Histological and Histometric Study of Testes in Albino Rats Treated with Convolvulus Microphyllus

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Abstract

Objective of this study is to observe the effect of alcoholic extract of *Convolvulus microphyllus* in male rats through histometric and histological studies of reproductive organs. It was observed that area of cross section of testes made from paraffin embedded tissues was unaffected after treatment. The area of seminiferous tubules per cross section was observed in mm^2 and percent area was also measured and found to be insignificantly changed. The area of interstitial space per cross section as well as percent area was also unaltered.

A significant decrease in the germinal epithelial cell height of seminiferous tubules. Reduction in the cytoplasmic and nuclear area of leydig cells was noticed. Mature leydig cells counts was decreased significantly with the increase in the number of degenerating cells was resulted after ethanolic extract of *Convolvulus microphyllus* administraion for 60 days to adult male rats.

Epithelial cell height (μm) of caput and cauda epididymis and seminal vesicle was reduced significantly. This shows antiandrogenic nature of drug as it is evident from testicular histormetry as well as histological observations.

INTRODUCTION

Medicinal plants are considered as one of the main source for developing new drugs with potential therapeutic effects. Thus study of traditional plant that been used as antispermatogenic activity should still be seen as a logical search strategy *Convolvulus microphyllus* is an herb that has been extensively investigated for its pharmacological and therapeutic effects. The plant contains alkaloid (Shakhapushpine), volatile oil, flavonoids (Kampeferol derivatives), phytosterol (beta sitosterol), carbohydrates (glucose, rhammose and starch), ceryl alcohol and scopoletin (1). This herb is believed to be powerful memory enhancer and brain tonic to improve the intelligence levels and brain function of a person. The plant named Shankhpushpi because of its Shank or conch shaped flowers. In ayurvedic formulation, these can be used to make decoctions and tonics for memory.

Fertility regulation with plant and plant preparations have been reported in the ancient literature of herbal medicine in India. A μo of plant species have been tested for fertility regulation beginning

about 50 years ago and were subsequently fortified by national and international agencies (2, 3). The role of plant products in the induction of male and female infertility in experimental animals has drawn the attention of researchers over the turn of the century (4. 5, 6).

In the light of this fact this work was conducted to monitor the effects of *Convolvulus microphyllus* on the reproductive system and fertility in adult male rats.

MATERIALS AND METHODS

Plant extract:-

Convolvulus microphyllus whole plant was collected from Jhalana Dungri, Jaipur. Shade dried plant was subjected to soxhalation in 50% ethanol.

Animal:-

Healthy male albino rats weighing about 200gms were used. Males were cohabited with proestrous females in the ratio of 1:3 only. The fertile males whose sire delivered average number of litter were used.

Experimental design:-

The males were grouped 5 in number, identified and kept in plastic cages. The daily dose of plant extract was freshly prepared dissolved in 5ml of distilled water and administered every morning for 60 days.

Gr. I	Vehicle treated controls - The group received vehicle only i.e. distilled water.
Gr. II	100mg/day/rat alcoholic extract of *Convolvulus microphyllus* was orally fed to rats.
Gr. III	200mg/day/rat alcoholic extract of *Convolvulus microphyllus* was orally fed to rats.
Gr. IV	300mg/day/rat alcoholic extract of *Convolvulus microphyllus* was orally fed to rats.

The whole spermatogenic process requires 53 days in rats, out of which spermatozoa spend last 6-7 days in the final transit through epididymis (7). The plant extract was administered for one complete spermatogenic cycle.

Histological analysis :-

The bouins fixed reproductive (testes, epididymis seminal vesicle, ventral prostate and vas deferens) along were cut into small pieces and processed for histological slides. After dehydration using different concentration of alcohol, specimens were embedded in paraffin blocks and sectioned 3 μm, placed on a clean histological studies and stained using hematoxylin and eosin stains. The structure of these organs were studied under light microscope.

Histometry:-

With the help of camera lucida one hundred of circular appearing seminiferous tubules were traced at 80x magnification and diameter of each tubule was measured separatly diameter of lydig cell were drawn and measured at 800x. Germinal epithelial height of seminiferous tubule were traced at x360. Epithelial cell heights of caput, cauda epididymis and seminal vesicle were traced at x360.

Testicular dynamics:-

Mature leydig cell, fibroblast like cell and degenerating cell population was estimated applying a differential count over 200 cells of this cells population and statistically verified by binomial distribution (8).

Testicular morphology:-

Area of cross section was calculated by drawing lengths and breadth of the section from vesopan. Similarly area covered by siminiferous tubules and blood vessely were also measured by vesopan drawings. Out of these data interstitial space (area) covered per cross section was also derived.

Statistical calculations:-

All the values of histome analysis and testicular dynamics were expressed in terms of mean II standard deviation. The different treatment groups were compared with control group using chi square test and student's t-test (9).

RESULT AND DISCUSSION

To evaluate type of action of the drug histometric results have also attempted to throw more light on histological observations. Reduction in the mean of germinal epithelium of seminiferous tubules and lumen were observed in the treatment groups. Intertubular stroma was significantly increased in the test groups. These findings further explains the poor differentiation of the germinal epithelium in the experimental groups. The significant increase in the inter tubular stroma infers the most of the interstitial tissues and cells of leydig. Cytoplasmic as well as nuclear area were lost and it also supports our report on morphological observation. The negative correlation observed between the lumen and seminiferous tubules in the control group was expected. This supports the work of (10). Positive correlation observed in the test groups indicates a deviation from the normal that shows that the test drug had effects on these groups. This further explains the wider lumen observed in the seminiferous tubules of the experimental groups. Other investigators have reported different histometric values, (11).

The result obtained by (12) demonstrate that androgens or gonadotropins are required for the maintenance of interstitial fluid volume in the adult rat testes. Which is found depleted by the plant extract treatments. This further supports the antiandrogenic nature of plant extract of *Convolvulus microphyllus*.

Sharpe and Cooker (13) and (14) have provided evidence for the presence in testicular fluid of a compounds which stimulates testosterone and pregnenolone synthesis in vitro. Therefore the reduction in the seminiferous tubule and leydig cell diameters and numbers is indication of reduction in FSH androgen action.

The number and area of leydig cells (calculated by diameter) also decreased after treatment with *Convolvulus microphyllus,* which further indicated decreased androgen levels. Purandare at el (15) reported that circulating testosterone levels are reduced whereas LH levels remain unaffected following, feeding of Embelia berris to bonnet monkeys. There is evidence that antiandrogens might cause reduction in circulating androgen levels inspite of unchanged gonadotrophin secretion (16). Hall (17) examined leydig cell at an ultrastructural level during secretory and non secretory conditions and highlighted the role of lipid droplets, mitochondria and smooth endoplasmic reticulum in steroidogenesis. The visible changes in these three organelles can be correlated with peaks of testosterone concentrations (18). Talka (19) suggested that antiandrogens possible affected the paracrine and autocrine regulatory mechanism which in turn affected leydig cell function.

CONCLUSION

The histological studies of male albino rats was observed post administration of *Convolvulus microphyllus* at all the three dose levels (100, 200 and 300 mg/day/rat). Testicular area of cross section of seminiferous tubules was non significantly changed. But germinal epithelial height of seminiferous tubule was decreased significantly. The leydig cell's cytoplasmic as well as nuclear area was also reduced noticeably. Number of interstitial differential counts was also altered. Mature leydig cell number decreased although degenerating cell number has increased significantly. The epithelial cell height of caput and cauda epididymis was reduced significantly. All the above observations shows the antiandrogenic nature of the drug.

OBSERVATION:-

TABLE NO. 1 : TESTICULAR MORPHOLOGY OF CONTROL AND CONVOLVULUS MICROPHYLLUS TREATED INTACT MALE RATS

(Mean ± SEM of 5 Animals)

GROUPS	TREATMENT	AREA OF CROSS SECTION	AREA OF SEMINIFEROUS TUBULE/CROSS SECTION		AREA OF INTERSTITIAL SPACE/CROSS SECTION		AREA OF BLOOD VESSEL/ /CROSS SECTION	
		mm^2	mm^2	%	mm^2	%	mm^2	%
I.	Control	0.5270 ±0.05	0.2403 ±0.001	45.98 ±2.04	0.2729 ±0.06	51.01 ±2.93	.00147 ±0.008	2.98 ±0.88
II.	100mg/day for 60 days	0.4012ns ±0.003	0.1141** ±0.006	28.46** ±0.81	0.2761ns ±0.01	68.80ns ±0.89	0.0109** ±0.006	2.71ns ±0.07
III.	200mg/day for 60 days	0.5966ns ±0.015	0.2405ns ±0.005	40.38ns ±1.58	0.3473ns ±0.01	57.28ns ±1.28	0.0086ns ±0.001	1.47ns
IV.	300mg/day for 60 days	0.5066ns ±0.15	0.1793ns ±0.04	34.43ns ±7.27	0.3208ns ±0.02	63.77ns ±7.21	0.0064ns ±0.001	1.27ns ±0.058

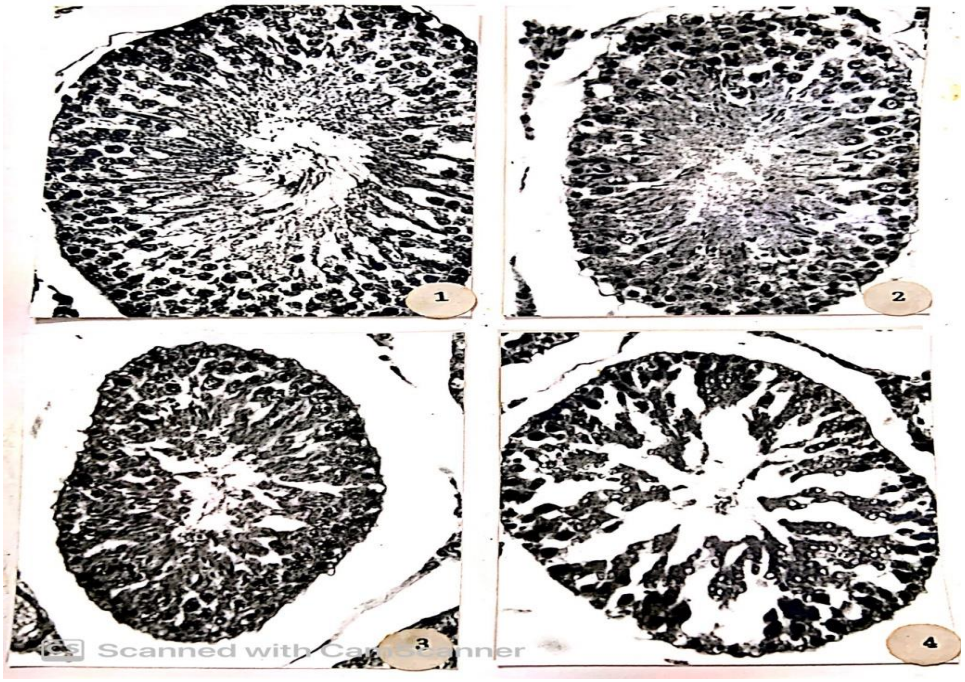
TABLE NO. 2 : EPITHELIAL CELL HEIGHTS OF CONTROL AND CONVOLVULUS MICROPHYLLUS TREATED INTACT MALE RATS

(Mean ± SEM of 5 Animals)

GROUPS	TREATMENT	LEYDIG CELL AREA (um^2)		INTERSTITIAL DEFFERENTIAL COUNTS (%)			GERMINAL EPITHELIAL HEIGHTS	EPITHELIAL CELLS HEIGHTS		
I.		Cytoplas-mic	Nuclear	Mature Leydig cell	Fibroblast like cells	Degenerating cells	(um)	Caput epididymides	Cauda epididymides	Seminal vesicle
II.	Control	82.21 ±0.09	19.88 ±0.05	43.35 ±2.5	43.8 ±2.7	14.7 ±0.50	102.88 ±1.83	42.37 ±0.39	31.84 ±0.62	18.71 ±0.21
III.	100mg/day for 60 days	43.09** ±0.05	10.38** ±0.04	31.35** ±1.29	38.32ns ±1.49	30.33** ±2.18	77.52 ±0.53	22.92** ±0.34	20.00** ±0.19	15.41** ±0.21
IV.	200mg/day for 60 days	56.11** ±0.16	12.56** ±0.04	29.88** ±1.14	35.16ns ±0.38	34.92** ±5.74	73.00 ±1.81	18.74** ±0.12	17.62** ±0.15	13.2** ±0.25
V.	300mg/day for 60 days	48.46** ±0.09	13.01** ±04.04	28.69** ±1.72	34.25** ±0.21	37.83* ±6.72	59.3* ±2.27	13.74** ±0.50	10.62** ±0.12	8.85** ±0.17

ns = nonsignificant * = P 0.01 compared with control

** = P 0.001 compared with control



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