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Duration dependent effect of plants extract on Hematology Histopathology Hormonal profile and Sperm parameters of rats: An approach for male contraceptive development

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Abstract:

Fertility control is an issue of global and national public health concern. Through increase public awareness, statements supporting research on male methods and greater involvements of men reproductive health have been forthcoming from several quarters. The clinical and scientific basis for the research has been reviewed in recent years. Apart from research for finding harmeless chemical druges as effective oral contraceptive in the wastern countries, the crude herbal drug used by tribal people are being closely looked into for their possible efficiency to find out safe and effective oral drugs for controlling human fertility. In present study our aim was to investigate the effect of extracts of *Saccharum officinarum*, *Momerdica diocia* and *Ocimum santum* plants on hematology, histopathology and cauda epididymis sperm parameters, its subsequent recovery and fertility of male albino rats. Male Wistar strain weighing between 180±30 gm were treated with plants ethanolic extracts of, root of *Momordica diocia*, leaves of *Saccharum officinarum* and *Ocimum sanctum* at the dose level of 20, 500, 250 mg/kg. b.wt./rat/day. The test groups were subdivided in two groups A and B, at the 60th day of experiment group A rats were autopsied while B group rats were allowed to recover for another 60 days without any drug administration. The plants extracts diminished reproductive organ weight significantly ($p<0.001$). Decrease number of germ cells and the less number of spermatozoa was found in lumen of all extract treated group, which was recovered after withdrawal of treatment. The hematological

parameters were all found with in normal range, this indicates that the ethanolic extract of these plants has no toxic effect on physiology of rats. Significant ($p<0.001$) reduction in the serum testosterone as well as in estradiol level in extract treated rats were observed in comparison to control and very low reduction observed in *Ocimum sanctum* treated animals. The effect brought by these plant extracts are non-toxic and transient.

Key words: Antifertility, *Saccharum officinarum*, *Momerdica diocia*, *Ocimum santum*, epididymis spermatozoa, reversible male contraceptive.

Introduction

Rising human population throughout the word more particularly in developing and underdeveloped parts has now area of concern. Fertility regulation comprising contraception and management of infertility forms an important component of reproduction health. Though considerable progress has been made in the development of highly effective, acceptable and reversible methods of contraception among females, progress and possibilities on males are still limited. With recent progress in male contraception research, there is a need to develop new contraceptive modalities for male .

Male reproduction is a multifaceted process that involves the testes, epididymis, accessory sex glands and associated hormones. Testes perform two highly organized and intricate events, called spermatogenesis and steroidogenesis, which are vital for the perpetuation of life. Spermatogenesis, a highly dynamic and synchronized process, takes place within the seminiferous tubules of the testis with the support of somatic sertoli cells, leading to the formation of mature spermatozoa from undifferentiated stem cells. The interstitial compartment, which comprises leydig cells, are the site of site of steroidogenesis in the testis. The male reproductive system is extremely sensitive to various environmental factors such as life style, drug, radiation, pollution and toxicants, the result of which could be congenital abnormalities in infants and functional alteration in adults. Several natural and synthetic products are reported to target the testis at the hormonal level or spermatogenesis or both.

Despite the availability of various contraception modalities, one of the most challenging pursuits in the realm of pharmaceutical and medical sciences is research for newer, more potent, safe and less expensive methods that require infrequent and self administration and should have long lasting but complete reversible antifertility effect.

In developing countries, 80% of the population continues to use medicinal plants and plant products in handling primary medical problems due to their accessibility and affordability. In these countries a variety of plants are claimed to have fertility regulation properties and a few have been tested for such effects [1,2,3,4,]. The attention paid buy health authorities to use of herbal medicines has increased considerably both because they are often the only medicine available in less developed areas and because they are becoming a popular alternative medicine in more developed areas.

To date a number of plants with antifertility effects have been screened[5,6]. *Saccharum officinarum*, *Momerdica diocia* and *Ocimum santum* are commonly known plants which have also posses antifertility activites as reported prevesiouly. In our research laboratory the antifertility effect of ethanol extracts of these plants being investigated to explore comparative possibilities of contraceptive efficacy of these plants in male albino rats.

Results

The data shown in the table (1) reveled that plant extracts induced significant increase in body weight, but the reproductive organ weight diminished significantly ($p \leq 0.001$) as compare to control rats.

The control rat testis showing normal size of seminiferous tubule with all successive stages of spermatogenesis, lumen was filled with sperm. Rats treated with extracts regressed size of seminiferous tubule. Decrease number of germ cells and the less number of spermatozoa was found in lumen. After recovery of drug treatment seminiferous tubular size restored almost normal and sperm concentration was also increased in rats.

The haematological parameters were all found with in normal range. This indicates that the ethanolic extract of these plants has no toxic effect on physiology of rats as shown in table (2). A significant ($p \leq 0.001$) reduction in the serum testosterone as well as in estradiol levels in extracts treated rats were observed in comparison to control, but very low reduction observed in *Ocimum sanctum* treated animals.

Analysis of sperm parameters, such as total sperm count, total number of motile sperm, forward velocity of the sperm and percentage of abnormal sperm of the cauda of epididymal plasma were carried out in the control and all the treated animals. All parameters are significantly change compare to control and after withdrawal of drug were found to be measured significantly (table 4).

All treatments with extracts was highly effective in producing reversible functional sterility. Weight reduction of the reproducing organs of treated male rats clearly indicates that the drug caused structural and functional alteration in testes, epididymides, seminal vesicle and ventral prostrate. Plant extracts in present experiment lead to change the normal status of the reproduction in rats and thus produce significant antifertility effect.

Discussion

The present study shows these plant extracts suggested a possible role of these plants as a potential agent in the field of male fertility regulation. Treatment with these plant extract was highly effective in producing reversible functional sterility. Weight reduction of the reproduction organs of treated male rats clearly indicate that the drug caused structural and functional alteration in testes, epididymides, seminal vesicle and ventral prostate.

Decrease number of spermatozoa may affect the level of sialic acid in testes. The reduced sialic acid content might alter the structure integrity of acrosomal membrane, ultimately affect the metabolism, motility and fertilizing capacity of spermatozoa [7], which could not peretrate the cervical mucus and

thus failed to fertilize the ova [8,9]. All these factors thus brought about functional sterility in the extract treated rats. However, the induced infertility was completely reversed after withdrawal of the treatment. Non-toxicity of ethanol extract of these plants is supported by the data obtained after examination of hematological parameters, which remain unaltered. Thus, it can be concluded that extracts from these plant possible exert a reversible antifertility effects mediated through testes and /or epididymis. Result of these present study clearly shows that these plant extracts brings about a reduction in sperm count. The result suggested that infertility in male rats seems to be due to impairment of spermatogenesis as well as changes like decrease in pH, hypotonic environment, and chemical substances like mucoproteins, alkaline phosphatase and acid phosphatase in spermatogenic cells leading to formation of non-viable spermatozoa [10].

Pharmacological effects of many plants have been studied. However, there are many limitations regarding safety and efficacy of these preparation. Knowledge about the active principles of plant preparations is not well defined and information on toxicity and adverse effects of these plant preparations are lacking. Information regarding pharmacokinetics and bioavailability is not available. Assurance of safety, quality and efficacy of medicinal plants and plant preparations are key issues, which need to be addressed. Selection of plant should be based quality, standardization of methods of preparation, enforcement of regulation regarding appropriate labels are measures, which will improve the quality and acceptability of herbal preparations. Ecotype pharmacological evaluation is very essential when the plants are used in crude form. The relative proportion of phytochemical present in medicinal plants can vary in different ecotypes. Keeping in view the importance of plants extracts as contraceptive in national regional land and international perspective there is an urgent need to locate collect and study its diversity. At the same time it is also essential to undertake ethno botanical studies to link these plants contraceptive efficacy with ethnic/folklore remedies to evaluate how different tribes use these plants. There is a dire need to documented this folklore traditional knowledge, which is vanishing rapidly due to lack of awareness in these people, also effective measure are required to document available diversity and bring out systematic information for wider dissemination and utilization of world's genetic diversity in these plants for exploring its therapeutic potential further. In conclusion future research effort should be directed towards the safety, quality and efficacy of medicinal plants and plant preparations used as natural contraceptive.

Materials and Methods

Animal model:

Colony bred, healthy adult male albino rats of the wistar strain, weighing between 180±30 g were used. The rats were hosed in plastic cage under standardized conditions (natural light). Standard rat feed and tap water provided *ad libitum.* Body weight of each animal in all groups was measured weekly to see the possible weight loss throughout the experiments. Indian national sciences academy(I.N.S.A.), New Delhi [11] guidelines were followed for maintenance and use of the experimental animals.

Preparation of plant extract:

Plant materials were shade dried, powdered and subject to soxhelet extraction with 50% ethanol [12]. The ethanol was evaporated under reduced pressure to obtain the crud extract. Extracts were dissolved in distilled water to use in desired concentrations. Root of *Momordica diocia*, leaves of *Saccharum officinarum* and *Ocimum sanctum* were used in antifertility examination.

Dose and duration of treatment:

The male rats of the experimental group were divided in control (group I) and test (II, III, IV) groups. First group of 6 rats served as vehicle treated control, while other groups of 6 rats each, were treated with ethanol extracts of, root of *Momordica diocia*, leaves of *Saccharum officinarum* and *Ocimum sanctum* at the dose level of 20, 500, 250 mg/kg. b.wt./rat/day. The test groups were subdivided in two groups A and B, at the 60^{th} day of experiment group A rats were autopsied while B group rats were allowed to recover for another 60 days without any drug administration.

Body weights:

Body weight of rats were recorded before treatment and at end of the treatment.

Autopsy Schedule:

The rats were autopsied within 24h of the last dose and at the end of withdrawal period, under ether anesthesia. The testes, epididymides, seminal vesicle, ventral prostate were excised dissected and freed of fat, blood vessels and weighed.

Histological studies:

Histological studies were carried out by collecting the specimens from testes and epididymis. These specimens were fixed in 10% neutral formalin and bouin fixatives. 5 micron thick paraffin section were prepared, stained with hematoxylin-eosin and examined microscopically.

Hematological:

Blood samples were collected from retro-orbital plexus [13]. An aliquot from blood sample was collected into tubes with EDTA for determination of hematological parameters, which were estimated by using Heamocytometer. Haemoglobin was determined by using Haemometer, the rest of the blood samples used for serum for bioassay of reproductive hormones.

Radioimmunoassay of Hormones:

Blood samples were also collected for estimations of serum testosterone, estradiol. Serum samples were separated by standard procedures and stored at -20^0C for subsequent analysis.

Sperm analysis:

The cauda epididymis was chopped into phosphate buffered glucose saline (PBGS). The debris was removed and clear suspension, the epididymal plasma was used for the analysis of total sperm count, sperm motility, forward velocity and relative percentage of abnormal sperms in male albino rats. The total sperm count and motility were calculated according to the method of Besley[10] using Neubauer's haemocytometer. Briefly, to increase the accuracy of sperm count, the epididymal plasma was diluted with a spermicidal solution, prepared by dissoloving 5 g of sodium bicarbonate ($NaHCO_3$) and 1 ml of 40% formaldehyde in 100 ml of normal saline.

A twenty times dilution was made using W.B.C pipette, which was thoroughly mixed and one drop was added to both sides of Neubauer haemocytometer. The spermatozoa were allowed to settle down in the haemocytometer by keeping them in a humid chamber for one hour. The sperm count was done in R.B.C counting 5 major squares. The total number of sperms were counted in all the major squares and calculated as follows.

$$\text{Total number of sperms/ml plasma} = \frac{\text{Total number of sperms per square (x)}}{\text{Total volume per square } (10^{-4})} \times \text{dilution factor (20)}$$

Similarly the total number of motile sperms was calculated, using phosphate buffer saline instead of spermicidal solution. The forward velocity of the sperm was calculated according to the method of Ratnasoorya[11]. Briefly, the epididymal plasma was suspended in phosphate buffer saline, cleared the tissue debris and a clear solution was used for the assessment of average forward velocity of sperms. The assessment was made under light microscope, fitted with a movable mechanical stage and a calibrated ocular micrometer, at 400 X magnification. A drop of sperm suspension was transferred to a clean glass slide and the initial place and time of each sperm was recorded. The time taken for forward movement of sperm from the initial place within microscopic field was recorded using a stop watch. The procedure was repeated for 10 spermatozoa in each sample and the average forward velocity of sperm was calculated and expressed as µm/sec. The relative proportion of abnormal sperms was analyzed according to the method of Bauer *et al* [14]. Briefly, equal volume of cauda epididymal plasma and 5% NaHCO3 were taken in a centrifuge tube, mixed well and centrifuged for 5 minutes at 4000g. The supernatant was discarded and 5 ml of normal saline was added to the precipitate, mixed well and centrifuged again. The procedure was repeated 2 to 3 times and a clear precipitate was obtained. To the final precipitate few drops of normal saline were added, mixed thoroughly and a smear was prepared on a clean slide. The smear was dried at room temperature, fixed by heating it over the flame for two to three seconds. Then the smear was flushed with 95% alcohol, drained and dried. It was stained in Ziehl Neelson's Carbol Fuchsin diluted with equal volume of 95% alcohol for 3 minutes and counter stained with 1:3 (v/v) aqueous solution of Loeffer's methylene blue for 2 minutes. After staining, the smear was rinsed in water and dried in air. The abnormal sperms included categories like double tailed, detached

head, detached tail, mid piece bending and irregular head. The relative proportion of the normal and abnormal sperms was from the smear and expressed in terms of percentage.

Statistical analysis:

The data are expressed as mean± SEM. Statistical analysis was carried out by one-way analysis of variance (ANOVA) and the comparisons between the group were done by Student's t-test. Difference were considered to be statistically significant when $p<0.001$.

Table 1: Effect of plant extracts on the body weight and organ weight in rats

Group/Test		Body Weight (g)	Organ Weight (mg/100g b. wt.)			
			Testies	Epididymides	Seminal vesicle	Ventral prostate
Group I		185 ±3.2	1250.5±21	428.3±6	760.1±3	360.3±8
Group II	A	190.5±3.2	1008.2±18	380.2±5	690±5	322.2±6
	B	200.4±4.5	1200.4±14	419±4	720±3	348.2±3
Group III	A	198.5±4.5	980±22	370±5	610±4	308±3
	B	208.7±6.4	1129±17	395±3	678±7	350±6
Group IV	A	197.7±5.8	972±18	400±5	680±5	310±3
	B	218.8±6.8	1108±17	415±6	730±8	329±5

Values are statistically significant at $p<0.001$.

Table 2: Effect of plant extract on haematological parameters in rat

Test/Group	Group I	Group II		Group III		Group IV	
		A	B	A	B	A	B
RBC (mill/mcl)	8.21±0.2	9.5±0.4	8.8±0.4	9.2±0.4	9.6±0.6	10.6±0.6	8.8±0.4
WBC(thous/mcl)	9.30±0.2	10.5±0.5	8.5±0.7	12.5±0.5	10.5±0.4	9.3±0.5	10.6±0.7
Hb (g/dl)	15.9±0.2	12.5±0.5	9.5±0.6	13.8±0.6	17.6±0.7	14.5±0.4	17.5±0.3
Lymphocytes(%)	43.40±2.9	48.30±4.5	40.30±2.5	48.9±3.5	38.8±4.6	46.5±4.4	45.7±39.7
Monocytes (%)	3.30±0.6	4.3±0.3	4.0±0.6	3.2±0.8	3.8±0.6	4.3±0.7	3.9±0.4
Neutrophils(%)	30.90±1.9	28.4±0.4	32.5±0.5	30.4±0.5	33.5±0.2	27.7±0.2	32.3±0.8
Eosinophil(%)	2.40±0.7	3.3±0.2	2.80±0.3	1.3±0.4	2.2±0.2	1.0±0.8	3.5±0.8
Basophil(%)	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0

Table 3: Effect of plant extracts on hormonal levels

Test/Group	Group I	Group II		Group III		Group IV	
		A	B	A	B	A	B
Testosterone (ng/ml)	1.3±0.08	0.5±0.02	2.2±0.05	0.8±0.05	2.3±0.04	1.0±0.3	2.5±0.08
Estradiol(pg/ml)	4.4±0.6	2.4±0.06	5.4±0.03	3.4±0.07	4.8±0.03	2.6±0.07	4.3±0.5

Testosterone values are statistically significant ($p<0.001$).

Table 4: Effect of plant extracts on various sperm parameters

Group/Test		Sperm motility	Sperm count	Forward velocity (µm/sec)	Abnormal sperm (%)
Group I		62.40±2.36	67.47±1.48	127.34±3.5	11.78±0.78
Group II	**A**	31.4±1.4	31.4±1.6	34.36±1.3	21.4±0.6
	B	57.7±1.8	62.3±1.8	102.4±2.2	13.6±0.8
Group III	**A**	34.28±2.5	37.4±1.6	80.71±2.9	34.6±1.3
	B	51.8±1.6	65.64±1.6	134.5±1.9	17.4±2.3
Group IV	**A**	26.24±1.2	30.00±1.7	15.6±1.3	65.7±2.4
	B	67.7±2.7	65.3±1.7	138.7±2.3	13.85±2.4

Values are statistically significant at $p<0.001$.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research report. The present work is part of first author's Integrated Doctor of Philosophy program in Biomedical Sciences at Bundelkhand University, Jhansi, India.

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