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EFFECT OF CRUDE DRUG COMBINATIONS ON FERTILITY IN MALE ALBINO RATS

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ABSTRACT: The extracts of bark, leaves and stem of A. indica, fruits of P. longum, berries of E. officinalis and seeds G. indicum were prepared using different solvents. Six different combinations of these extracts were tried on male albino rats to study their effects on sperm production on histological changes in the tests of the rats here was no significant change in production and viability of sperms. However, the rats treated with combinations I, II and III exhibited histological changes in the seminiferous tubules of testes, due to high degree of testicular edema.

Introduction

In Ayurvedic texts, references, are available of various plant extracts used as good antifertility agents without side effects. Norman et al (1) have reviewed the potential value of plants as source of new antifertility agents. The antifertility activity was found to be due to their adverse action on spermatogenesis or sperm motility, because of the inhibition of testicular steroidogenesis (2-8). The antifertility activity of berries of E. ribes in albino rats has been reported earlier (9-11). Kholkute et al (12-13) screened the dried fruits of P. longum and its various extracts for antifertility activity in female albino rats. The antifertility activity of A. indica has been investigated (14, 15). (+) Gossypol was screened for postcoital anti-implantation activity on female albino rats (16). The antifertility, antiovulatory, mitodepressive and clastogenic activity of crude drug combinations have been reported earlier by the authors (17-21).

Though, literature is available on the effects of individual vegetative extracts as antifertility agents in male and female rats, the research pertaining to antiertility screening of different combinations of extracts prepared from crude drugs is lacking. In the present investigation, an attempt has been made to study the cumulative effect of different plant extracts in combination on fertility in male albino rats.

Materials and methods

A. Preparation of Crude Drug Combinations

The extracts of the crude drugs were prepared by percolation technique with different non-polar and polar solvents. The leaves, stem and bark of Azadirachta indica (Meliaceae), berries of Emblica

officinalis (Euphorbiaceae), and seeds of Gossypium indicum (Malvaceae) were used for the preparation of extracts.

Combination I: pet ether (60-80C) extract of leaves of A. indica; pet ether nails; and ethanolic extract of fruits of P. longum (1:1:1).

Combination II: Pet. ether (60-80*C) extracts of leaves, bark and stem of A. indica in equal proportions; pet ether (60-80*C) extract of berries of E. officinalis; and pet ether (60-80*C) extract of fruits of P. longum (2:1:1).

Combination III: Ethanolic extracts of bark, stem and leaves of A. indica in equal proportions; ethanolic extract of fruits of P.longum and methanolic extract of seeds of G. oindicum (2:1:1).

Combination IV: Ethanolic extract of leaves and stem of A. indica in edual proportions; ethanolic extract of fruits of P. longum; ethanolic extractof berries of E. officinalis and ethanolic extract of seeds of G. indicum (1:1:1:1).

Combination V: Aqueous extract of leaves and stem of A. indica in equal proportions; aqueous extract of fruits of P. longum; aqueous extract of berries of E. officinalis; and aqueous extract of seeds of G. indicum (1:1:1:1).

Combination VI: Benzene extract of leaves of A. indica; solvent ether extract of berries of E. officinalis; pet. Ether (60-80 C) extract of seeds of G. indicum; and pet ether (60-80C) extract of fruits of P longum (1:1:1:1).

B. Effect on Fertility of Male Albino Rats

The healthy male albino rats (Wistar strain) of proven fertility obtained from National Institute o Nutrition, Hyderabad were acclimatized to laboratory conditions for a period of fifteen days prior to experimentation. The rats were grouped into 7 groups of 8 animals each. First six groups of rats received drug combinations I-VI orally at a dosage of 200 mg/Kg body weight for 21 days and seventh group of rats served as control and received 1% corboxy methyl cellulose (CMC) for the same period of experimentation. On the 22^{nd} day, the rats were individually weighed and sacrificed. The testes of each animal were carefully dissected out and weighed to calculate Gonadosomatic index (GSI). The cauda epididymis of each testis was finely teased in 20 ml of normal saline and the sperm motility saline and the sperm motility and viability were observed and also the unit sperm count was recorded using haemocytometer. The weighed testes were labeled and fixed in Bauins fluid and further processed for histological studies. The sections of testes (5µ) were obtained using ERMA rotator type microtome. The selected sections of the tissues from all the 7 groups of tats were stained with haematoxylin and eosin and mounted with DPX mountant on microscopic slides. Microphotographs were taken with 100X resolution with the aid of photographic attachment to the Meopta research microscope. The seminiferous tubular density of each section was calculated using the microscopic calibration scale.

Results and Discussion

Different parameters pertaining to the studies on fertility of the rats due to crude drug combinations in comparison to the control are given in Table-I. The body weight changes of rats before and after treatment of drug combinations were not significant. Gonadosomatic index values of rats fed with combinations I,IV and VI are in the order of 1.08, 1.15 and 1.29 respectively in comparison to 0.69 for control groups. There is no dignificant change in count, motility and viability of sperms in all the tested groups. The histological studies of the testes sections recealed high dregree fo testicular edema (Figs. 2-4) in rats treated with combnations I,II and III. Combinations IV and VI, however, revealed only mild edema. Combination V showed minimum edema almost resembling that of control group (Fig.1). the histological sections revealed no abnormal effect on the spermatogenic cycle and on the sperm production. All the drug treated rats showed similar structure of tubular epithelium of cells (4-6 layers) with normal differentiation as in the control group. There was no variation in the phases of spermatogenesis or in their relative frequency. The different combinations of phytochemicals derived from higher plats did not exhibit any significant antifertility activity in male albino rats. It was however, interesting to rots that combinations I, II and III exhibited significant histological changes in he testes due to high degree o testicular edema. Administration of combinations I. II and III could be further elucidated for the physioloicalbasis responsible or the variation in histological behavior of testes.

Fig. 1: Control.
Cross section of seminiferous tubules (X100) showing tubules of differentiating spermatozoa giving wheel spoke appearance. Type B spermatogonia are clearly seen at the peripheri of the tubules. The tubules are closely set giving a compact look (Phase-6).

TABLE –I Effect of crude drug combinations on fertility in male albino rats

Group No	No. of Animals In each	Combi-nation	Route dose mg/kg	Average Body wt. before Treatment (Mean ± S.D)	Average Body wt. after Treatment (Mean ± S.D)	Average Testes Weight (Mean ± S.D)	GSI values (Mean ± S.D)	Average No. of sperms/0.1 ml of normal saline*	Average** Diameter of tubules (u)	Average** No. of Tubules For unit Area (mm)
1	8	I	Oral/200	259.80 ± 25.66	246.60 ±28.25	2.70 ±0.11	1.08± 0.12	291± 13	315.81 ± 7.00	8.12 ±1.60
2	8	II	Oral/200	283.60 ±42.90	285 .60 ±45.07	2.65 ±0.23	0.87 ±0.09	253 ±12	291.61 ±6.17	8.51 ±2.70
3	8	III	Oral/200	261 .60 ±44.05	260.20 ±42.90	2.62 ±0.11	1.03 ±0.17	300 ±13	307.92 ±6.32	7.80 ±2.45
4	8	IV	Oral/200	255.40 ±26.33	249.60 ±24.66	2.86 ±0.27	1.15 ±0.12	299 ±14	269.10 ±6.11	9.60 ±2.70
5	8	V	Oral/200	291.00 ±17.50	293.20 ±19.51	2.76 ±0.31	0.94 ±0.13	273 ±15	276.15 ±5.02	11.41 ±1.98
6	8	VI	Oral/200	233.20 ±17.50	234.00 ±69.04	2.85 ±0.23	1.29 ±0.32	296 ±12	307.71 ±8.74	10.22 ±2.85
7	8	Control (1% CMC)	Oral/200	290.00 ±29.47	300.00 ±35.53	2.46 ±0.61	0.69 ±0.16	297 ±13	281.47 ±6.06	12.31 ±2.31

*Each cauda epididymis of testis teased in 20 ml or normal saline for sperm count.

** Average of 25 readings.

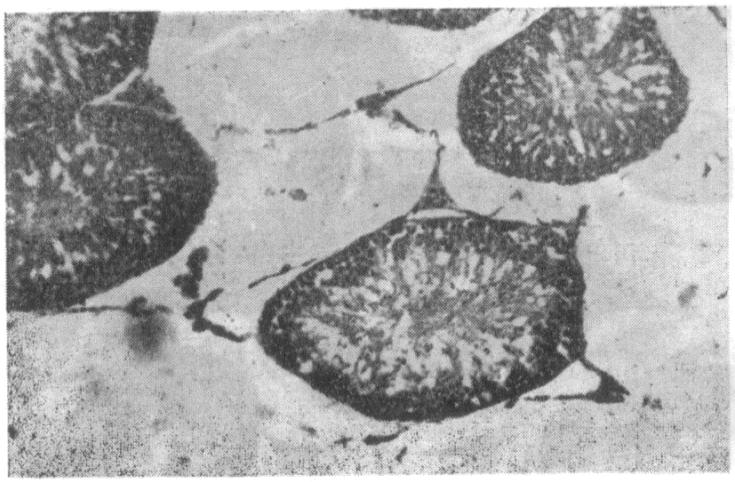

Fig. : Comboination I.
Cross section of seminiferous tubules (X100) with spermatozoa free in the lumen with their tails forming a vortex. The dark granules at the peripheri of the lumen represents cellular detritus. The tubules are widely set apart due to interstitial edema (Phase-8).

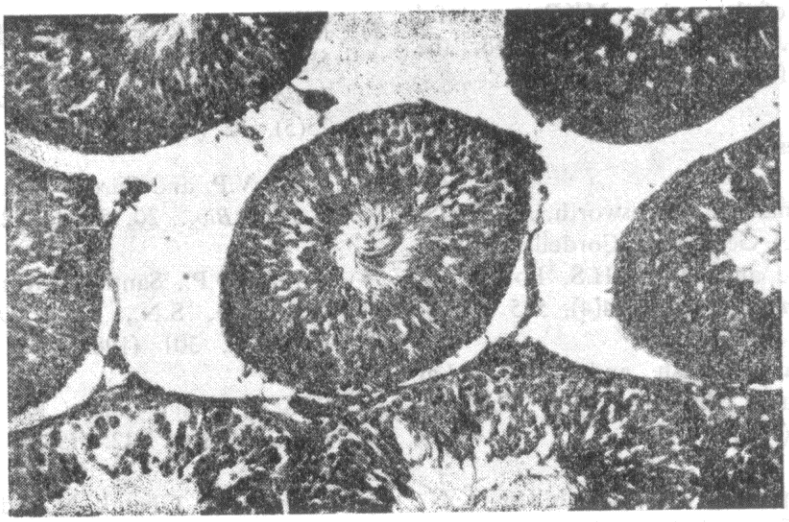

Fig. 3: Combination II.
Gross section of seminiferous tubules (X100) showing spermatozoa lying in the lumen with characteristic vortex. There is moderate separation of the tubules from interstitial edema (Phase-8).

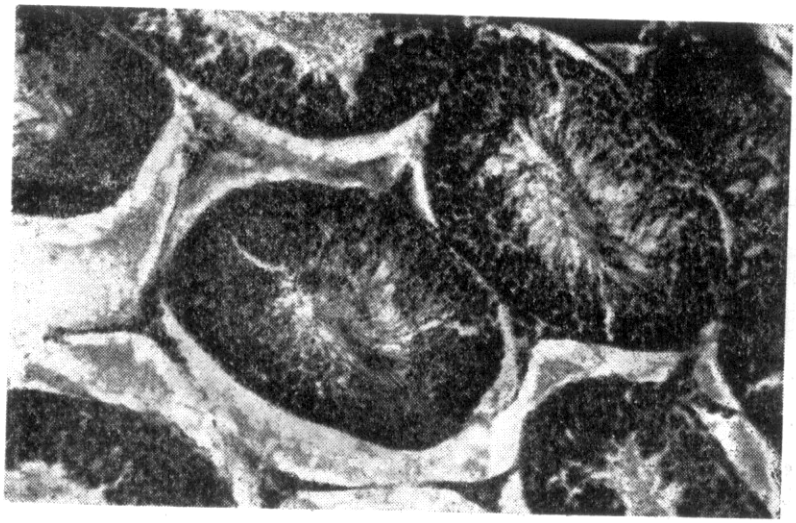

Fig. 4: Combination III.
Cross section of seminiferous tubules (X100) showing spermatozoa having hardly any bundles. Spermatozoa forming a complete outer layer. The tubules are moderately set apart due to interstitial edema (Phase-7).

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