IJPSR (2018), Volume 9, Issue 10 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 17 January, 2018; received in revised form, 19 April, 2018; accepted, 13 May, 2018; published 01 October, 2018

FERTILITY EFFECT OF *CYCAS CIRCINALIS* AND *IONIDIUM SUFFRUTICOSUM* IN SENILITY INDUCED STERILITY OF MALE WISTAR RATS - A HISTOMORPHOMETRIC STUDY

B. Senthil Kumar [*1] and J. Vijaya Kumar [2]

Department of Anatomy [1], Vinayaka Mission's Kirubananda Variyar Medical College and Hospital, Vinayaka Mission's Research Foundation, (Deemed to be University), Salem - 636308, Tamil Nadu, India.
Department of Anatomy [2], Saveetha Medical College and Hospital, Chennai - 602105, Tamil Nadu, India.

Keywords:

Cycas circinalis, *Ionidium suffruticosum*, Sterility, Infertility, Senility

Correspondence to Author:
Dr. B. Senthil Kumar

Assistant Professor, Department of Anatomy, Vinayaka Mission's Research Foundation, (Deemed to be University), Salem - 636308, Tamil Nadu, India.

E-mail: skdrchinu88@gmail.com

ABSTRACT: Ageing is associated with the diminished function of various tissues in the body. The gonadal function declines with age. In male, there is progressive atrophy of the sperm producing elements of the testes, resulting in diminished spermatogenesis. Many natural nontoxic herbs were found to enhance the fertility in male. The study is done to evaluate the effect of *Cycas circinalis* (Cc) and *Ionidium suffruticosum* (Is) in improving the fertility of senility induced sterility of male albino rats. A total of 24 healthy young male albino rats were selected and six animals were randomly distributed into 4 groups. The groups include normal control, positive control, experimental I and Experimental II. Normal control was administered sterile water orally; positive control was administered testosterone hormone subcutaneously (10 µg/kg body weight), experimental I group was administered ethanolic extract of *Cycas circinalis* (200 mg/kg body weight) and Experimental II group was administered ethanolic extract of *Ionidium suffruticosum* (200 mg/kg body weight) orally for 30 days. Various parameters such as sexual behaviour, weight of animals, dimension and weight of testes, hormone, semen and histological analysis were compared among the groups. Restitution of fertility was compared with the normal fertile controls rats. The drug's efficacy was compared by one way ANOVA among the groups and both the herbs showed significant improvement in all the parameters in experimental rats when compared to control rats. The herbs were found to be effective on the gonads of senility induced sterility of male Albino rats.

INTRODUCTION: Ageing is associated with the diminished function of various tissues in the body. This decline in the organism's capacity for optimal functioning may be attributed to changes arising out of involution, wear and tear of the tissues. With age, there are also changes occurring in the cell membrane and cellular enzymes.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.9(10).4267-72

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(10).4267-72

The gonadal function declines with age. In male, there is progressive atrophy of the sperm producing elements of the testes, resulting in diminished spermatogenesis [1]. The trend for parenthood at an older age has also been seen in men. Since 1980, the infertility rate for men in their 30s has increased by 21% and for men aged 40 years, the rate has increased nearly 30%. In contrast, the fertility rate in men below the age of 30 years has decreased by 15%. Because motility is acquired during the sperm transit through the prostate and the epididymis, the decrease in motility is suspected to be due to age related decline in the function of these post testicular glands [2].

Age related changes also cause alterations in sperm mitochondrial functioning [3]. Seminal volume evidence suggests there is a mild decrease in the volume with increasing age, although the clinical significance of this finding is marginal. The decrease in volume may be related to seminal vesicle insufficiency, because seminal vesicle fluid composes most of the ejaculate volume. Prostatic changes, including smooth muscle atrophy, may also affect semen volume and sperm motility [4].

There is a remarkable increase in the use of herbs over the past few years and research interests have focused on various herbs. According to Indian system of Medicine many herbs were used for treating male sexual disorders since ancient times. Several non-hormonal herbal preparations have been used to correct such sexual disorders. Apart from these preparations some individual herbs also have the effect of treating such sexual disorders. The present research was undertaken to study the effect of *Cycas circinalis* Linn. and *Ionidium suffruticosum* (Ging) on fertility of senile (old) male albino rats.

MATERIAL AND METHODS: The study was approved by Institutional Animal Ethical Committee of Saveetha University, Chennai, Reference number SU/ BRULAC /RD /009 /2013. A total of 24 senile male Wistar rats (12 -14 months old) were taken for the study, six animals were randomly distributed into 4 groups. The groups include normal control, positive control, Experimental I and Experimental II. Normal control was administered sterile water orally; positive control was administered testosterone hormone subcutaneously (10 µg/kg body weight), Experimental I group was administered ethanolic extract of *C. circinalis* (200 mg/kg body weight) and Experimental II group was administered ethanolic extract of *Ionidium suffruticosum* (200 mg/kg body weight) orally using oral gavage tube for 30 days. Sexual behaviour of all the group rats was observed. Sample collection, measuring the dimension of testes, sperm count, morphological analysis of the sperm, testosterone hormone estimation, histomorphometric analysis of testes were done according to the general procedure of our earlier studies [5, 6].

RESULTS: Restitution of fertility was compared with the normal fertile controls rats. The drug's efficacy was compared by one way ANOVA among the groups and both the herbs showed significant improvement in all the parameters in experimental rats when compared to control rats.

TABLE 1: FERTILITY PARAMETERS OF THE SENILE RATS

S. no.	Parameters	NC	PC	E I (Cc)	E II (Is)	F	P value
1	MI	7.85 ± 1.68	8.72 ± 1.72	7.42 ± 1.24	8.43 ±1.59[#]	0.308	0.82
2	TSB	198.2 ± 0.97	207 ± 0.91	204 ± 0.91	210.30 ± 0.98*	29.67	0.001
3	BW (gm)	361.17 ± 1.53	362 ± 1.81	359.17 ± 1.2	365.33 ± 1.78[#]	2.56	0.083
4	VT (cu.cm)	1.21 ± 0.09	1.62 ± 0.14	1.59 ± 0.09	1.38 ± 0.10*	3.289	0.002
5	WT (gm)	1.06 ± 0.02	1.45 ± 0.02	1.28 ± 0.01	1.42 ± 0.02*	96.08	0.001
6	GSI	0.29 ± 0.01	0.41 ± 0.01	0.33 ± 0.01	0.39 ± 0.01*	45.5	0.001
7	SC (millions/ ml)	27.9 ± 0.33	48.2 ± 0.71	38.13 ± 0.71	46.91 ± 0.64*	231.1	0.001
8	TH (ng/ml)	1.83 ± 0.16	3.8 ± 0.26	2.4 ± 0.21	3.26 ± 0.22*	16.33	0.001

MI – Mounting Index, TSB – Total Sexual Behaviour, BW – Body Weight, VT – Volume of Testes, WT – Weight of Testes, GSI – Gonado Somatic Index, SC – Sperm Count, TH – Testosterone Hormone. Normal control (NC) – administered sterile water, Positive control (PC) – administered testosterone hormone, Experimental I (E I) – administered *C. circinalis* extract (Cc), Experimental II (E II) – administered *I. Suffruticosum* (Is) extract. Values are expressed as Mean ± SEM, n=6, [#] - non significant, * - significant, * *P<0.05*, control groups compared to Experimental groups, Statistical analysis - One Way ANOVA.

TABLE 2: MORPHOMETRY OF TESTES OF SENILE RATS

S. no.	Parameters	NC	PC	E I (Cc)	E II (Is)	F	P value
1	LCN dm (µm)	4.02 ± 0.18	8.63 ± 0.12	6.52 ± 0.18	8.32 ± 0.04 *	232.8	0.001
2	STdm (µm)	238.9 ± 3.4	282.2 ± 8.1	274.20 ± 5.2	276.2 ± 8.6*	8.65	0.001
3	EH (µm)	63.17 ± 0.40	83.67 ± 0.95	112.33 ± 1.7	122.67 ± 1.2*	543.02	0.001
4	SCN dm (µm)	5.5 ± 0.09	7.53 ± 0.09	7.85 ± 0.11	8.55 ± 0.09*	189.06	0.001
5	SN dm (µm)	4.38 ± 0.11	5.73 ± 0.04	8.20 ± 0.04	6.40± 0.09*	451.67	0.001
6	PSN dm(µm)	6.12 ± 0.12	7.48 ± 0.08	7.95 ± 0.1	8.73 ± 0.11*	104.44	0.001
7	SSN dm (µm)	4.93 ± 0.24	5.73 ± 0.12	6.22 ± 0.08	7.68 ± 0.08*	65.31	0.001

LCN dm – Leydig Cell Nuclear diameter, ST dm – Seminiferous Tubule diameter, EH – Epithelial Height of seminiferous tubules, SCN dm – Sertoli Cell Nuclear diameter, SN dm – Spermatogonium Nuclear diameter, PSN dm – Primary Spermatocyte Nuclear diameter, SSN dm – Secondary Spermatocyte Nuclear diameter. Normal control (NC) – administered sterile water, Positive control (PC) – administered testosterone hormone, Experimental I (E I) – administered *C. circinalis* extract (Cc), Experimental II (E II) – administered *I. suffruticosum* (Is) extract. Values are expressed as Mean ± SEM, n=6, [#] - non significant, * - significant, **P<0.05*, control groups compared to Experimental groups, Statistical analysis - One Way ANOVA.

TABLE 3: SPERM MORPHOLOGY OF SENILE RATS

Sperm Morphology	NC	PC	EI (Cc)	EII (Is)	Total
Abnormal Sperm	372	168	174	134	848
Normal Sperm	528	732	726	766	2752
Total	900	900	900	900	3600

Chi-Square Value – 216.36, df – 3, Significant (P< 0.001), Normal Control (NC) – administered sterile water, Positive control (PC) – administered testosterone hormone, Experimental I (EI) – administered *C. circinalis* extract (cc), Experimental II (E-II) – administered *I. suffruticosum* extract (Is).

DISCUSSION: The present study shows the effect of *Cycas circinalis* (Cc) and *Ionidium suffruticosum* (Is) on the hypothalamo-pituitary-gonadal axis [8] which increases the secretion of testosterone by acting on the Leydig cells. Testosterone has been proven to have anabolic effects, [8] thus causing an increase in the general body weight. The aged rat's (Senile) gonadal function declines, with progressive atrophy of the sperm-producing elements of testes resulting in diminished spermatogenesis [1]. When all other parameters were comparatively high in senile rats, there was only a gradual increase in the body weight in all groups **Table 1**, a positive finding of the study, because a sudden and remarkable increase of weight in senile rats, may result with deleterious effects of obesity in these aged rats. The sexual behaviour was found to be increased in PC and Is infused rats EI when compared to Cc infused rats EII **Table 1**.

The weight and volume of the testes is one of the markers of a possible alteration in androgen status [9], increase in weight of gonads [10] along with an increase in diameter of the seminiferous tubules [8] and the present study also shows an increase in the testicular weight of the rats treated with is followed by Cc **Table 1**. The GSI is a better way to assess the damage of the testes in relation to the body. The increase in GSI of Is and testosterone infused group (PC) was due to increased level of serum testosterone, as androgen exerts its major role in sex organs [10].

There was an increase in GSI of Is and testosterone infused group **Table 1**. Sekar Suresh *et al*., (2009) have worked on aged rat's sperm by orally infusing some herbal drugs on senile rats and observed that it increased the sperm count to a higher level [11]. Shivraj *et al*., (1971) and Mitra *et al*., (1996) also added to the above finding, but they used a patent drug [12, 13]. The concentration of the spermatozoa in the cauda epididymis of the senile Wistar rats was found to be increased in PC and EII (Is infused), because of increase testosterone which might have induced spermatogenesis **Table 1**. Serum testosterone level was found to be increased in is infused group followed by positive control. The Cc infused group showed only a moderate increase in testosterone level **Table 1**.

Histomorphometric Analysis of Testes: The spermatogonium and spermatocytes were counted from randomly selected 30 round sections of seminiferous tubules from each group. The average of the spermatogonium and spermatocytes were calculated. In normal control the spermatogonium (79.4 ± 2.1) was less with a difference in count when compared to PC (88.2 ± 2.3), followed by EII (85.4 ± 2.4) and EI (84.2 ± 2.6), simultaneously the spermatocytes count showed a wide range of difference among the groups PC (198 ± 6.2), EII (189.8 ± 5.9), EI (183.7 ± 5.1) when compared to the control (104.3 ± 5.2). The data's were analysed by one way ANOVA and found to be statistically significant (P<0.05).

The histomorphometric analysis of the testes showed an increase in all the nuclear diameter of EII when compared to other groups **Table 2**. The Leydig cells nuclear diameter was increased in EII than the EI and control group rat testes, these cells are responsible for secretion of testosterone hormone. All the cells of seminiferous tubules nuclear diameters were increased in both the experimental groups which show the increased cellular activities of all these cells. The sertoli cells nourish the developing spermatogenic cells. The size and the number of sertoli cells were increased in experimental groups when compared to control group **Table 2**.

The nuclear diameter of the sertoli cells was also increased in EII and EI. All the parameters were analyzed by One way ANOVA which proved to be highly statistically significant (P<0.001) except mounting index, body weight and volume of testes which was not significant **Table 1** and **2**. The Post Hoc Pair wise comparison shows the significance of all the parameters among the groups **Table 4**.

Sperm Morphology: The sperm motility and viability was compared among the groups. The normal control group rat's sperm motility was 48.4% which was increased to 84.2% in PC, followed by EI 78.2% and EII 82.6%. The abnormal sperms were found to be less in EII than EI, further the data were analyzed using Chi-Square test and found to be highly significant (P<0.001) **Table 3**. The progressive atrophy of the gonads due to senility results in decreased secretion of testosterone [1] and this study has proved to be having a great efficacy in improving the fertility parameters and other sexual functions of senile rats. The hypertrophy of seminiferous tubules (ST) found in EII could be possibly by androgenic effect of the drug Is on testes in general and was responsible for increased production of testosterone which in turn was responsible for the increased size of tubules **Fig. 1** and the spermatozoal count [8].

TABLE 4: POST HOC PAIR WISE COMPARISON OF THE SENILE RATS

S. no.	Parameters	NC *vs.* PC	NC *vs.* EI	NC *vs.* EII	PC *vs.* EI	PC *vs.* EII	EI *vs.* EII
1	MI	n/s	n/s	n/s	n/s	n/s	n/s
2	TSB	S	S	S	n/s	n/s	S
3	BW	n/s	n/s	n/s	n/s	n/s	n/s
4	VT	n/s	n/s	n/s	n/s	n/s	n/s
5	WT	S	S	S	S	n/s	S
6	GSI	S	S	S	S	S	S
7	SC	S	S	S	S	n/s	S
8	TH	S	S	S	S	n/s	n/s
9	EH	S	S	S	S	S	S
10	LCN dm	S	S	S	S	n/s	S
11	ST dm	S	S	S	n/s	n/s	n/s
12	SCN dm	S	S	S	n/s	S	S
13	SN dm	S	S	S	S	S	S
14	PSN dm	S	S	S	S	S	S
15	SSN dm	S	S	S	n/s	S	S

MI – Mounting Index, TSB – Total Sexual Behaviour, BW – Body Weight, VT – Volume of Testes, WT – Weight of Testes, GSI – Gonado Somatic Index, SC – Sperm Count, TH – Testosterone Hormone, EH – Epithelial Height of seminiferous tubules, LCN dm – Leydig Cell Nuclear diameter, ST dm – seminiferous Tubule diameter, SCN dm – Sertoli Cell Nuclear diameter, SN dm – Spermatogonium nuclear diameter, PSN dm – Primary Spermatocyte Nuclear diameter, SSN dm – Secondary Spermatocyte Nuclear diameter. n/s - non significant, S -significant, *P<0.05*, Normal control (NC) – administered sterile water, Positive control (PC) administered testosterone hormone, Experimental I (E I) – administered *C. circinalis* extract (Cc), Experimental II (E II) – administered *I. suffruticosum* (Is), control groups compared to EI group (Cc) and EII group (Is).Statistical analysis – Post Hoc pair wise comparison.

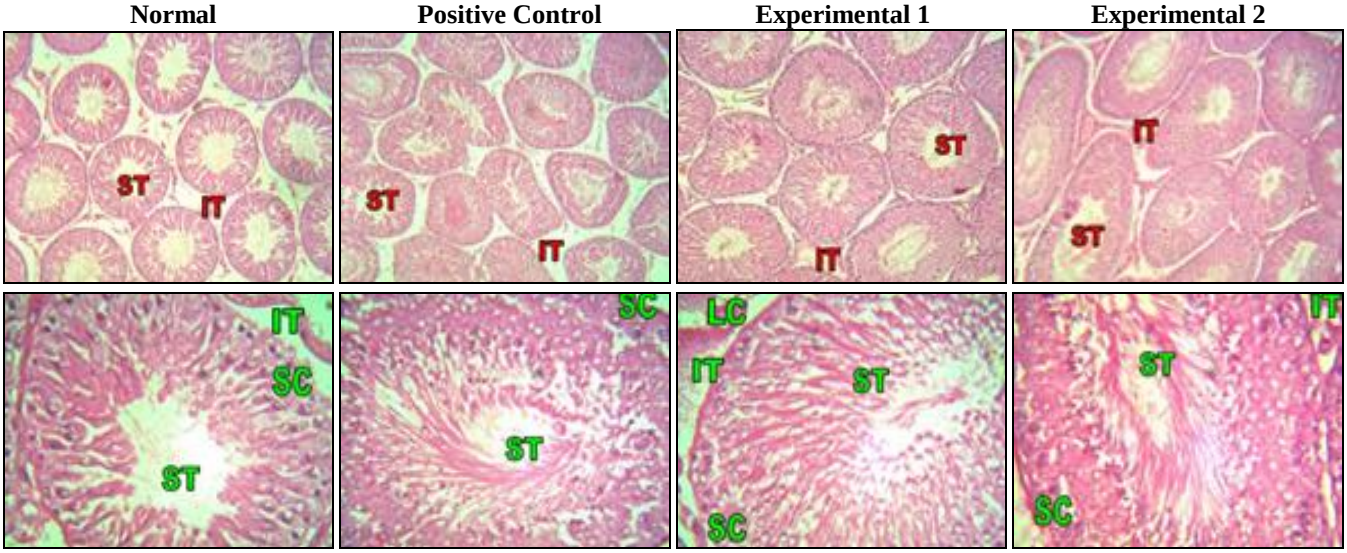

FIG. 1: HISTOLOGICAL ANALYSIS (SENILITY INDUCED STERILE RAT TESTES) H AND E STAIN 10x AND 40X ST - Seminiferous tubule , IT - Interstitial tissue, SC – Sertoli Cells. Normal control - administered sterile water, Positive control - administered testosterone hormone, Experimental I – administered *C. circinalis* extract and Experimental II – administered *I. suffruticosum*.

Histological Analysis: Each lobule of the testis contains one or more highly convoluted seminiferous tubule and as the tubules are highly convoluted, each tubule was cut several times in any sections through the testis and thus the tubules assume various shapes. Between this layer and the tubular lumen there are various germ cells that include spermatogonia, spermatocyte, spermatid, spermatozoa, along with the sustentacular cells (sertoli cells).

The senile rat's testes showed the seminiferous tubules with less number of germinal epithelium and the spermatogenesis cell series were completely condensed, their number is decreased. sertoli cells show only fewer changes and also they were less in number. The leydis cells and interstitial spaces were much reduced when compared to normal young rat's testes.

After infusing Cc and Is the seminiferous tubules of senile rats showed more histological changes, with more number of spermatogenesis cells, increased Leydig cells in interstitial space in between the tubules. The hypertrophy of seminiferous tubules was found in E I and E II could be possibly by androgenic effect of the drugs Cc and Is on testes in general. E I group showed the following normal pattern on examination of a histological section of testes, basement membrane with normal epithelium, simultaneously showed increased tubular volume and with long cylindrical narrow spermatozoa.

The spermatozoa completely filled the lumen of seminiferous tubules of E I (75-80%); whereas in the E II (82-87%) and PC (85-90%) and the remaining tubules are either empty or filled with few scattered spermatozoa when correlated with control (50 - 60%) filled with sperm cells **Fig. 1**. The percentage of changes in the tubules was not very satisfactory, on comparing with the microscopic structure of testes, so a morphometric analysis of testes was also carried out. The increase in testosterone hormone induces spermatogenesis, thus increase in spermatogenic elements of testes. The androgenic effect of the *C. circinalis* and *I. suffruticosum* restitutes the fertility back to normal in senile rats which was substantiated by increased sperm count, increase in weight of testes and increased nuclear diameters of spermatogenic elements.

The phytoconstituents and secondary metabolites of the herbs might have been responsible for all these fertility changes in senile rats. To conclude the *I. suffruticosum* was more effective in regaining the fertility of old rats back to nearly normal when compared to that of synthetic testosterone hormone and *C. circinalis.*

CONCLUSION: The gonadal function declines with age. In male there is progressive atrophy of the sperm producing elements of the testes resulting in diminished spermatogenesis. *I. suffruticosum* (Is) and *C. circinalis* (Cc) has proved to be having a great efficacy in improving the fertility parameters and sexual behaviour of senile rats. The androgenic effects of the herbs were responsible for increased testosterone hormone secretion which in turn increased the size of the seminiferous tubules, induced spermtogenesis and thus raised the sperm count. The testosterone infused positive control showed increased fertility parameters when compared to Cc and Is infused rats. The Is was more effective than Cc in improving the fertility of senile rats.

ACKNOWLEDGEMENT: Nil

CONFLICT OF INTEREST: Nil

REFERENCES:

1. Ganeriwal SK, Tiwari BV, Reddy BV and Kher JR: Effect of geriforte on male reproductive organs in albino rats. Indian Drugs 1998; 3(19): 102.
2. Sartorius GA and Nieschlag E: Paternal age and reproduction. Hum Reprod Update 2010; 16: 65-79.
3. Aitken RJ, Nixon B and Lin M: Proteomic changes in mammalian spermatozoa during epididymal maturation. Asian J Androl 2007; 9: 554-564.
4. Kidd SA, Eskenazi B and Wyrobek AJ: Effects of male age on semen quality and fertility: a review of the literature. Fertil Steril 2001; 75: 237-248.
5. Kumar BS: Fertility effect of *cycas circinalis* and *ionidium suffruticosum* in alcohol induced sterility of male wistar rats -An histomorphometric study, International Journal of Pharmaceutical Sciences and Research 2017; 8(2): 825-30.
6. Kumar BS and Kumar JV: Fertility effect of *Cycas circinalis* and *Ionidium suffruticosum* on stress induced sterility of male wistar rats, International Journal of Scientific Research 2017; 6(7): 15-16.
7. Vermes I, Toth EK and Telegdy G: Effect of drugs on brain neurotransmitter and pituitary testicular function in male rats. Horm. Res 1979; 10: 222-32.
8. Arora HL, Subbaro VV and Gupta ML: Effect of Speman on testes- A histological study. 1973; Probe XII (4): 197-00.

9. Latif R, Lodhi GM and Aslam M: Effects of amlodipine on serum testosterone, testicular weight and Gonado-Somatic Index in adult rats. J Ayubcollabhottabad 2008; 20(4): 8-10.
10. Patki PS: The role of indigenious drugs in the management of male sexual disorders. 1988; Probe (XXVIII): 25-28.
11. Suresh S, Prithiviraja E and Parkas S: Dose- and time-dependent effects of ethanolic extract of *Mucuna pruriens* Linn. seed on sexual behaviour of normal male rats. Journal of Ethno pharmacology 2009; 122 (3): 497-501.
12. Shivraj S, Jaddhav and Bahga HS: Effect of Tentex forte and speman individually and in combination of gonadal structure in rats. Journal of the Indian Medical Profession 1971; (17): 8055.
13. Mitra SK, Muralidhar TS and 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.

How to cite this article:
Kumar BS and Kumar JV: Fertility effect of *Cycas circinalis* and *Ionidium suffruticosum* in senility induced sterility of male Wistar rats - a histomorphometric study. Int J Pharm Sci & Res 2018; 9(10): 4267-72. doi: 10.13040/IJPSR.0975-8232.9(10).4267-72.