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# FERTILITY EFFECT OF CYCAS CIRCINALIS AND IONIDIUM SUFFRUTICOSUM IN STRESS INDUCED STERILITY OF MALE WISTAR RATS

Anatomy

**B. Senthil kumar** Assistant Professor, Department of Anatomy, VMKV Medical College and Hospital, Salem - Correspondence Author

**J. Vijaya Kumar** Professor, Department of Anatomy, Saveetha Medical College, Chennai.

## ABSTRACT

In our day to day life the stress and strains is one of the causes of male infertility. Many natural non-toxic herbs were found to enhance the fertility in male. To study the fertility effect of the herbs *Cycas circinalis and Ionidium suffruticosum* in stress induced infertile male albino rats. A total of 36 healthy young male albino rats were selected and are subjected to stress by immobilization for 4 hrs/ day for 14 days, to check the stress induced status serum cortisol was analyzed. *Cycas* and *Ionidium* extract 200mg/kg body weight were administered orally to the experimental albino rats. The drug's efficacy was proved by the restitution of fertility by comparing with the normal fertile controls. The herbs were found to be effective on the gonads of stress induced sterile male albino rats and it has restituted the fertility back to normal.

## KEYWORDS:

Cycas circinalis, Ionidium suffruticosum, Sterility, Male infertility, Stress

## INTRODUCTION:

Stress affects the sexual desire in men and women and can cause impotence in men. In men, both the physical and psychological stress has an adverse effect on the reproductive capacity; activation of the Hypothalamic-pituitary-adrenal axis by stressors is a presumable mechanism for the inhibition of male reproductive functions through a depression in the Hypothalamic-pituitary-testicular axis (1,2). Several reports have suggested the stress related decline in semen quality, low sperm concentration, abnormal sperm morphology and low percentage of sperm motility. The sperm concentration and fertility potential of males are correlated with one another (3). Cortisol is the hormone produced in response to stress and increased level of cortisol may reduce the functional activity of Luteinizing Hormone (LH), thereby reducing testosterone level (4). Psychological stress leads to low testosterone level due to reduction in LH pulse frequency. Reduced testosterone in turn reduces libido and leads to oligosper matogenesis (5). The present study was done to find out the fertility effect of *Cycas circinalis and Ionidium suffruticosum* in stress induced sterility of male Wistar rats.

## MATERIALS AND METHODS:

A total of 30 male Wistar rats were taken for the study, six animals were randomly distributed into 5 groups. The groups include normal control, sterile control, positive control, Experimental I and Experimental II. Sterility was induced in young rats of all the four groups by stress due to immobilization for 4 hrs/ day for 14 days except the normal control group (6). The stress induced was confirmed by estimating serum cortisol. The sterility induced in all groups was confirmed by testicular biopsy, sexual behaviour and testosterone hormone analysis as per the standardized procedure. After confirmation of alcohol induced sterility the respective groups were given drugs. The study was approved by Institutional Animal Ethical Committee of Saveetha University, approval number ANAT.005/2012. Normal control and sterile control were administered sterile water orally; positive control was administered testosterone hormone subcutaneously (10µg / kg body weight), Experimental I group was administered ethanolic extract of *Cycas circinalis* (200 mg/kg body weight) and Experimental II group was administered the extract of *Ionidium suffruticosum* (200 mg/kg body weight) orally using oral gavage tube for 30 days. Sexual behaviour of the rats of different groups were observed and compared. The rats were anesthetized by. Blood sample was collected and testosterone hormone estimation was done, followed by removal of testes and measuring the dimension of testes. The semen analysis and morphological analyses of sperm were carried out. The histo-morphometric and histological analysis of testes were done. Sample collection, measuring the dimension of testes, semen analysis, morphological analysis of the sperm, testosterone hormone estimation, histomorphometric analysis of testes were done according to the standardized procedure of our earlier studies (7,8).

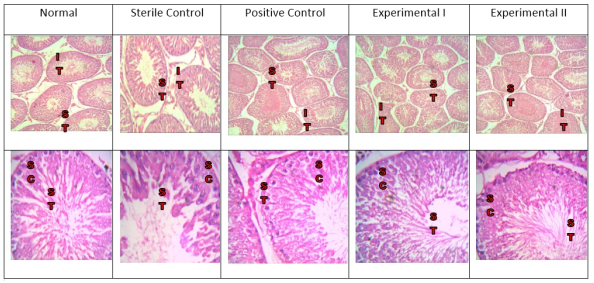
## RESULTS:

The serum cortisol was found to be increased in stressed rats than normal control rats. This confirms that the stress was induced in experimental rats. Cortisol is the hormone produced in response to stress and increased level of cortisol may reduce the functional activity of LH, thereby reducing testosterone level (4). Reduced testosterone in turn reduces libido and leads to oligospermatogenesis. The histopathological interpretation of testicular biopsy showed decrease in diameter of seminiferous tubules and shrinkage of some tubules. The Leydig cells were normal but the stroma was considerably reduced in the stress induced rats (9). Sertoli cells were disrupted and detached from the basement membrane (Figure.1). The normal rats did not show any histopathological changes in the tubules (Figure.1). This confirms that sterility was induced in stress induced experimental rats.

**Table 1: Fertility parameters of the stress induced sterile rats**

| S.no | Parameters | NC | SC | PC | E I (Cc) | E II (Is) |
|---|---|---|---|---|---|---|
| 1 | MI | 6.58 ± 1.80 | 5.5±0.22 | 7.67±0.42 | 7.05±0.3 | 8.82±0.36* |
| 2 | TSB | 186.2 ± 0.67 | 104.3±4.9 | 196.7±3.4 | 188.5±2.2 | 191.8±1.8 * |
| 3 | BW (gm) | 181.17± 1.9 | 156.17±1.6 | 221.7±7.0 | 205±3.4 | 211.7±2.7 * |
| 4 | VT (cu.cm) | 0.83± 0.05 | 0.58±0.14 | 0.93±0.02 | 0.90±0.01 | 1.17±0.08 * |
| 5 | WT (gm) | 0.95 ± 0.01 | 0.71±0.04 | 1.19±0.06 | 1.14±0.1 | 1.26±0.07 * |
| 6 | GSI | 0.48 ± 0.01 | 0.46±0.03 | 0.54±0.02 | 0.57±0.3 | 0.59±0.03 * |
| 7 | SC (millions/ ml) | 30.86 ± 0.40 | 25.15±0.85 | 55.78±2.1 | 46.03±0.8 | 54.7±2.63 * |
| 8 | TH (ng/ml) | 2.26 ± 0.05 | 1.47±0.09 | 4.20±0.3 | 3.37±0.08 | 4.02±0.18 * |
| 9 | EH (µm) | 82.50±2.19 | 67.8±2.4 | 106.8±1.01 | 107.83±1.4 | 118±1.0* |
| 10 | LCN (µm) | 5.03±0.07 | 4.52±0.11 | 6.68±0.13 | 7.05±0.1 | 7.6±0.29* |
| 11 | ST (µm) | 258.6± 3.2 | 195±5.63 | 288.17±6.2 | 289.50±1.5 | 292.67±1.8* |
| 12 | SCN (µm) | 7.08±0.08 | 6.2±0.24 | 7.95±0.20 | 8.1±0.15 | 8.4±0.07* |
| 13 | SN (µm) | 4.9±0.17 | 4.75±0.16 | 5.43±0.15 | 5.41±0.15 | 5.71±0.09* |
| 14 | PSN (µm) | 7.22±0.04 | 6.2±0.17 | 7.95±0.18 | 8.5±0.19 | 8.8±0.06* |
| 15 | SSN (µm) | 5.43±0.09 | 3.78±0.18 | 5.82±0.31 | 6.53±0.12 | 7.2±0.26* |

**Figure 1 - Histological analysis (Stress induced sterile rat testes) H & E stain 10x & 40X**




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 – Leydig Cell Nuclear diameter, ST – Seminiferous Tubule diameter, SCN – Sertoli Cell Nuclear diameter, SN – Spermatogonium Nuclear diameter, PSN – Primary Spermatocyte Nuclear diameter, SSN – Secondary Spermatocyte Nuclear diameter. Normal control **(NC)** and Sterile control **(SC)** – 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, * - significant, control groups compared to Experimental groups, Statistical analysis – One Way ANOVA.

## DISCUSSION:

In men, stress interferes adversely with the reproductive capacity, resulting in inhibition of male reproductive functions due to depression in the Hypothalamic-pituitary-testicular axis (1,2). Immobilization stress can decrease testosterone secretion in males, resulting in deficit of sexual performance and sexual act (2). Many research findings stated, stress related decrease in semen quality, sperm concentration, morphology and sperm motility (10). The sexual behaviour of SC was compared to other groups. The Mounting index and TSB was found to be considerably increased in PC and EII than EI (Table 1). The body weight of SC group was decreased whereas in PC, EI and EII it was moderately increased (Table 1). The volume and weight of testes were found to be increased in EII than EI and PC. The GSI of all experimental groups did not show much significant changes, only a border line increase (Table 1) when compared to NC. The sperm count of PC showed raised counts when compared to experimental groups, but the sperm counts were considerable more than the SC and NC (Table 1). The testosterone hormone level was found to be elevated in EII followed by EI group rats. An increase in nuclear diameter of spermatogonium, spermatocytes and spermatids in testosterone treated groups (PC) followed by EII and EI indicates that testosterone restores male reproductive function (Table 1). All the fertility parameters were analyzed by one way ANOVA, proved to be highly statistically significant ($P<0.001$). The Post Hoc Pair wise comparison shows the significance among the groups.

Histo-pathological damage was observed in 45% tubules. Some of them were shrunken while others showed anastomosis. Some of them completely lost their tubular appearance. The Leydig cells were normal but the stroma was considerably reduced. Spermatids and spermatocytes showed severe reduction in their number (9). After Cc and Is drug administration the tubules showed better organization of germ cells was observed, although all the tubules did not attain normal structure most of the tubules contained spermatozoa, but some debris were also seen in some tubules. Abnormal spermatozoa in the tubules were much less in number. Stages of spermatozoa could be identified in peripheral tubules. The Leydig cells showed normal structure and distribution (Figure.1).

The combined activity of testosterone and estrogen is probably essential for spermatogenesis and improving the male sexual function as estrogen has been widely used to abrogate male climacteric symptoms (11). In phytochemical analysis, presence of phytosterols, alkaloids, saponins, glycosides, carbohydrates, and saponins were traced out. From the present study, the phytosteroids undergo absorption and metabolism in the gastric tract and get converted into phytoestrogen metabolites (12). These phytoestrogens metabolites act in a way similar to estrogen derivatives and produce their effects through activation of estrogenic receptors. Estrogenic receptors may be present in testis, sertoli cells and Leydig cells, thereby improving sexual performance and male reproductivity (6).

## CONCLUSION

Cycas Circinalis and Ionidium Suffruticosum has some important phytoconstituents such as alkaloids, flavonoids, terpenoids and phytosterols (Phytochemical analysis earlier studies) which might have been beneficial in promoting male sexual behavior in normal rats as well as stress induced sexual dysfunction. The I.suffruticosum was more effective in regaining the fertility in stress induced rats than the synthetic testosterone hormone and C.circinalis administered male rats.

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