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FERTILITY EFFECT OF *CYCAS CIRCINALIS.L* EXTRACT ON MALE WISTER RATS

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ABSTRACT

Infertility is a major public health concern. In Siddha System of Medicine many herbs were used for treating male sexual disorders. The use of herbs remarkable increased over the past few years and researcher now focuses on herbs. The present study was taken to analyze the fertility effect of an herb *Cycas circinalis* on male albino rats. A total of 18 healthy adult male albino rats were taken and divided into 3 groups with 6 rats in each group. One group of animal was administered orally *Cycas circinalis* extract (200mg/kg bodyweight) and compared to the normal control and positive control albino rats given testosterone 10µg/kg body weight subcutaneously. Various parameters were compared among the groups and the drug's efficacy was analyzed. The administration of the drug showed significant positive results in positive control followed by experimental group. Since the synthetic hormonal preparation have grave side effects it's better to go with herbal aphrodisiacs for better results without any side effects.

KEYWORDS: Infertility, *Cycas circinalis*, Testes, Sperm, Histomorphometry, Seminiferous tubules

INTRODUCTION

Infertility is a major public health concern. Management of infertility therefore requires the keenest insight, the tactfulness and utmost compassion.[1] The treatment of certain sexual disorders with pituitary hormones have not yield favorable results. [2, 3] There is a remarkable increase in the use of herbs over the past few years and research interests have focused on various herbs. Many herbs were used for treating male sexual disorders traditionally since ancient times. *Cycas circinalis .L* (Family –Cycadaceae), a sago palm commonly known as Madana Kaman in Tamilnadu. The genus is native of eastern and southeastern asia and is cultivtated in many tropical and subtropical areas for ornamental purpose.[4] The male sago cone has aphrodisiac activity.[5] The present research was undertaken to study the aphrodisiac effect of *Cycas circinalis*. The study shows some interesting results on the effect of *Cycas circinalis* on the fertility of male albino rats.

MATERIALS & METHODS

a) Animal selection and drug administration

A total of 18 male albino rats (Rattus *norvegicus albinus*) of wister lineage with an average weight of 130 – 140 gm were housed in the experimental animal unit. The rats were fed with standard rat feeds and given fresh water, acclimatized on a 12 hour light & 12 hour dark schedule [6]. The study was approved by Saveetha University Animal ethical committee approval reference no- ANAT.005/2012. Rats were kept in cages which were cleaned twice a week. The rats were distributed with 2 rats in each cage which was selected randomly 6 for control, 6 for positive control and 6 for experimental group. The cages were labeled with group, weight of the animal and dosage of the drug. All the rats were placed under the same environment and management conditions. [7] After a week of stabilization the C.circinalis extract was administered orally using infants feeding tube (oral gavage) to the experimental group (200mg/Kg body weight). The positive control was given testosterone

10µg/kg body weight subcutaneously, biweekly on alternate days. The normal control rats were fed with the same amount of sterile water. The drug was administered orally once a day regularly in the morning at a fixed time for 30 days for all the experimental animals. Rest period of about 10 days were given to all the animals after feeding the drug.

b) Sample collection

The rats were anaesthetized using Xylazine & ketamine [8]. The jugular vein was traced out and using disposable syringe about 2ml of blood was withdrawn. The rat was cut open by midline thoraco-abdominal incision and the heart was perfused with buffered formalin. After perfusion a midline incision was made on scrotum and the testicles were removed. The spermatic cord was exposed. The testes were then separated from the epididymis with the scalped blade. [6, 9]

c) Measuring dimension of testes

The lengths, breadth, height of the testes were measured using vernier caliper and the volume of the testis were calculated using the Lambert's formula [10] (Volume = Length x Breadth x Height x 0.71 cu.cm) and the testes were weighed and dropped into gendre's fluid for 48 hrs of fixation. The relative weight of testes was also known as gonado-somatic index (GSI) were calculated with the help of following formula (GSI = Weight of testes I grams / Body Weight in grams x 100) where GSI (Gonado Somatic Index), weight of Testes (Absolute weight in gram) and body Weight (weight of rats on the 40th day in gm). [11, 12] After calculating GSI, the values were tabulated.

d) Semen analysis

The semen samples were there after collected from the cauda epididymis by milking out on a glass slide and mixed with one drop of 2.9% sodium citrate. [6] The semen was collected from any one epididymis, either right or left randomly selected. The other side epididymis was utilized for smear preparation. The sperm suspension in the slide was drawn into a white blood cell pipette and diluted to 1:10 with normal saline. Improved double Neubauer ruling Chamber (Depth 1/10 mm) was used for counting the spermatozoa.

Smears were prepared from the spermatozoal samples. The slides were stained by Papanicolaou stain Fig 1. [13] The morphological characteristic of the sperm cells in all the smears were observed under oil immersion (100 X). The following abnormalities were noticed both in the control and experimental groups as described by headless tail, rudimentary tail, curved mid piece, curved tail, looped tail, bent mid piece, tailless head, bent tail. [6, 14] The percentage of normal and abnormal sperm cells were tabulated for both control and experimental group, by counting 200 sperm cells per smear and three smears per rat. The data's were analyzed by Chi-Square test.

e) Hormone analysis

Serum was separated; hormone analysis was done using ELISA analyzer.

f) Histomorphometry of testes

The stained slides were carefully observed for histological changes and morphometric analysis was done. The mean diameter was taken from the 50 seminiferous tubules per section of testes. The final average diameter of the seminiferous tubules of each animal was tabulated. The mean, standard deviation, Standard error mean were calculated and tabulated .Further the data's were analyzed by ANOVA using Graph pad software quick cals online calculator for scientist.

RESULTS

The mean weight of albino rats, volume and weight of testes, gonadosomatic index, sperm count, testosterone hormone level and diameter of seminiferous tubules were tabulated for control and experimental rats separately (Table 1) and analyzed by ANOVA to prove that the data's were statistically significant.

DISCUSSION

The study shows the steroidogenic activity on weight of the experimental rats. The positive control and experiment animal shows a marked increase in testosterone hormone. The anabolic effect induces an increase in the body weight of the animal. The initial weights of all the animals were noted down on day 1. After a period of 40 days, the mean body weight of control rats 220± 1.88, experimental rat's 219.33 ±1.71 gm was found to be increased, when compared to control 181.17±1.90 gm Table 1. As the data were analyzed by ANOVA, the P value was found to be highly significant on day 40 (0.0001) this was due to the anabolic effect of synthetic testosterone hormone and Cycas circinalis which was administered.

The histological analysis of testes showed a significant hypertrophy and increase in size of seminiferous tubules which resulted in increase of weight of the gonads Fig 3 & 6. The mean volume and the weight of control and experimental rats were compared, and found to be increased in both positive control experimental (1.36±0.12 cu.cm and 1.42±0.02

gm) and experimental (219.33± 1.71 and 1.28± 0.05 when correlated with the control 0.83±0.05 cu.cm and 0.95±0.01 gm Table 1. The reason for increase is due to an elevated testosterone level which in turn increases the diameter of the seminiferous tubules that induces spermatogenesis resulting in an increase in sperm count. [15] The p value was 0.0001 highly significant for weight of testes and 0.0001 for volume which is also significant.

Gonado Somatic Index (GSI) was taken as one of the parameters to correlate the increase in body weight and gonadal weight of the rats. [11, 12] The GSI of experimental (0.56± 0.01), positive control (0.62±0.01) when compared to control (0.48±0.02) was less Table 1. The statistical P value of GSI (0.0193) which was significant as the weight of the animal and weight of the gonads are directly proportional to each other. Mitra et al reported that the count was 55.33±2.47 million\ml in single cauda epididymis of albino rats using phosphate buffer as a diluting fluid and the experimental animal showed increased counts up to 81.50±2.70 million\ml. [15] The present study showed with a mean sperm count of 30.86±0.40 million\ml in control, positive control 56.4±0.81 million\ml and 50.41±0.72 million\ml in experimental Table 1 which has brought out a marked increase in count, due to the effect of testosterone on sperm count via., the influence on spermatogenesis. The data analysis showed a P value of 0.0001 which is proven to be highly significant.

Oyeyemi et al studied the morphology of spermatozoa and categorized the abnormal and normal spermatozoa. [16] Sperm cell morphology also includes primary and secondaryabnormalities, according to the classification by Noarkes et al. In this study about 8% of abnormal spermatozoa were found in control, whereas a much less 2.9% in experimental group thus producing good quality of sperm and the data's analyzed by Chi-Square test proved to be highly significant (0.0001).

Experimental rats showed pronounced hypertrophy of seminiferous tubules in testes. The interstitial tissues had been reduced to a small extent, while the tubular volume was much increased among experimental Fig 3 & 6 but less in control Fig 2 & 5. The drug induced the spermatogenesis without causing any damage to the seminiferous tubules. The spermatozoa completely filled the lumen of seminal tubules of experimental (80-87%); the remaining tubules are either empty or filled with few scattered spermatozoa when correlated with control (54-60%) were filled with sperm cells or the remaining was found to empty. The mean diameter of the tubules was charted out and found to be 258.62±3.22µm in control, with a difference in experimental 271.80±7.37µm Table 1 and when further analyzed by ANOVA, showed a much significant P value of 0.001.

CONCLUSION

The fertility effect of *Cycas circinalis* was compared to that of a synthetic hormonal preparation and was found to be having almost an equal effect. Since the synthetic hormonal preparation have grave side effects it's better to go with herbal aphrodisiac for better results without any side effects. Even though *Cycas circinalis* may take a long period to show its effect on improving the fertility, drastic side effects can be prevented by avoiding synthetic hormonal preparations. The *Cycas circinalis* administration has shown significant positive results in improving various parameters involved in maintaining maleness. This study has given us a definite hope about the efficacy of the drug. This is a small part of vast PhD research work other research papers linked to this study were published in many journals.[17,18,19,20] The alkaloid specificity of the drug has to be further studied with more parameters with the aids of advanced technology. The study done in animal if extended in humans and if found to be equally effective; will turn out to be a boon for infertile couples who were anxious to conceive.

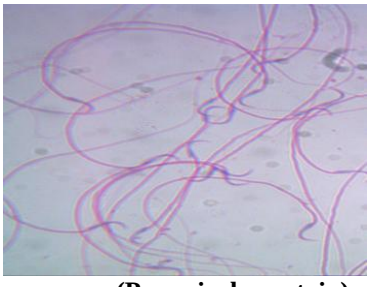

Figure 1. Semen smear (Papanicolaou stain)

Histological analysis - young rat testis low magnification 10 X

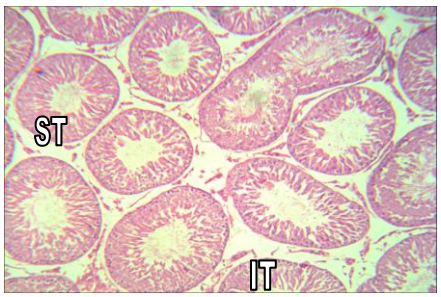

Figure 2. Control Rat testis

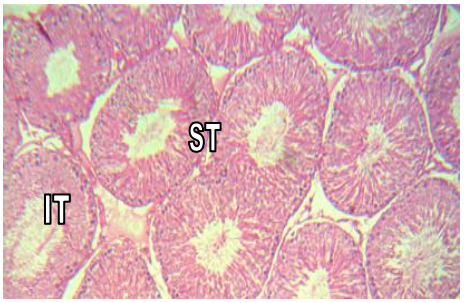

Figure 3. Experimental Rat testis

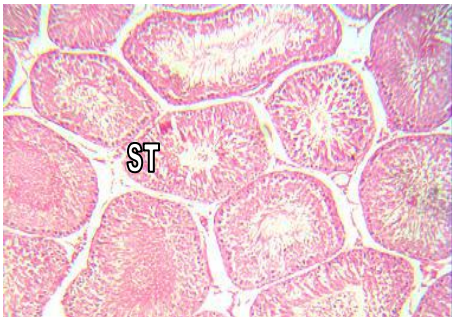

Figure 4. Positive Control Rat testis

Histological analysis - young rat testis high magnification 40 X

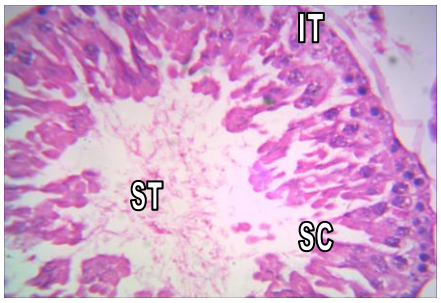

Figure 5. Control Rat testis

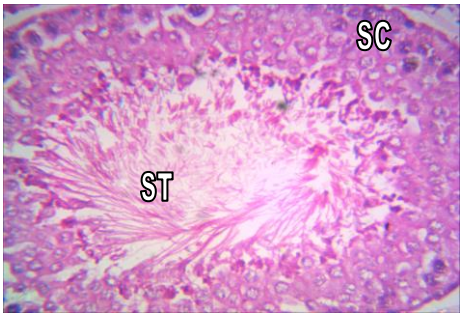

Figure 6. Experimental Rat testis

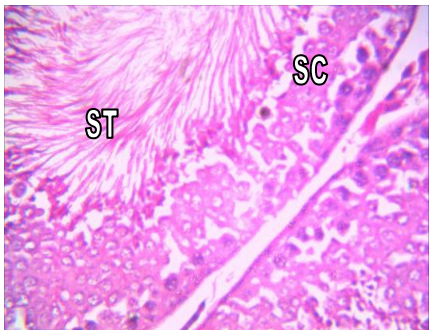

Figure 7. Positive control Rat testis

ST-Seminiferous tubule , IT-Interstitial tissues, SC-Sertoli cells, LC- Leydig cellss

Table 1: Various Parameters of the Study

S.no	Parameters	Normal Control	Positive Control	Experimental rats
1	Mounting Index(MI)	6.58 ± 1.80	7.34 ±1.24	7.21±1.41#
2	Total sexual behavior (TSB)	186.20 ± 0.67	203.40 ± 0.84	210±0.87***
3	Weight of rats (gm)	181.17± 1.90	220± 1.88	219.33 ± 1.71***
4	Volume of testes (cu.cm)	0.83± 0.05	1.36 ± 0.12	1.28 ± 0.05***
5	Weight of Testes (gm)	0.95 ± 0.01	1.42 ± 0.02	1.13 ± 0.02***
6	Gonado somatic index (GSI)	0.48 ± 0.01	0.62 ± 0.01	0.56 ± 0.01***
7	Sperm count (millions/ ml)	30.86 ± 0.40	56.4 ± 0.81	50.41± 0.72***
8	Testosterone hormone level (ng/ml)	2.26 ± 0.05	4.26 ± 0.22	3.01± 0.05***
9	Diameter of seminiferous tubule (μm)	258.62 ± 3.22	286.21 ± 8.6	271.80± 7.37*

*Values are expressed as Mean ± SEM, Number of animals per group was 6, *P<0.05, **P< 0.01, ***P<0.001, # - Statistically not significant, n – number of animals, SEM – Standard Error Mean*

REFERENCES

1. Arora HL, Subbaro VV, Gupta ML. Effect of Speman on Teates- A histological study. Probe, 1973; XII (4): 197-200.
2. Hotchkiss RS. Effect of massive doses of testosterone propionate upon spermatogenesis. J Clin Ender, 1944; 4: 117-120.
3. Grollman A. Clinical endocrinology and its physiological basis. JB Lippincott Company:Philadelphia; 1966: 122-143
4. Abeer Moawad, Mona Hetta , Jordan K Zjawiony, Melissa R Jacob, Mohamed Hifnawy, and Daneel Ferreira, Phytochemical Investigation of Cycas circinalis and Cycas revolute Leaflets: Moderately Active Antibacterial Biflavonoids, Planta Med, 2010; 76(8) :796–802.
5. Murugesa Mudaliar, Siddha Materia Medica (medicinal plant division),1996, Herb. 439, 706.
6. Ufaw. The Handbook on the management of laboratory animals. Churchillivingstone Edinburgh, London; 1976, pp.46.
7. Misra L, Wagner H. Extraction of bioactive principle from Mucuna pruriens. Indian J Biochem Biophys 2007; 44: 56-60.
8. Paul Flecknell. Laboratory animal anaesthesia. 3rd ed., Elsevier publication: 2009, pp.187
9. Gay IW. Methods of animal experimentation. Academic Press: London; 1966,pp. 147
10. Ming Li Hsieh, Shih Tsung Huang, Hsin Chieh Huang, Yu Chen and Yu-Chao Hsu. The reliability of ultrasonographic measurements for testicular volume assessment: comparison of three common formulas with true testicular volume. Asian Journal of Andrology 2009; 11: 261–265.
11. Qamar Hamid, LiagatAliminhas, Sadaf Hamid, Anjuman Gal. Influence of cimetidine and bromocriptine on weight of rats and its relation with fertility. Journal of clinical medicine and research 2010; 2(2): 015-021.
12. RabiaLatif, Ghulam Mustafa Lodhi, Muhammad Aslam. Effects of amlodipine on seram testosterone, testicular weight and Gonado-Somatic Index in adult rats. J Ayubcollabhottabad 2008; 20(4): 8-10.
13. Culling CFA. Handbook of Histopathological and Histochemical techniques. 3rd ed., 1975: 49, pp. 213, pp. 491-92.

14. Adebowale Bernard Saba, olayinka AO, Mathew Olubenga Oyeyemi. Spermatozoa Morphology and characteristics of male wistar rats administered with ethanolic extract of Lagenariabreviflora. African journal of Biotechnology 2009; 8(7): 1170-1175.
15. Mitra SK, Muralidhar TS, Rao DRB. Experimental assessment of relative efficacy of drugs of herbal origin on sexual performance and Hormone levels in alcohol exposed and Normal rats. Phytotheraphy Research,1996; 10: 296-299.
16. Oyeyemi MO, oluwatoyin O, AjalaLeigh OO, Adesiji T, Fisayo. The Spermiogram of male wistar rats treated with aqueous leaf extract of veronica amygdalina: Folia Veterinaria 2008; 52(2): 98-101.
17. Senthil Kumar B, Vijaya Kumar J, Selvaraj R. An Acute Oral Toxicity Study of Cycas circinalis and Ionidium suffruticosum in Wister Albino Rats, International journal of Pharmaceutical Sciences Review and Research 2012;17(2): 97-100.
18. Senthil Kumar B, Vijaya Kumar J, Selvaraj R, Preliminary Phytochemical Screening of *Cycas circinalis (l.)* and *Ionidium suffruticosum (ging.)* International Journal of Pharmacy, 2013; 3(3):510-513.
19. Senthil Kumar B, Vijaya Kumar J, Selvaraj R. Evaluation of Fertility Efficacy of Ionidium Suffruticosum Extract on Senility Induced Sterility of Male Albino Rats. IJCRR. 2013; 5(6): 98-103.
20. Senthil Kumar B, Vijaya Kumar J, GCMS Analysis of Bioactive constituents from the cycas circinalis and Ionidium suffruticosum, International Journal of Pharmaceutical sciences Review and Research. 2014; 28(2): 197-201.