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FERTILITY EFFECT OF *CYCAS CIRCINALIS* AND *IONIDIUM SUFFRUTICOSUM* IN ALCOHOL INDUCED STERILITY OF MALE WISTAR RATS – AN HISTOMORPHOMETRIC STUDY

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Keywords:

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

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ABSTRACT: Alcohol abuse is well known to impair reproductive performances due to impaired testosterone production and testicular atrophy. 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 alcohol induced sterile male albino rats. A total of 30 healthy young male albino rats were selected. A total of 24 rats were subjected to 3gm of ethanol/kg body weight/day orally for 4 weeks for experimental rats. Cc and *Is* extract 200mg/kg body weight were administered orally to the experimental albino rats E-I (n=6) and E-II (n=6) respectively and compared to the positive control albino rats PC (n=6) which was administered testosterone 10µg/kg body weight subcutaneously, biweekly with that of the alcohol induced sterile control rats SC (n=6) using various parameters such as sexual behavior, weight of animals, dimension and weight of testes, hormone, semen, and histological analysis. Restitution of fertility was compared with the normal fertile controls rats NC (n=6). 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 alcohol induced sterile male albino rats. The synthetic hormonal preparations have grave side effects it's better to go with herbal aphrodisiacs for better results without any side effects. 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.

INTRODUCTION: Infertility affects more than 80 million people around the globe. It is a ubiquitous phenomenon that transcends race and nationality [1]. Excessive use of alcoholic beverages results in a variety of medical and psycho-sociological disturbances that identify alcoholism as one of modern society's major problems [2]. Most studies of ethanol induced fertility alterations have been conducted with the male gender of both man and laboratory animals.

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The effects of ethanol on pubertal processes are poorly understood and only a few studies have been conducted in this respect. Some studies have however reported that ethanol delays certain aspects of sexual maturations [2]. *Cycas circinalis L* (Family –Cycadaceae) (Cc) a sago palm commonly known as Madana Kaman in Tamilnadu. The male sago cone has aphrodisiac activity. *Ionidium suffruticosum* Ging (Family–Violaceae) (Is), a perennial herb known as Orithazthamarai.

The whole plant has aphrodisiac activity and it is used as rejuvenating herb in Siddha system of Medicine. The present research work was done to find out the restitution of fertility in alcohol induced sterile male Wistar rats by administering *Cycas circinalis* and *Ionidium suffruticosum.*

MATERIALS AND METHODS: A total of 30 male Wistar rats (2 -3 months) 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 on young rats of all the four groups by administering 3 gm of ethanol / kg body weight / day orally for 4 weeks except normal control group. [3, 4] The sterility induced in all groups was confirmed by testicular biopsy, observing sexual behaviour and testosterone hormone analysis as per the standardized protocol of our earlier studies. After confirmation of alcohol induced sterility the respective groups were given drugs. [5, 6]

Normal control and sterile control groups 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* (200mg/kg body weight) and Experimental II group was administered ethanolic extract of *Ionidium suffruticosum* (200mg/kg body weight) orally using oral gavage tube for 30 days. Sexual behaviour of the male rats of different groups was observed. 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. [5, 6]

RESULTS:

Confirmation of alcohol induced sterility in young rats group: The parameters were compared between the normal control and sterile rats and found to be highly significant except mounting index (**Table 1**). The histopathological interpretation of testicular biopsy showed degeneration of seminiferous tubules with devoid of germ cells, increased luminal diameter, desquamation of the epithelium in the tubules were observed, the basement membrane was thin and only few germ cells attached to the basal lamina of the tubules, in the alcohol administered rats and the normal rats did not show any histopathological changes in the tubules. The sexual behaviour and testosterone hormone level was found to be decreased in sterile rats when compared to normal control (**Table 1**). This confirms that sterility was induced in alcohol administered experimental rats.

TABLE 1: PARAMETERS FOR CONFIRMATION OF ALCOHOL INDUCED STERILITY IN YOUNG RATS

Parameter	Normal control (Mean ± SEM)	Sterile rats (Mean ± SEM)	T	P value
Mounting Index (MI)	6.95 ±1.68	4.55 ± 1.10$^{\#}$	1.19	0.26
Total sexual behavior (TSB)	202 ± 0.967	134 ± 0.61***	59.47	0.001
Serum Testosterone Hormone (ng\ml)	2.1 ± 0.21	1.2 ± 0.12**	3.72	0.01

Values are expressed as Mean ± SEM, $^{\#}$ - non significant, * -significant, *$P<0.05$, **P<0.01 &***P< 0.001 control groups compared to Sterile group, Statistical analysis – Unpaired t test.

TABLE 2: FERTILITY PARAMETERS OF THE ALCOHOL INDUCED STERILE RATS

S.no	Parameters	NC	SC	PC	E I (Cc)	E II (Is)	F	P value
1	MI	6.58 ± 1.80	5.83±0.31	8.20±0.37	7.17±0.24	7.92±0.13*	15.2	0.001
2	TSB	186.20 ± 0.7	133.2±3.2	195±3.85	193±1.69	199.8±1.5 *	134.5	0.001
3	BW (gm)	181.17± 1.9	159.7±3.4	208.3±4.7	194.8±1.6	208±3.7*	39.01	0.001
4	VT (cu.cm)	0.83± 0.05	0.61±0.03	0.89±0.13	0.84±0.01	1.28±0.09 *	22.96	0.001
5	WT (gm)	0.95 ± 0.01	0.73±0.03	1.12±0.06	1.04±0.05	1.18±0.05 *	16.22	0.001
6	GSI	0.48 ± 0.01	0.46±0.02	0.54±0.02	0.53±0.25	0.56±0.02*	4.65	0.001
7	SC (millions/ ml)	30.86 ± 0.40	25.55±1.4	57.31±2.1	43.26±1.6	54.3±2.16 *	78.14	0.002
8	TH (ng/ml)	2.26 ± 0.05	1.37±0.08	4.02±0.22	2.87±0.12	3.48±0.14 *	58.85	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)** 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, $^{\#}$ - non significant, * - significant, *$P < 0.05$, control groups compared to Experimental groups, Statistical analysis – One Way ANOVA.

TABLE 3: MORPHOMETRY OF TESTES OF THE ALCOHOL INDUCED STERILE RATS

S.no	Parameters	NC	SC	PC	EI (Cc)	EII (Is)	F	P Value
1	EH (µm)	82.50±2.19	78.67±1.52	99.8±1.97	105.2±1.6	114.7±2.1	64.76*	0.001
2	LCN dm (µm)	5.03±0.07	3.9±0.13	7.18±0.12	7.35±0.14	8.35±0.07	290.35*	0.001
3	STdm (µm)	258.6 ± 3.2	222.3±5.01	276±5.7	281.2±5.4	303±1.24	46.07*	0.001
4	SCN dm (µm)	7.08±0.08	5.35±0.06	8.70±0.24	7.75±0.07	8.58±0.11	117.59*	0.001
5	SN dm (µm)	4.9±0.17	4.48±0.09	5.67±0.19	5.3±0.09	6.12±0.07	22.95*	0.001
6	PSN dm(µm)	7.22±0.04	6.10±0.09	8.12±0.13	7.53±0.08	8.6±0.09	112.8*	0.001
7	SSN dm (µm)	5.43±0.09	3.5±0.08	5.43±0.11	6.33±0.09	7.43±0.09	223.25*	0.001

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. 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, # - non significant, * - significant, *$P < 0.05$, control groups compared to Experimental groups, Statistical analysis – One Way ANOVA.

TABLE 4: SPERM MORPHOLOGY OF STERILE RATS

Sperm Morphology	NC	SC	PC	EI (Cc)	EII (Is)	Total
Abnormal Sperm	240	336	158	164	118	1016
Normal Sperm	660	564	742	736	728	3483
Total	900	900	900	900	900	4500

Chi-Square Value -189.6, df – 4, Significant (P< 0.001), Normal Control **(NC)**, Sterile control **(SC)**, Positive control **(PC)** – administered testosterone hormone, Experimental I **(EI)** – administered *C.circinalis* extract (cc), Experimental II **(E-II)** – administered *I.suffruticosum* extract (Is).

TABLE 5: POST HOC PAIR WISE COMPARISON OF THE ALCOHOL INDUCED STERILE RATS

S.no	Parameters	NCvs.SC	NCvs.PC	NCvs.EI	NCvs. EII	SCvs. PC	SCvs.EI	SCvs.EII	PCvs. EI	PCvs. EII	EIvs.EII
1	MI	n/s	S	n/s	S	S	S	S	n/s	n/s	n/s
2	TSB	S	n/s	n/s	S	S	S	S	n/s	n/s	n/s
3	BW	S	S	n/s	S	S	S	S	n/s	n/s	n/s
4	VT	S	n/s	n/s	S	S	S	S	n/s	S	S
5	WT	S	n/s	n/s	S	S	S	S	n/s	n/s	n/s
6	GSI	n/s	n/s	n/s	S	S	n/s	S	n/s	n/s	n/s
7	SC	n/s	S	S	S	S	S	S	S	n/s	S
8	TH	S	S	S	S	S	S	S	S	n/s	S
9	EH	n/s	S	S	S	S	S	S	n/s	S	S
10	LCN dm	S	S	S	S	S	S	S	n/s	S	S
11	ST dm	S	n/s	S	S	S	S	S	n/s	S	S
12	SCN dm	S	S	S	S	S	S	S	n/s	S	S
13	SN dm	n/s	S	n/s	S	S	S	S	n/s	n/s	S
14	PSN dm	S	S	n/s	S	S	S	S	S	S	S
15	SSN dm	S	n/s	S	S	S	S	S	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)** and Sterile control **(SC)** – administered sterile water, Positive control **(PC)** administered testosterone hormone, Experimental I **(EI)** – administered *C.circinalis* extract **(Cc)**, Experimental II **(EII)** – administered *I.suffruticosum* **(Is)**, control groups compared to EI group (Cc) and EII group (Is).Statistical analysis – Post Hoc pair wise comparison.

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 2, 3** and **4**). The herbs were found to be effective on the gonads of alcohol induced sterile male albino rats.

DISCUSSION: Chronic alcohol consumption results in disorders of spermatogenesis in human. [7] Male alcoholics undergo erectile dysfunction and infertility due to reduced testosterone level. [8] Ethanol is a primary testicular toxin. [9] Apoptosis was induced in ethanol treated animals indicating the tissue injury of testicles followed by testicular DNA fragmentation, and increased number of

apoptosis of spermatogonia and spermatocytes. [10, 11] Alcohol causes an adverse effect on the secretory function of sertoli cells. [10] Testicular atrophy occurs due to loss of sperm cells and decreased diameter of the seminiferous tubules. The present study showed desquamated seminiferous tubules and pronounced changes in the nuclear diameter of the germinal epithelium (**Fig. 1** and **2**). Ethanol ingestion in mice revealed degenerative changes of epithelial component of the seminiferous tubules and alcohol treated rats showed testicular lesions with decrease in the diameter of the seminiferous tubules and decrease in Leydig cell's number.[12, 13]

The sexual behavior was observed to be more in testosterone, Cc and Is infused rats when compared to sterile control. Alcohol induced alterations in testicular weight, sperm count, sperm motility, and sperm morphology in sterile rats (**Table 2**). There was an increase in body weight, testes weight and GSI, testosterone hormone in experimental rats when compared to sterile rats (**Table 2**). The sperm count was found to be increased almost twice when compared to sterile controls (**Table 2).**

Sperm morphology: The sperm motility and viability was compared among the groups. The normal control sperm motility was 46.2%, which was increased to 83.2% in PC, followed by EI 82.4% and EII 86.5%. The sterile control sperm motility (39.4%) was less than the normal control (46.2%). There was much reduction in abnormal sperms in experimental groups (**Table 4).** The data's were analysed by Chi-square test and found to be statistically significant.

Histo-morphometric 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. The mean values are expressed as mean ± SEM. In normal control the spermatogonium (64.2 ± 1.9) was much less in count when compared to PC (86.2 ± 2.2), followed by EII (81.2 ± 2.1) and EI (78.6 ± 21.9). The sterile control showed less count (42.4 ± 1.2) when compared to normal control (64.2 ± 1.9), simultaneously the spermatocytes count showed a wide range of difference among the groups PC (178 ± 5.2), EII (172.2 ± 5.1), EI (164.2 ± 4.2) when compared to the control (106.2 ± 4.8). The sterile control spermatocytes were much reduced (88.7 ± 3.4) when compared to normal control (106.2 ± 4.8). The data's were analysed by one way ANOVA and found to be statistically significant ($P< 0.05$). The histo-morphometry of the testes showed an increase in all the nuclear diameter of EII when compared to other groups (**Table 3**).

All the 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 NV vs. EII, SC vs. EI, SC vs. EII and SC vs. PC (**Table 5**), whereas in other group's comparison were not statistically significant.

Histological analysis: Testis has endocrine part which secretes hormones like testosterone, estrogen, inhibin by Leydig and sertoli cells. The exocrine part contains seminiferous tubules that produce spermatocytes. Each testicular lobule has one to four seminiferous tubules and which has two types of cells in it; sertoli cells and spermatosoid cells. The spermatosoid cells divided frequently in basal membrane and transform into spermatozoa after distinctive steps. Spermatosoid cells discriminate gradually from the basement membrane of the tubule towards the lumen and their reproduction causes the cells to be expelled into inner cavity of the lumen. During spermatogenesis process, spermatogonium cells were transformed into spermatocytes following necessary divisions and changes and eventually turn into primary spermatocyte cells. In first meiosis division, smaller cells called secondary spermatocytes emerge. By the end of the second meiosis division, the secondary spermatocyte turns into two smaller cells namely spermatids (**Fig. 1**).

Alcohol administered rats showed varying degrees of degeneration of seminiferous epithelium as well as presence of large sized multinucleated cells in the tubules and empty interstitial spaces when compared with testicular tissue from the control rats (**Fig. 1).** Cc and Is administered rats showed normal tubular epithelium, various stages of spermatogenic cells and more number of spermatids in the lumen of the tubules. A reduction in seminiferous tubule diameter and germinal epithelial height was observed in alcohol induced

sterile rats (**Table 3**). Moreover, the mean thickness of the tunica albuginea and basal lamina of the seminiferous tubules decreased in animals treated with alcohol in comparison with controls.

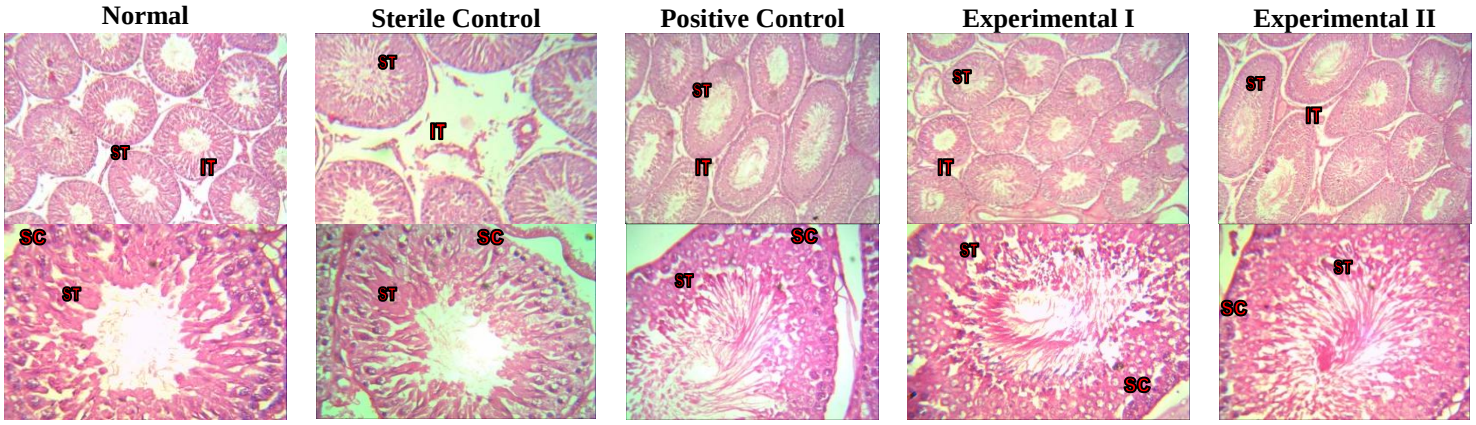

FIG. 1: HISTOLOGICAL ANALYSIS (ALCOHOL INDUCED STERILE RAT TESTES) H & E STAIN 10X & 40X
ST - Seminiferous tubule , **IT -** Interstitial tissue, **SC** – Sertoli Cells.
Normal control and Sterile control - administered sterile water, Positive control - administered testosterone hormone, Experimental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

Restoration of fertility may be due to the steroidogenic activity of *C.ciricnalis* and *I.suffruticosum.* The increase in sperm count, decrease in abnormal spermatozoa and increase in testicular weight in experimental groups showed the restitution of ethanol induces testicular dysfunction back to normal in Cc and Is administered rats.

The fertility effect of Cc and Is on alcohol induced sterility of male Wistar rats was the first study reported till date, no other scientific study has been carried out by the researchers on the fertility effect of the two herbs. Furthur the study will be extended in future to identify the specific phytoconstituents responsible for steroidogenesis and their androgenic effect.

CONCLUSION: The *I.suffruticosum* was found to be more effective in restituting the fertility of sterile male Wistar rats. The phytoconstituents such as alkaloids of *C.Circinalis* and *I. Suffruticosum* might have possibly triggered the production of testosterone which was proved by the increased testotosterone hormone level in Cc and Is infused group of rats, flavonoids might have acted by resisting the effect of alcohol on gonads which was proved by the restitution of fertility back to normal in sterile rats, tannins might have inhibited oxidation by acting as an antioxidant. The free radicals are harmful byproducts of many normal metabolic processes. To prevent the damage it must be quickly converted into other non-toxic substances. The antioxidants of the natural herbs inhibit the oxidation and prevent the production of free radicals. This study has given us a definite hope about the efficacy of the drug. *I.suffruticosum* was found to be more effective than *C.circinalis* in alcohol induced sterile rats.

CONFLICT OF INTEREST: Nil.

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