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Suppression of Fertility in Male Albino Rats Following the Administration of *Piper longum* Crude Extracts

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ABSTRACT

Fertility control is important to keep your reproductive health in good shape. There are many different ways to do this, including contraception and infertility care. Recently, scientists have learned a lot about how male reproductive systems work, and new methods of contraception are needed to help people prevent pregnancy. The study was designed to investigate the effect of *Piper longum* plant extract on fertility in male albino rats. The plant components were extracted effectively with boiling water using a Soxhlet extractor. The animals were fasted overnight and given just water, after which the extracts were given orally at a dose of 5mg/kg body weight. Body weight, sperm motility, sperm count and sperm morphology were measured. The testes, epididymis, vasdeferens, seminal vesicle, and ventral prostrate were then removed, trimmed, and weighed on a torsion balance. The scientists weighed the organs of different animals and found that the animals in the high dose group (600 mg/kg bwt) lost the most weight. The animals in the moderate dose group (300 mg/kg bwt) lost the most weight, and the animals in the low dose group (100 mg/kg bwt) lost the least weight. There were no changes in the vas deferens, seminal vesicle, or prostate in any of the groups. However, the high dose group had a big problem with their sperm. The sperm in the high dose group had a lot more damage to the tail area than the head region.

Key words - *Piper longum*, fertility, soxhlet, vasdeferens, rats, prostate, sperm.

INTRODUCTION

Oral contraceptives are also known as antifertility medicines since they control fertility (Krueger et al, 1974). Some medications affect and complicate female ovulation and the menstrual cycle. Birth control tablets are made of progesterone and oestrogen together (Raji et al, 1997). When the anti-fertility element prevents ovulation, implantation, terminates the zygote, or results in abortion in females, it is considered to be active.

Infertility can be brought on by a variety of different mechanisms, including hormonal, pharmacological, and immunological therapies (Chinoy et al, 1996). Many steroidal and non-steroidal drugs, including Depot Medroxy Progesterone Acetate (DMPA), Danazol, Melatonin, Cyproterone Acetate (CPA), Metapiron, Levenogestral, Seratonin, and Chlorohydrin, affect testicular function (Dwivedi et al, 1990). However, due to various hazards and evidence that they are harmful or atypical in both short and long-term usage, the use of all of these drugs in the reproductive organs has been carefully questioned (Prasad, 1972; Gurrapu et al, 2016). Despite the wide variety of contraceptive methods available, one of the most challenging experiments in pharmacology and medicine is the creation of newer, stronger, additionally safe, and less expensive contraceptive methods that call for infrequent and self-administration and should have a long-lasting but fully reversible antifertility effect.

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The purpose of this study was to examine if the administration of crude extracts of *Piper longum* would reduce fertility in male albino rats.

MATERIAL AND METHODS

Selection of the Plant

Field trips to the villages of Gannaram, Panthini, and Uduthagudem in the Telangana district of Warangal's Vardhannapeta mandal were conducted often during the study period. Local names and parts used for fertility control were disclosed by traditional healers from the aforementioned villages. The collected plants were accurately identified with assistance from senior academics from Kakatiya University's Botany Department. Also, some plants were photographed while on the field visits. A large number of conventional healers were questioned to understand more about these medicinal herbs. Further information about their culture, way of life, and—most significantly—gynecological care were also provided. The tables in the findings section provide a summary of the data collected.

The author noticed that tribal people employ a variety of medicinal herbs as contraceptives in the rural Vardhannapeta neighbourhood in Telangana's Warangal district. There has been no scientific study done on the fertility-controlling abilities of the drug plant Piper Longum, though. In order to evaluate its possible antifertility action in male albino rats, this medicinal plant was chosen.

Collection of Plant Material

Informal discussions with traditional healers revealed that the preparation of this herb varied from community to community. Various herbalists had unique ways of preparing their products; some preferred to dry the ingredients first before mixing them finely, while others preferred to utilise freshly harvested stuff blended together. The solutions are always given orally after being boiled, after which they are allowed to cool.

The whole plant was placed in brown paper bags, dried in the drying room, and ground into a fine powder for extraction.

Plant extract preparation

The plants were washed and given a good water rinse. They were then ground into a fine powder and dried for 5 days at 25°C in the shade without sunlight. Using a Soxhlet extractor, the powdered plant material (almost 30 g) was successfully extracted with boiling water, chilled, and then filtered through Whatmann No 1 filter paper. Centrifuging the filtrate at 10,000 rpm and ambient temperature resulted in the removal of the sediment (250C). For use in the experiment, the supernatant was concentrated on rotavapour and ground at low pressure.

Animals used in research

Healthy male Wistar albino rats weighing between 180 and 240 grammes were the rodents used in this study. The animals were kept in routine environments with 25°C temperature, light, and darkness (12:12h). Rats had unrestricted access to water and were fed a standard pellet diet (NIN, Hyderabad, India).

Studies on Acute Toxicity

To determine the intrinsic toxicity of a plant as well as the effects of acute overdose, it is required to evaluate the toxic activity of plant extracts before deeming a treatment safe. Laboratory mice become sensitised to toxic compounds contained in plants. By giving the extracts in escalating doses, the toxicity limits can be found.

According to the OECD recommendations, the examination into acute oral toxicity was conducted. Six albino rats of either sex were chosen at random for the investigation (OECD, 2002). After being given only water for a whole night, the animals were then fasted. Following this, stomach intubations were used to administer the extracts orally at a dose of 5 mg/kg body weight. The animals were then observed for 14 days. The dose was deemed dangerous if two out of every three animals perished. The same amount was administered to the other animals if one animal perished in order to verify the hazardous dose. The procedure was repeated with higher doses of 50, 100, 1000, and 2000 mg/kg body weight if there was no sign of death.

Design of Experiments

Each plant was subjected to its own experiment. Male rats were put into four groups (total 16 groups) in each plant trial, with six animals in each group.

The male rats in each trial were separated into four groups, each with six animals.

- For 14 days, rats in Group I were given normal saline (control).
- Group II: For 14 days, rats were given an aqueous crude extract of a selected plant at a dose of 100 (hundred) mg/kg bwt (low dose).
- Group III: For 14 days, rats were given an aqueous crude extract of a selected plant at a dose of 300 (three hundred) mg/kg bwt (moderate dose).

Table-1: The effect of *Piper longum* aqueous crude extract on the body weight and reproductive organ weight of adult male albino rats

Treatment Groups	Body wt (gm)		Testis (gm)	Epididymis (mg)		VD (mg)	SV (mg)	Prostate (mg)
	Before	After		Caput	Cauda			
Group-I	255.14±10.3	263.42±11.2	1.685±0.55	131.13±2.6	276.41±12.0	99.26±10.2	340.36±12.8	166.46±8.1
Group-II	237.19±11.4	249.21±9.0*	1.412±0.30*	116.43±4.0*	242.12±7.4*	87.14±1.3	315.43±16.2	153.56±7.2
Group-III	215.15±10.0	234.14±13.1	1.300±0.80**	113.26±2.0**	229.46±4.2**	93.21±1.3	310.56±11.2	165.26±7.4**
Group-IV	213.25±8.1	211.32±11.1	1.008±0.11***	95.43±1.5***	212.72±1.6***	90.46±2.2	315.23±13.2	150.33±7.4**

Each Value is SEM of 6 animals *p<0.05, **p<0.01, ***p<0.001 Control vs Treated

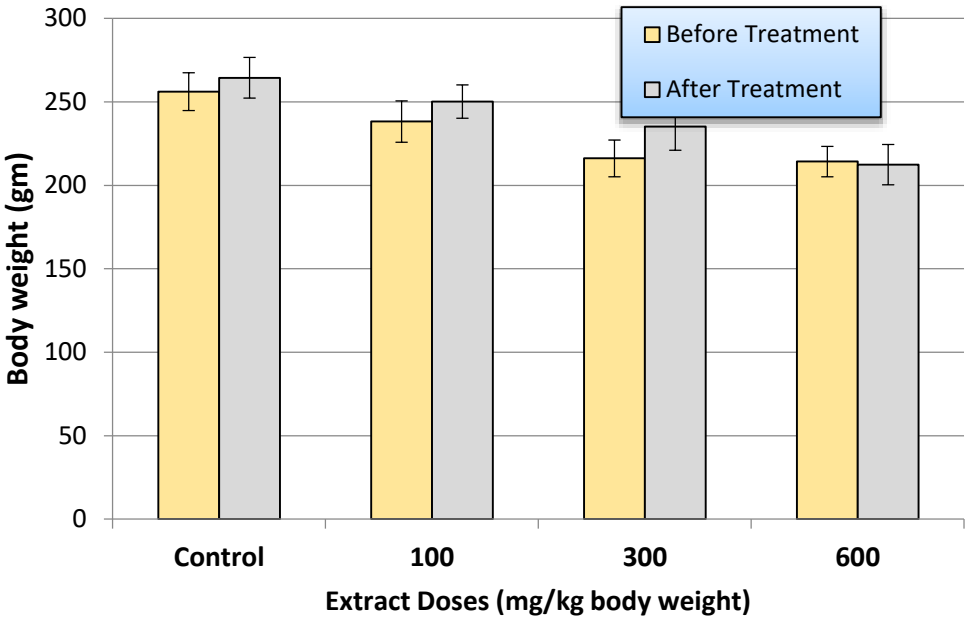

Figure-1 Effect of aqueous crude extract of *Piper longum* on the body weight of control and treated male albino rats

- Group IV: For 14 days, rats were given 600 (six hundred) mg/kg bwt of aqueous crude extract of a selected plant (High dose).

Each trial's ultimate weight was calculated 24 hours after the last treatment, and the animals were then put to death by being beheaded. Before being used in various biochemical assays, serum was separated by centrifugation at 3000g for 10 minutes. The ventral prostrate, seminal vesicle, vas deferens, testes, and epididymis were then dissected, trimmed, and weighed on a torsion balance. The weights of the organs were expressed as milligrammes for every 100 grammes of body weight.

Sperm Count:

To count epididymal sperm, the WHO Laboratory Manual's protocol was used (1999). 24 hours following the last treatment, the rats were given an ether anaesthesia, and the caudal and caput epididymis were immediately removed.

A 1mm incision was then made in the caudal and caput epididymis after that. 95 microliters of diluents were used to dilute a 5 microliter portion of epididymal sperm. The counting chambers of the modified Neubauer type hemocytometer were

Table-2: The effect of *Piper longum* aqueous crude extract on sperm concentration and motility in the epididymis of adult male albino rats.

Parameter		Treatment Groups							
		Group-I		Group-II		Group-III		Group-IV	
		Caput	Cauda	Caput	Cauda	Caput	Cauda	Caput	Cauda
Sperm Concentration (Counts x 10^6 millions)		474.14 ±28.4	502.13±32.03	203.12±13.4*	196.10±21.1*	192.22±13.4**	170.11±15.4**	155.5±12.9***	103.3±17.2***
Sperm Motility (in %)		----	81.0±2.60	----	55.0±3.4*	----	51.0±3.6**	----	41.0±2.0***
Sperm abnormality (in %)	Head	----	4.11±0.12	----	42.5±2.39*	----	67.89±4.10**	----	94.21±5.3***
	Tail	----	5.77±0.45	----	61.33±1.45*	----	87.98±3.13**	----	97.54±3.68***

Each Value is SEM of 6 animals *p<0.05, **p<0.01, ***p<0.001 Control vs Treated

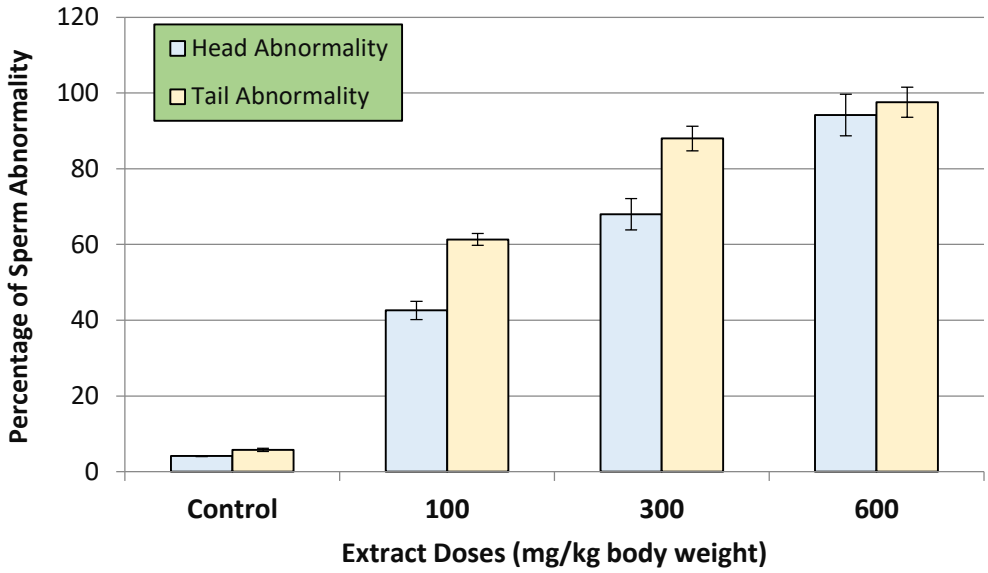

Figure-2 Effect of different doses of aqueous extracts of *Piper longum* on sperm head and tail abnormality in male albino rats

covered with a coverslip. 10 microliters of correctly mixed, diluted material were added to each of the hemocytometer's counting chambers and left to stand for 5 minutes in a humid environment to prevent drying out. Sperm cells settled at this time and were counted under a light microscope with a 200x magnification. It was determined how many spermatozoa were complete (head and tail). In millions per millilitre of suspension, the numbers were then transformed.

Sperm Motility and Morphology

A technique for assessing the motility of epididymis sperm was described by Linder et al. (1986) and Cooke et al. (1986). (1991). A small incision was made in the caudal epididymis with a sharp razor. The fluid that leaked from the cauda epididymis was gathered using a pipette tip and diluted to 2 mL in Ham's F12 medium at 35°C. Motile and non-motile sperm were counted in a hemocytometer using an aliquot of this

solution. Prior to counting the motile sperm, the non-motile sperm were counted first. Calculating epididymal sperm motility involved dividing the number of sperm measured by the proportion of motile sperm.

A study was done on the morphology (abnormality) of sperm from the cauda epididymis. Spermatozoa are made up of a head, neck, primary piece, middle piece (midpiece), and end piece. Due to the difficulty of observing the terminal piece under a light microscope, the cell can be separated into a head (and neck) and a tail (midpiece and principal piece). A spermatozoon is said to be normal if both the head and tail are normal. It is best to regard any forms that are on the verge of being aberrant as such.

Statistic evaluation

By comparing the values of the various treatment groups to the values of the individual controls, the data were reviewed. The outcomes are shown as the mean plus standard deviation. The most recent computer software programme was utilised to search for statistically significant differences between values using one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Effect of *Piper longum* Plant Extract on Fertility In Male Albino Rats

Body & Reproductive Organ Weight

To test for antifertility effects, different doses of an aqueous extract of *Piper longum* were administered to male Wistar albino rats. Rats receiving aqueous extracts of the aforementioned plant had no influence on their body weight (Table 1 and Figure 1) or libido, but their testicular and other accessory sex organ weights were significantly reduced (p 0.01) after administration (Table-1). The accessory sex organs' caput and caudal epididymal segments have significantly lost weight. The weight loss was dose-dependent, with the highest decline occurring in the high dose (600 mg/kg bwt) treated groups (Group IV), followed by the moderate (300 mg/kg bwt) and low dose (100 mg/kg bwt) treated groups (Group III) (Group II). The prostrate, seminal vesicle, and vasdeferens did not change.

Sperm count and Sperm motility

Male Wistar albino rats were administered various doses of an aqueous extract of *Piper longum* to test for antifertility efficacy. The administration of aqueous extracts of the aforementioned plant had no effect on the rats' body weight (Table 2 and Figure 2) or libido, but it significantly decreased the weight of the testes and other accessory sex organs (p 0.01). (Table-3). The caput and caudal epididymal segments of the accessory sex organs had lost a considerable amount of weight. High dose (600 mg/kg bwt) treated groups (Group IV) shed the greatest weight, followed by moderate (300 mg/kg bwt) and low dose (100 mg/kg bwt) treated groups (Group II). The vas deferens, seminal vesicle, and prostrate did not change.

Aberrant sperm

In the caput and caudal areas, *Piper longum* extract greatly decreased sperm abnormalities (p 0.05). The high dose group among the three dose treatment groups displayed the most severe abnormalities in sperm morphology, with the tail portion of the sperm being significantly more affected than the head region in all treated groups.

It is recognised that the accessory sex organs, including the epididymis and vas deferens, are androgen-dependent target organs with a range of androgen sensitivity levels. The internal environment of the epididymis is known to be impacted by any change in the levels of circulating androgens, which can alter sperm metabolism and motility (Khan and Awasthy, 2003).

CONCLUSIONS

The negative effects of these herbs on male reproduction were confirmed when *Piper longum* was administered as a medicine. The negative impact of these 4 plants on sperm may be required as a study benefit because male reproductive toxicology and male contraception are two sides of the same coin. The study's management had no negative effects on the liver or kidneys, nor did it directly affect the development or functionality of the male reproductive system or reproductive organs. In the current study, treatment with *Piper longum*, aqueous crude extracts over an extended length of time resulted to noticeable changes in the male reproductive organs. When therapy is over, will the alterations be permanent or reversible? More research is required to pinpoint the precise procedure.

Conflicts of Interest

Authors declare that there is no conflict of interests regarding the publication of this paper.

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