone- 22.5mg/kg body wt.

Spermatogenesis activity;

Albino rats were randomly grouped into 5 groups of six animals each. Group I served as control. Group II serve as Standard with administration of testosterone once in month, orally in the calculated dose of 22.5 mg/kg once in 7 days for 60 days. Group III (EMG), Group IV(EMP), Group V(EMS) serve as the Test Group with administration of 1.81 gm/kg body weight, test drug collected in three different seasons. The above scheduled drugs were administered for a period of 60 days¹⁴.

After 60 days of drug administration animals of all groups were weighed and anaesthetized with ether. After anesthetization incision was made in the inguinal region and cauda epididymal tissue was identified. Cauda epididymal tissue was excised out carefully and transferred to normal saline(0.5 ml) and teased gently with forceps to liberate spermatozoa. Cauda epididymis suspension was incubated at 38°C for 5 minutes before testing and was examined for sperm count, motility, and sperm morphology assessment. Testis and seminal vesicles separated and send for histopathological study¹⁵.

Methodology¹⁶:

Sperm count:

Count was done with a Hem cytometer (Neuberger-improved counting chamber). After proper cleaning of the chamber, the cover slip placed in position and the thoroughly mixed semen solution was charged in between. The twenty-five smaller squares (400 smallest) were used for counting ie RBC chamber. Calculations were made to make the count in million/caudal epididymal tissue suspension.

Sperm motility:

Before doing the counting, the sperm motility was observed by simply viewing the sperm under microscope and counting the number of live cells in percentage under high power microscope. They were categorized into sperms showing rapid linear progressive, slow linear progressive and immotile.

Next the animals sacrificed by severing jugular vessels; their testis, seminal vesicles and ventral prostate dissected out and placed in normal saline.

Organs weighed in a monoplane balance and transferred to 10% formalin solution and processed for histopathological study following standard procedure (NIN Manual, 1993).

The data obtained from the sperm count, motility and cytoarchitecture of the testis, seminal vesicle, prostate from the test drugs groups were compared with those of control and standard group to arrive at an inference.

Statistical Analysis¹⁷:

The data were expressed as Mean $\pm$ SEM. Results were analyzed statistically by one-way analysis of variance (ANOVA) followed by Dunnett and Tukey's test. P value <0.05 was regarded as statistically significant.

RESULTS:

The results of the spermatogenetic activity of the *Erandamoola* (*Ricinus cumunis*) collected in Greeshma ruthu (EMG) and Pravrut ruthu (EMP) and Shishira ruthu (EMS)

Table 1: Effect of test drug on sluggish motility:

Groups		% change
Group 1 (Normal control)	12.37 $\pm$ 1.03	--
Group 2 (Standard)	25.71 $\pm$ 2.48**	107.84 $\uparrow$
Group III (EMG),	21.66 $\pm$ 4.61*	75.10 $\uparrow$
Group IV (EMP),	19.16 $\pm$ 1.57	54.89 $\uparrow$
Group V (EMS)	20.83 $\pm$ 1.37	68.39 $\uparrow$

Data: MEAN $\pm$ SEM,*P<0.05, **P<0.01

Table depicts the effect of Test Drug on Sluggish motility in Albino Rats. The standard drug showed 107.84% increase in sluggish motility parameter of sperm count. Whereas Group 3(EMG) showed 75.10%, Group 4(EMP) showed 54.89% increase on sluggish motility, while Group 5(EMS) showed 68.39% increase.

Table 2: Effect of test drug on non-motility:

Groups		% change
Group 1 (Normal control)	87.62 $\pm$ 1.03	--
Group 2 (Standard)	74.57 $\pm$ 2.45**	14.89 $\downarrow$
Group III (EMG),	78.33 $\pm$ 4.61*	10.60 $\downarrow$
Group IV (EMP),	80.33 $\pm$ 1.57	8.32 $\downarrow$
Group V (EMS)	79.16 $\pm$ 1.37	9.65 $\downarrow$

Data: MEAN $\pm$ SEM,*P<0.05, **P<0.01

Table depicts the effect of test Drug on non-motility of sperm in Albino Rats. The standard drug showed 14.89% decrease while Group 3 (EMP) showed 8.32% decrease on non-motility. Group 5(EMS) showed 9.65% decrease in non-Motility.

Table 3: Effect of test drug on sperm count:

Groups		% change
Normal control	2.24±0.42	--
Standard	5.42±0.61**	141.96↑
Sample 1(EMG)	3.68±0.78	64.28↑
Sample 2(EMP)	5.83±0.92**	160.26↑
Sample 3(EMS)	5.01±0.92*	123.66↑

Data: MEAN ± SEM, *P<0.05, **P<0.01

Table depicts the effect of Test Drug on Sperm count in Albino Rats. The standard drug showed 141.96% increase, while Group 2(EMG) showed 64.28%, whereas Group 3(EMP) showed 160.26% increase on sperm count. Group 3 (EMS) showed 123.66% increase in Sperm count in testing animals.

Table 4: Effect of test drug on normal morphology:

Groups		% change
Group 1 (Normal control)	80.12 ±0.58	--
Group 2 (Standard)	82.28 ±0.56*	2.69↑
Group III (EMG),	78 ±0.81	2.64↑
Group IV (EMP),	80.66 ±0.61	0.67↑
Group V (EMS)	81.16 ±0.30	1.29↑

Data: MEAN ± SEM, *P<0.05

The table depicts the effect of Test Drug on normal morphology of sperm in experimental animals. The standard drug showed 2.69% increase, whereas Group 3(EMG) showed 2.64%. Group 4 (EMP) showed 0.67% increase on normal morphology while Group 5 (EMS) showed 1.29% increase.

Table 5: Effect of test drug on amorphous head morphology:

Groups		% change
Group 1 (Normal control)	2.25±0.25	---
Group 2 (Standard)	1.57±0.29	30.22↓
Group III (EMG),	2.33±0.42	3.55↑
Group IV (EMP),	2.66±0.42	18.22↑
Group V (EMS)	1.83±0.30	18.66↑

Data: MEAN ± SEM

The table depicts the effect of Test Drug on amorphous head morphology in Albino Rats. The standard drug showed 30.22% decrease while Group 3(EMG) showed 3.55% increase. Group 3 (EMP) showed 18.22% increase on amorphous head morphology while Group 4 (EMS) showed 18.66% increase.

Table 6: Effect of test drug on hook less morphology:

Groups		% change
Group 1 (Normal control)	16.25±0.59	---
Group 2 (Standard)	14.85±0.63	8.61↓
Group III (EMG),	17.66±0.66	8.67↑
Group IV (EMP),	15.66±0.49	3.63↓
Group V (EMS)	15±0.73	7.69↓

Data: MEAN ± SEM

Table depicts the effect of Test Drug on hook less morphology in Albino Rats. The standard drug showed 8.61% decrease whereas Group 3(EMG) showed 8.67% increase. Group 4(EMP) showed 3.63% decrease on hook less morphology whereas Group 5(EMS) showed 7.69% decrease in hook less morphology Motility in Albino Rats.

Table 7: Effect of test drug on curved tail morphology:

Groups		% change
Group 1 (Normal control)	1.37±0.26	---
Group 2 (Standard)	1.28±0.18	6.56↓
Group III (EMG),	1.16±0.16	15.32↓
Group IV (EMP),	0.66±0.33	51.82↓
Group V (EMS)	1.33±0.21	2.91↓

Data: MEAN ± SEM

Table depicts the effect of Test Drug on curved tail morphology in Albino Rats. The standard drug showed 6.56% decrease whereas Group 3(EMG) showed 15.32% decrease. Group 4 (EMP) showed 51.82% decrease while Group 5(EMS) showed 2.91% decrease in curved tail morphology Motility in Albino Rats.

Table 8: Effect of test drug on testis weight:

Groups		% change
Group 1 (Normal control)	3.00±0.09	---
Group 2 (Standard)	2.23±0.11**	25.66↓
Group III (EMG),	2.53±0.20	15.66↓
Group IV (EMP),	2.49±0.14*	17↓
Group V (EMS)	2.50±0.07*	16.66↓

Data: MEAN ± SEM, *P<0.05, **P<0.01

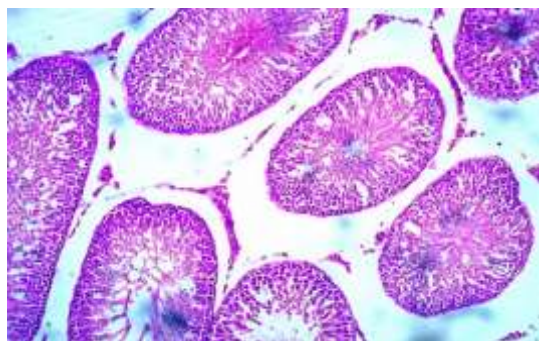
The table depicts the effect of Test Drug on testis weight motility in Albino Rats. The standard drug showed 25.66% decrease whereas Group 3(EMG) showed 15.66% in weight of testis, whereas Group 4(EMP) showed 17% decrease and Group 5(EMS) showed 16.66% decrease in testis weight.

Table 9: Effect of test drug on seminal vesicle weight:

Groups		% change
Group 1 (Normal control)	0.9±0.09	--
Group 2 (Standard)	0.58±0.02*	35.55↓
Group III (EMG),	0.61±0.05*	32.22↓
Group IV (EMP),	0.61±0.05*	32.22↓
Group V (EMS)	0.57±0.08*	36.66↓

Data: MEAN ± SEM, *P<0.05

The standard drug showed 35.55% decrease in weight of seminal vesicle, whereas Group 3 (EMG) showed 32.22% decrease and Group 4 (EMP) showed 32.22% decrease on seminal vesicle weight. Group 5 (EMS) showed 36.66% decrease in this study.

Histopathological study:**PHOTOMICROGRAPH OF TESTIS**

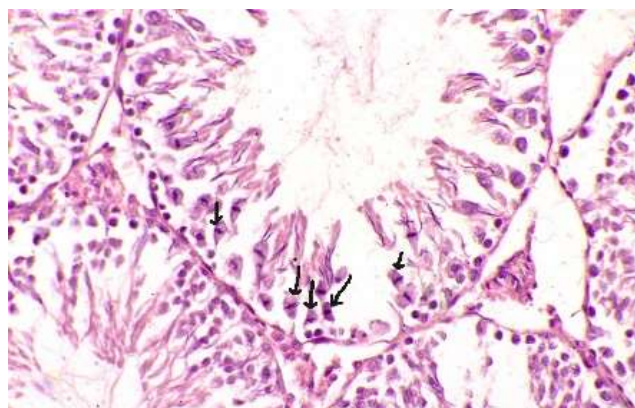
Control R1



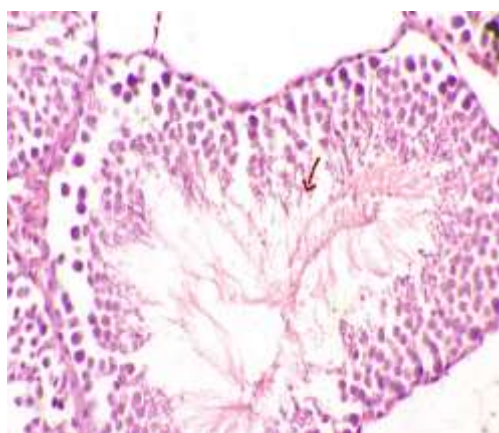
Standard



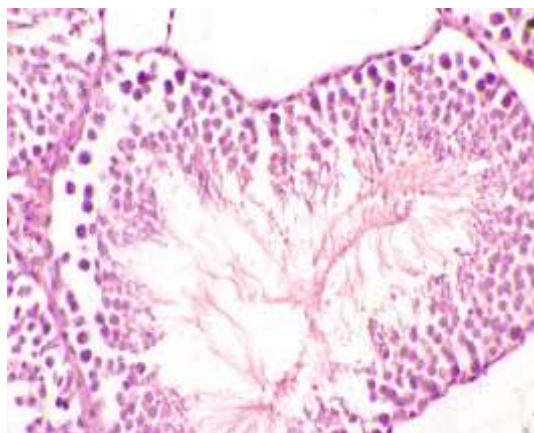
Group 3



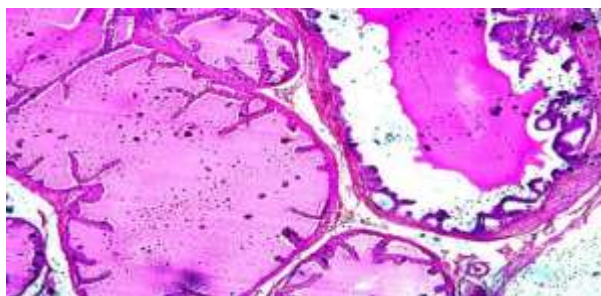
Group4



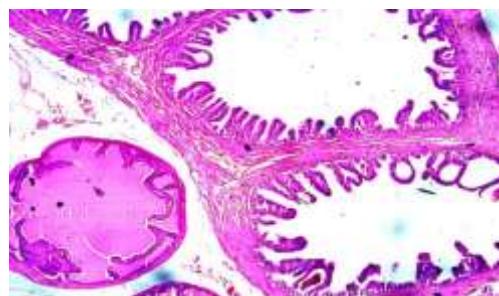
Group 5



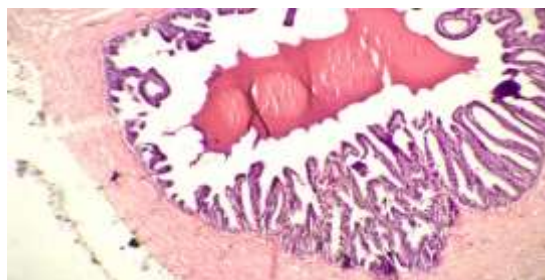
Group 5

PHOTOMICROGRAPH OF SEMINAL VESICLE

Control R1



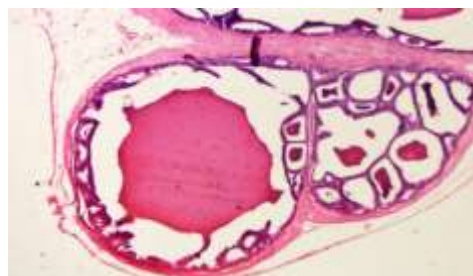
Standard



Group 3



Group 4



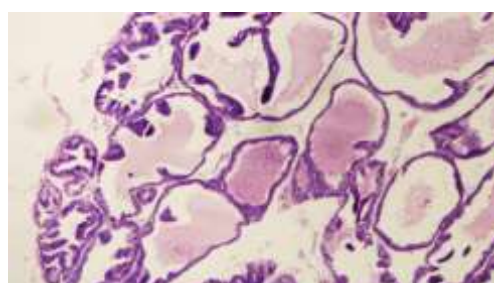
Group 5



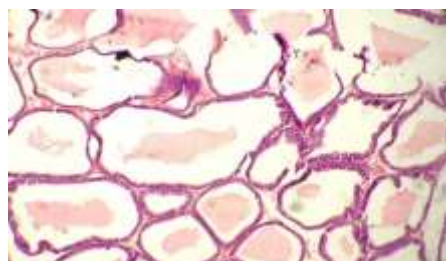
Group 5

PHOTOMICROGRAPH OF PROSTATE

Group 1 Control



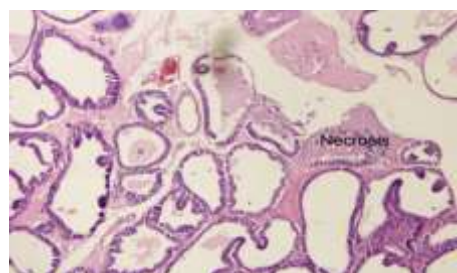
Group 2 Standard



Group 3



Group 4



Group 5



Group 5

Report on Histopathology:

Standard group of testis showed normal histology, whereas other three test groups showed small and medium tubules. Histology of prostate in standard group showed epithelium and secretions, whereas all test groups presented dilated acini with increased secretion. Standard group of seminal vesicle showed branched epithelium and secretion in the lumen, whereas test groups filled with secretions.

DISCUSSION:

The present research work has been carried out with a view to provide a scientific basis for the claims made in various Ayurvedic texts regarding the VrshyaKarma of Eranda moola (*Ricinus cummunis*) collected in different season as per traditional drug collection practices.

Albino rats were randomly grouped into 5 groups of six animals each. Group I served as control. Group II serve as standard with administration of testosterone once in week, orally in the calculated dose of 22.5 mg/kg once in 7 days for 60 days. Group III (EMG), Group IV (EMP), Group V (EMS) serve as the Test Group with administration of 1.81 gm/kg body weight, test drug collected in three different seasons. The above scheduled drugs were administered for a period of 60 days.

After 60 days of drug administration animals of all groups were weighed and anaesthetized and Cauda epididymis suspension was was examined for sperm count, motility, and sperm morphology assessment. Testis, prostate and seminal vesicles separated and histopathological study conducted.

Among Sluggish motility standard drug showed 107.84% increase in sluggish motility parameter of sperm count. Whereas Group 1(EMG) showed 75.10%, Group 2 (EMP) showed 54.89% increase on sluggish motility, while Group 3(EMS) showed 68.39% increase.

Non-motility of sperm Group 3(EMP) showed 8.32% decrease on non-motility while Group 4(EMS) showed 9.65% decrease in non-Motility.

The standard drug showed 141.96% increase, in sperm count while Group 3(EMG) showed 64.28%, whereas Group 4(EMP) showed 160.26% increase on sperm count. Group 5 (EMS) showed 123.66% increase in Sperm count in testing animals. The standard drug showed 2.69% increase, in normal morphology whereas Group 3(EMG) showed 2.64%. Group 4 (EMP) showed 0.67% increase on normal morphology while Group 5 (EMS) showed 1.29% increase.

The standard drug showed 30.22% decrease in amorphous head morphology while Group 3(EMG) showed 3.55% increase. Group 3 (EMP) showed 18.22% increase on amorphous head morphology while Group 4 (EMS) showed 18.66% increase.

The standard drug showed 8.61% decrease in hook less morphology whereas Group 3(EMG) showed 8.67% increase. Group 4(EMP) showed 3.63% decrease on hook less morphology whereas Group 5(EMS) showed 7.69% decrease in hook less morphology Motility in Albino Rats.

The standard drug showed 6.56% decrease among curved tail morphology whereas Group 3(EMG) showed 15.32% decrease. Group 4 (EMP) showed 51.82% decrease while Group 5(EMS) showed 2.91% decrease in curved tail morphology Motility in Albino Rats.

The standard drug showed 25.66% decrease in weight of testis whereas Group 3(EMG) showed 15.66% in weight of testis, whereas Group 4(EMP) showed 17% decrease and

Group 5(EMS) showed 16.66% decrease in testis weight.

The standard drug showed 35.55% decrease in weight of seminal vesicle, whereas Group 3 (EMG) showed 32.22% decrease and Group 4 (EMP) showed 32.22% decrease on seminal vesicle weight. Group 5 (EMS) showed 36.66% decrease in this study.

CONCLUSION:

Erandamoola (roots of *Ricinus communis*) has shown best spermatogenesis activity in experimental animals proving our ancient quotation as Vrishya. Also roots collected in different seasons have shown their difference among activity. Histopathological study of genital organs also shown positive result among spermatogenic activity.

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