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EVALUATION OF EFFECT OF CATHARANTHUS ROSEUS, SOLANUM XANTHOCARPUM AND MOMORDICA CHARANTIA EXTRACT ON REPRODUCTIVE SYSTEM OF MALE RATS

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ABSTRACT

To determine the effect of ethanolic extract of *Catharanthus roseus, Solanum xanthocarpum* and *Momordica charantia* on the fertility of male albino rats. 25 healthy Male rats weighing between 150-200g were selected. Each category (male and female groups) contains 5 groups of 5 each. First group received distilled water and considered as control. The second and third, fourth and fifth group of animals were received ethanolic extract of *Catharanthus roseus, Solanum xanthocarpum* and *Momordica charantia* orally at a dose of 100, 200 and 400 mg/kg body weight daily for 56 days (8 weeks) respectively. Group V rats were administered 400 mg/kg body weight as in group IV but was allowed a recovery period of eight weeks. In male rats group, there was a substantial decrease in the sperm count, motility and a substantial reduction in sex hormones and fertility capacity reduction were observed. The decreased levels of sperm count, fertility capacity, in male rats reveals the antifertility activity of all three plant extracts in dose dependent manner.

KEYWORD: *Catharanthus roseus, Solanum xanthocarpum* and *Momordica charantia*, Sperm count and Antifertility.

INTRODUCTION

Population control is an issue of global and national public health concern. Birth control is an essential part of our life. A variety of synthetic contraceptive agents are available in the market only for women and their use is associated with severe side effects, the progress and possibilities on male are still slow and limited.[1] The World Health Organization suggested

that practice of usage of traditional medicine for the control of fertility, instead of synthetic drugs, as cost effective management for Birth control.[2] 80% population in the world opting for plant products to treat the diseases.[2] 90% of African countries people depends on the plant products for treating health problems.[3,4] Traditionally, plant medicines were used for the regulation of fertility in the past.[5,6] Since then, many numbers of medicinal plants have been screened for their antifertility effect and their use in female fertility regulation,[7,8] Many plants have been examined for their antifertility effect previously. *Catharanthus roseus, Solanum xanthocarpum* and *Momordica charantia* are the valuable medicinal plant and examined for their potential anti-fertility activity. They contains the various phytochemicals such as alkaloids, tannins, flavonoids.

Cataranthus contains wide varieties of chemicals including the glycosides and alkaloids. The indole-indoline alkaloids are very important constituents. About 60 alkaloids have been isolated so far from different parts of Cataranthus. The important alkaloids with anticancer property are vincristine and vinblastine. Vincristine is used in treatment of leukemia. Vinblastine is used for the treatment of generalized Hodgkin's disease and chorionepithelioma. It also exhibits hypotensive and antifertility activities. Kantkari is used in Ayurveda, Siddha and Unani to treat variety of diseases. It is useful in treating worms, cough, hoarseness of voice, fever, painful urination, enlargement of the liver, muscular pain, and stone in the urinary bladder. In migraine, asthma and headache, leaves juice of Kantkari is administered through nasal. It is also having male anti-fertility actions. The paste of whole plant is applied on swollen and painful joints in arthritis. This gives relief in the swelling and pain. In India, Momordica charantia used by tribal people for abortions, birth control, increasing milk flow, menstrual disorders, vaginal discharge, constipation, food, diabetes, hyperglycemia, jaundice, stones, kidney, liver, fever (malaria), gout, eczema, fat loss, hemorrhoids, hydrophobia, intestinal parasites, skin, leprosy, pneumonia, psoriasis, rheumatism, scabies, snakebite, vegetables, piles, tonic, anthelmintic, purgative. In the present study, we intended to evaluate its anti-fertility effect in male rat's reproductive hormones and was compared its potency.

MATERIALS AND METHODS

Collection of Plant Material

The whole plants of *Catharanthus roseus, Solanum xanthocarpum* and *Momordica charantia* used for antifertility activity were collected fromthe area around Amroha. The plant materials

were authenticated by Botanical Survey of India and Voucher specimens were deposited at the same organization.

Methods

a. Preparation of plant material and Extraction

The parts were washed with clean water, air dried at room temperature, pulverized using pestle and mortar, and then stored in air-tightcontainers at 4°C. The pulverized materials were soaked in either distilled water or 70% ethanol and stirred intermittently for 48 hours at room temperature. The crude extracts were filtered using sterile cotton wool and then Whatman filter paper (No.1). The residues were resuspended in the same amount of solvent and filtered three more times. The pooled aqueous filtrates were concentrated over water bath (50^{o}C) to dryness, whereas the aqueous ethanolic filtrates were dried under the electric fan. The dried extracts were stored at 4^{0}C until required.

b. Phytochemical screening

Analysis of major phytoconstituents was carried out qualitatively using standard procedures as described by Odebiyi and Sofowora (1978). One gram of the extract of each plant was dissolved in 100 ml of its own solvent to obtain a stock concentration (1%).

c. Acute Toxicity

This segment of the study is the first step in identifying clinical effects of the extract following oral administration and in establishing dosage regimen for sub-acute toxicity (repeated dose study). The acute toxicity study involved the revised Up – and - Down procedure (9, 10). Five female rats, one rat at a time, were administered by gavage 5000 mg/kg body weight of all the three plant extracts and were monitored for signs of toxicity and mortality within 48 hours of treatment. Thereafter, observations were made daily for 14 days in case of delayed toxicity. At the end, the animals were weighed, humanely sacrificed and necropsied.

d. Fertility studies: **Effect of Extract of C. roseus, S. xanthocarpum and M. charantia on male reproductive functions**

Twenty-five sexually mature male Wistar rats weighing 120 g - 170 g were randomly divided into five groups of five animals each as follows: Group I received the vehicle (0.5 ml distilled water) daily for 56 days and served as the control, Groups II - IV were administered the extracts orally at 100, 200 and 400 mg/kg body weight daily for 56 days (8 weeks)

respectively. Group V rats were administered 400 mg/kg body weight as in group IV but was allowed a recovery period of eight weeks.

The body weight of each animal was recorded at the commencement of the study and at the time of autopsy. At the end of the eighth week, all the rats in groups I – IV were autopsied 24 hours after the last dose of treatment respectively. Rats in group V were autopsied at the end of the sixteenth week. Blood samples were collected by cardiac puncture into sterile plain tubes and allowed to clot at room temperature. Serum samples were aspirated after centrifugation at 3000 rpm for 10 minutes and stored at 20^{o} C until use for testosterone assay.

Reproductive organ weights: At autopsy, the testes, epididymides, vas deferens, seminal vesicles and ventral prostates were unraveled, dissected out, blotted free of blood and cleared of connective tissue or fat. The organs were weighed (to the nearest 0.01mg) using a metler balance (11, 12).

Fertility test: Ten days to the end of the experiment (i.e. at the 46th day of extract administration), the male rats were paired with normal cycling females in a ratio of 1:1. The mated females were allowed to deliver at full term and numbers of litters were recorded. Percentage fertility was calculated as number of pregnant female rats divided by the number of mated females multiplied by 100.

Analysis of epididymal spermatozoa: Semen samples were prepared by mincing the content of 100 mg of cauda epididymis of each rat in 2 ml of physiological saline (13, 14)

a) ***Sperm motility*:** One drop of the diluted semen was applied to a clean glass slide and covered with cover slip. Sperm motility was assessed by counting both motile and immotile spermatozoa per unit area at the magnification of x 40. The right epididymis was used for sperm count and viability, whereas the left epididymis was used for sperm motility and morphology.

b) ***Sperm count*:** The semen from each rat was diluted with a solution (1: 100) containing 5g NaHCO3, 25 mg eosin and 1 ml 35% formalin in 100 ml distilled water. Each counting chamber of the improved Neubauer's haemocytometer was charged with 10 micro litres of diluted semen and allowed to stand for 5 minutes. The spermatozoa were counted under the light microscope at x 40 magnification. (Semen from each rat was counted twice). The count was expressed as million /ml of suspension.

c) ***Sperm viability*:** This was done by using one step Eosin-Nigrosin staining method of Bjorndal *et al* (2003). A smear was made after mixing a drop of the stain and a drop of semen for 30 seconds. The smear was air dried and viewed under the light microscope. The percentages of live and dead spermatozoa were calculated by counting about 200 spermatozoa per sample.

d) ***Sperm morphology*:** Morphological appearance of normal and abnormal (headless tail, curved tail, bent tail, bent midpiece, tailless head) spermatozoa was determined by examining stained smears under the microscope at x 400 magnification and their percentages were calculated.

e) ***Histopathology*:** Tissues were prepared for histopathology by taking small pieces of testis, epididymis, prostate, seminal vesicle and vas deferens. Portions of the organs were removed and preserved in 10% formalin for histopathology. The tissues were dehydrated in graded ethanol, cleared in xylene and embedded in paraffin wax. Serial sections (5µ thick) were cut using rotary microtome. Slides were prepared from these tissues, dewaxed, passed through absolute alcohol and then water for 5 minutes. Slides were stained with haematoxylin (H) and eosin (E) dye and examined under the microscope (Olympus, Japan). Photomicrographs were taken in bright field.

Statistical analysis

Statistical evaluation of data was done using one –way analysis of variance (ANOVA). Means found to be significantly different at $P < 0.05$ were separated 50 using Duncan multiple range test. The results were expressed as mean ± S.E.M. using Graph Pad Prism Version 3.0 for Windows (Graph Pad Software, San Diego, California).

RESULT AND DISCUSSION

Phytochemical screening of *C. roseus, S. xanthocarpum and M. charantia* revealed the presence of alkaloids, glycosides, flavonoids, saponins and carbohydrates as shown in Table 1. Anthraquinones and phlobatannins were not detected in the extract.

Table 1: Phytochemical screening of hydroethanolic extract of *C. roseus, S. xanthocarpum and M. charantia* for alkaloids, glycosides, flavonoids, saponins, tannins, terpenoids, phlobatannins and anthraquinones.

S. No.	Chemical Constituent	*C. roseus,*	*S. xanthocarpum*	*M. charantia*
1	Alkaloids	+++	+++	++
2	Anthraquinones	-	-	-
3	Glycosides	++	++	+++
4	Flavonoids	+++	+++	-
5	Saponins	+++	+++	+++
6	Tannins	-	-	-
7	Phlobatannins	-	-	-
8	Terpenoids	+	-	-
9	Steroids	+	+	++
10	Carbohydrate	+++	+++	+

Key: + + + = highly present, + + = moderately present, + = lightly present - - = absent

Acute Toxicity Study: There was no mortality in the rats when the dose of 5000 mg/kg body weight was administered orally. The aqueous ethanolic extract of *C. roseus, S. xanthocarpum and M. charantia* had no untoward effect on the nervous system since the animals did not convulse. Also no adverse changes in behaviour, breathing, stool, urine and mucous membranes were observed within the period.

Effect of extracts on male reproduction

The effect of all three extract on sperm motility, Sperm Count, Viability, Morphology, Fertility Test and body and organ weights are presented in Table 2, 3, 4, 5, 6 and 7.

Table 2: Effect of *C. roseus* on sperm characteristics of Wistar rats. Values are expressed as means + S.E.M. (N = 5).

Groups	Sperm motility (%)	Sperm Count	Viability (%)	Morphology (%)		Fertility Test	Litter Size
				Normal	Abnormal		
I (Control)	78.00 ± 3.74	70.50 ±4.78	80.80 ± 3.22	83.85 ±3.4	16.15 ± 3. 94	100	6. 40 ± 1. 34
II (100 mg/kg)	28.80 ± 3.80	31.80 ±4.65	22.00 ± 3.52	46.18± 3.96	53.82 ±3. 96	0	0. 00 ± 0. 00
III (200 mg/kg)	23.60 ± 4.41	26.60 ± 3.37	27.80 ± 4.62	43.80 ±2. 77	56.20 ± 2. 77	0	0. 00 ± 0. 00
IV (400 mg/kg)	23.00 ±5. 15	20.00 ±3.65	16.40 ± 3.44	43.90 ± 3. 82	56.10 ± 3. 82	0	0. 00 ± 0. 00
V (Recovery)	65.20 ±4.33	63.33 ±4.41	60.20 ± 3.90	65.35 ± 2. 87	34.65 ± 2. 87	66	4.20 ± 1.80

Table 3: Effect of *S. xanthocarpus* on sperm characteristics of Wistar rats. Values are expressed as means + S.E.M. (N = 5).

Groups	Sperm motility (%)	Sperm Count	Viability (%)	Morphology (%)		Fertility Test	Litter Size
				Normal	Abnormal		
I (Control)	78.00 ± 3.74	70.50 ±4.78	80.80 ± 3.22	83.85 ±3.4	16.15 ± 3. 94	100	6. 40 ± 1. 34
II (100 mg/kg)	27.50 ± 5.50	29.90 ±4.44	21.02 ± 2.32	43.13± 3.36	54.84 ±4. 46	0	0. 00 ± 0. 00
III (200 mg/kg)	21.11 ± 1.41	21.20 ± 2.17	19.22 ± 2.62	42.20 ±3. 17	52.10 ± 1. 17	0	0. 00 ± 0. 00
IV (400 mg/kg)	22.00 ±3. 11	19.00 ±3.45	16.40 ± 3.44	43.90 ± 3. 82	56.10 ± 3. 82	0	0. 00 ± 0. 00
V (Recovery)	64.24 ±2.34	63.44 ±3.23	59.89 ± 3.89	64.99 ± 2.65	36.66 ± 2.64	64	4.34 ± 1.33

Table 4: Effect of *M. charantia* on sperm characteristics of Wistar rats. Values are expressed as means + S.E.M. (N = 5).

Groups	Sperm motility (%)	Sperm Count	Viability (%)	Morphology (%)		Fertility Test	Litter Size
				Normal	Abnormal		
I (Control)	78.00 ± 3.74	70.50 ±4.78	80.80 ± 3.22	83.85 ±3.4	16.15 ± 3. 94	100	6. 40 ± 1. 34
II (100 mg/kg)	26.60 ± 4.40	29.90 ±4.44	21.02 ± 2.32	43.13± 3.36	54.84 ±4. 46	0	0. 00 ± 0. 00
III (200 mg/kg)	22.31 ± 1.21	23.24 ± 3.13	18.23 ± 2.33	42.30 ±3. 13	53.14 ± 1. 18	0	0. 00 ± 0. 00
IV (400 mg/kg)	22.00 ±3. 11	19.00 ±3.45	16.40 ± 3.44	43.90 ± 3. 82	54.14 ± 3. 82	0	0. 00 ± 0. 00
V (Recovery)	64.24 ±2.34	64.44 ±3.23	54.83 ± 3.84	63.83 ± 2.33	34.64 ± 2.34	64	4.34 ± 1.33

Table 5: Effect of *C. roseus* extract on body and reproductive organ weights. Values are expressed as means ± S.E.M. (N = 5).

Parameters	Control	100mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Body Weights					
Initial Wt	148.00 ± 5.82	144.20±3.95	149.20 ± 3.26	145.34 ± 0.10	144.90 ±0.39
Final Wt	196.25 ± 4.15	166.00 ± 4.42	162.40 ± 4.01	166.86± 0.20	167.75 ± 0.28
Testes					
Absolute Wt	2.92±01^{a}	2.16±0.02^{ab}	1.95±0.06^{b}	1.92±0.03^{b}	1.86 ± 0.05^{b}
Relative Wt	1516.18±40.33^{a}	1348.80±44.17^{ab}	1219.20±47.81^{b}	1189.61±49.01^{a}	1176.58±52.04
Epididymis					
Absolute Wt	0.99± 0.04^{a}	0.48 ± 0.09^{ab}	0.31± 0.05^{b}	0.25± 0.02	0.19 ± 0.01
Relative Wt	486.53± 31.97^{a}	322.32±24.37^{ab}	202.84 ± 29.81^{b}	188.92 ± 25.07	175.99 ± 21.45
Ventral Prostate					
Absolute Wt	0.24 ± 0.01^{a}	0.17 ± 0.05^{b}	0.14±0.02^{ab} 83.74	0.12± 0.06	0.10 ± 0.03
Relative Wt	122.32 ± 9.95^{a}	97.02 ± 6.58^{b}	± 6.26^{ab}	78.33 ± 6.04	71.30 ± 6.02
Seminal Vesicles					
Absolute Wt	0.89 ±0.01 ^{a}	0.32±0.04^{ab}	0.27 ±0.05^{ab}	0.25 ±0.02^{ab}	0.19 ± 0.01^{ab}
Relative Wt	436.91± 11.23^{a}	146.01 ± 12.28^{ab}	126.71±10.42^{ab}	118.43±11.32 ^{ab}	108.39±21.21^{ab}
Vas deferens					
Absolute Wt	0.32±0.02^{a}	0.21±0.01^{ab}	0.16±0.01^{b}	0.12±0.02^{b} 98.67	0.10±0.02^{a} 0.86
Relative Wt	144.34 ± 4.81^{a}	131.81 ± 3.07^{ab}	107.36 ± 4.79^{b}	± 5.51^{b}	±6.21^{b}

Table 6: Effect of *S. xanthocarpus* extract on body and reproductive organ weights. Values are expressed as means ± S.E.M. (N = 5).

Parameters	Control	100mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Body Weights					
Initial Wt	146.00 ± 6.55	145.25±3.75	145.20±3.24	144.34±0.14	143.43±0.34
Final Wt	194.52 ± 5.14	176.00 ± 6.14	169.67 ± 3.45	166.46± 0.24	165.74 ± 0.45
Testes					
Absolute Wt	3.01±0.33^{a}	2.13±0.03^{ab}	1.95±0.06^{b}	1.92±0.03^{b}	1.86 ± 0.05^{b}
Relative Wt	1514.14±40.54^{a}	1343.83±43.13^{ab}	1213.23±43.31^{b}	1183.63±43.03^{a}	1173.53±53.03
Epididymis					
Absolute Wt	0.98± 0.05^{a}	0.47± 0.08^{ab}	0.34± 0.04^{b}	0.24± 0.04	0.18 ± 0.05
Relative Wt	484.54± 34.47^{a}	324.34±44.47^{ab}	204.84 ± 24.51^{b}	185.95 ± 24.07	173.94 ± 24.44
Ventral Prostate					
Absolute Wt	0.21 ± 0.02^{a}	0.18 ± 0.04^{b}	0.15±0.03^{ab}	0.14± 0.04	0.11 ± 0.04
Relative Wt	121.31± 9.15^{a}	96.88 ± 6.48^{b}	84.75 ± 6.56^{ab}	77.34 ± 6.54	71.34 ± 6.55
Seminal Vesicles					
Absolute Wt	0.87 ±0.04^{a}	0.32±0.04^{ab}	0.27 ±0.05^{ab}	0.25 ±0.02^{ab}	0.19 ± 0.01^{ab}
Relative Wt	435.95± 15.25^{a}	145.03 ± 13.58^{ab}	127.74±15.45^{ab}	117.53±12.12^{ab}	107.43±22.11^{ab}
Vas deferens					
Absolute Wt	0.33±0.03^{a}	0.22±0.02^{ab}	0.15±0.02^{b}	0.13±0.03^{b}	0.11±0.02^{a}
Relative Wt	143.54 ± 4.51^{a}	132.82 ± 3.02^{ab}	105.35 ± 5.74^{b}	98.55 ± 4.61^{b}	0.85 ±4.25^{b}

Table 7: Effect of *M. charantia* extract on body and reproductive organ weights Values are expressed as means ± S.E.M. (N = 5).

Parameters	Control	100mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Body Weights					
Initial Wt	141.00 ± 6.11	142.25 ± 2.72	143.30 ± 3.23	145.44 ± 0.13	142.13 ±0.31
Final Wt	191.51± 5.11	172.02 ± 6.12	169.63 ± 3.334	166.36± 0.32	163.34 ± 0.35
Testes					
Absolute Wt	2.89±0.13^{a}	2.13±0.03^{ab}	1.95±0.06^{b}	1.92±0.03^{b}	1.86 ± 0.05^{b}
Relative Wt	1511.11±51.54^{a}	1342.82±42.12^{b}	1211.24±41.32^{b}	1181.52±41.23^{a}	1171.52±51.03
Epididymis					
Absolute Wt	0.89± 0.02^{a}	0.47± 0.08^{ab}	0.34± 0.04^{b}	0.24± 0.04	0.18 ± 0.05
Relative Wt	472.34± 32.33^{a}	321.31±41.17^{ab}	202.23 ± 25.11^{b}	182.25 ± 25.02	171.32 ± 22.42
Ventral Prostate					
Absolute Wt	0.22 ± 0.03^{a}	0.17 ± 0.05^{b}	0.14±0.04^{ab}	0.13± 0.03	0.10 ± 0.03
Relative Wt	124.31± 9.13^{a}	92.83± 3.41^{b}	85.55 ±1.46^{ab}	75.44 ± 2.44	72.33 ± 3.53
Seminal Vesicles					
Absolute Wt	0.84 ±0.04^{a}	0.31±0.01^{ab}	0.25 ±0.03^{ab}	0.23 ±0.03^{ab}	0.17 ± 0.02^{ab}
Relative Wt	434.45± 14.25^{a}	141.01±12.52^{ab}	126.64±16.46^{ab}	114.43±14.11^{ab}	108.83±28.41^{ab}
Vas deferens					
Absolute Wt	0.32±0.02^{a}	0.26±0.06^{ab}	0.16±0.02^{b}	0.14±0.04^{b}	0.12±0.03^{a}
Relative Wt	144.14 ± 4.61^{a}	135.81 ± 3.01^{ab}	103.33 ± 4.74^{b}	96.56 ± 5.51^{b}	0.83 ±4.35^{b}

The ability of the animals to successfully fertilize the female and produce viable offspring showed 100% fertility for the control and 40%, 0% and 0% fertility for rats that received doses of 100, 200 and 400 mg/kg body weight of the extract respectively. However, the animals that received the highest dose of extract (400 mg/kg body weight) but allowed to recover for the same period of treatment recorded 60% fertility (Table 2, 3 and 4).

Histopathology: Photomicrograph of the untreated control showed normal histo-architecture of the testis and epididymis whereas necrosis of the seminiferous tubules, leucocytic infiltration into the interstitum, pyknosis of the nuclei and general disorganization of histoarchitecture as well as eosinophilic epididymitis and concretions in ductuli epididymis were observed in the extract treated rats. The disrupted histoarchitecture of the testis was restored to normal following withdrawal of the extract for eight weeks. There were no treatment related adverse effects on prostate gland, seminal vesicles and vas deferens as revealed by their normal histoarchitecture relative to the control.

CONCLUSION

Evaluation of new drugs with lowest side effect which can act both on male reproductive system is necessary in the present era. Because, the present available anti fertility drugs are causing many unwanted effects on long term usage. Male contraceptives are not available for clinical use. So, to produce herbal drugs without side effects in Male reproductive systems, the present study was undertaken. The main purpose of the present study was to evaluate the anti-fertility effect of *Catharanthus roseus, Solanum xanthocarpum* and *Momordica charantia* extracts in Male rats simultaneously. In Male rats, sperm concentration, motility and abnormality in the epididymis, fertility capacity was assessed. Our study revealed that ethanolic extracts of all three plant extracts effectively reduced the sperm count, motility, fertility in Male rats. After treatment with plant extracts in Male groups, found that there is a significant decrease in sperm count of epididymis, increase motility and abnormality of sperm.

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