Antifertility effect of aqueous and ethanolic extract of *Piper longum* on male albino rat.

Richa Sharma[1], Varsha Anand[2], Atul Samiran[3] & D.N. Choudhary[4]

Research Scholar[1,2,3], Associate Professor[4]
University Dept of Zoology, T.M.B.U. Bhagalpur

Abstract

Aqueous and ethanolic extract (100mg/kg of body weight) of fruit of Piper longum were used as treatment in male albino rat to show its antifertility effect. The healthy, adult and fertile rats were divided into three groups, one control group and two experimental groups. Experimental groups were provided ethanolic and aqueous extract of fruit of Piper longum for 21 days. After the treatment fertility as well as other fertility related tests were performed. The obtained result indicates that both types of extract of Piper longum extract cause antifertility effect in albino rat. It significantly reduces the primary as well as secondary reproductive organs weight and alters other reproductive parameters.

Keywords: *Fructose, Piper longum, Protein, Reproductive organs, Seminal Vesicle, Testis.*

Introduction

Rising human population throughout the world especially in developing and underdeveloped countries has detrimental effects on life supporting system on earth. Traditionally, plants have been used to treat different kinds of ailments. The growing importance of phytochemicals in males has been reported. Contraceptive ability of plants has been reported in several animal models. The reversibility of the anti-fertility effects of plants and its active compounds are of potential clinical relevance in the development of male contraceptive. (Princewill *et al*, 2011). Plants have served as a natural source of antifertility substances. (Chouhan , 2007). At present, one of the social problems regarding world health is the stability of population growth. The study of contraception is very important, but is a secondary issue. Many methods have been devised for women, while men have not received enough attention in this respect (Shafik, 1994). Globally, men have not shared equally with women the responsibility for fertility regulation; the lack of male involvement may also reflect the limited options available to men. (Chauhan 2010). The increase in population is becoming a comprehensive dilemma, causing much pressure on economic, social, and natural assets. The population explosion is a leading cause of poverty and pollution in developing countries. (Zade, 2013). Exponentially growing population has been adversely affecting the social, economic and technological development of human race. (Agarwal A 2010). This overpopulation can be checked through biological means with reference to modulation in the human fertility rate. Along with the advancement in the reproductive biomedicine different hormonal contraceptive pills are developing but all have side effects. (Ghosh 2015). Herbal remedies, since ancient times , have been used in almost all human races as a source of medicine. Nearly 80% of the world's population still relies upon medicinal herbs for basic health care needs. (Kong JM 2003). Although they are often considered as natural and relatively harmless, phyto preparations are not always free from toxicity. (Dalsenter, 2004). Herbal products have been used frequently without proper evaluation of their actual efficacy in the treatment or the occurrence of undesirable side effects. Currently little information is available on the reproductive effects of popular medicinal plants. There has been growing interest over the safety of natural products in clinical use and evaluation of side effects on reproductive system has been considered as part of the safety studies of widely used medicinal plants. (Schilter, 2003). Hence, there is a need for efficient drugs to oppose these problems. (Kumar, 2013). In the present study, we have studied the effect of alcoholic and aqueous extract of fruits

of *Piper longum* on the reproductive organs and fertility of rat, Albino male, which were used as an animal model. Several male reproductive end points such as organs weight, biochemical parameters and fertility indices were evaluated.

Material and Methods

Plant Material

Plant: *Piper longum* L. is a climber that grows wild in India, Malaysia, Nepal, Sri Lanka and Vietnam. The stems are flexuous, pubescent and terete. The leaves are simple. The petiole has grooves, varies in length, and can grow to a maximum of 10 cm. The blade is ovate, 6–12 cm 3–12 cm, papery, with an acuminate base an0d two–three pairs of secondary nerves emerging from the base near the apex. The fruits are utilised in pickles, meals, beverages, liquors, and medications in addition to being used as spices. Long pepper is most commonly used as a therapeutic component in Ayurveda, Siddha, and Unani, three of the most prevalent medical systems in India. It uses dried roots as well as fruit. Along with the spikes, thicker stem and roots are used to make "piplamool," an ingredient in Ayurvedic, Sidha, and Unani treatments.

Plant extract preparation

Alcoholic extract

The plant extract was prepared by the method adopted by Agokei et.al,2010. The dried piper fruit was taken for preparation of alcoholic extract. The dried plant parts were powdered by using mixer grinder. 20gm of plant powder of each test plant was poured into a conical flask containing 150ml of 50% of ethanol. The mixture was stirred, allowed to settled, and kept covered. At the end of second day the extract was filtered with no.1 Whatman filter paper. The filtrate was taken on a petri dish and evaporated at room temperature. The residue remained in the petri dish was ready for experiment.

Aqueous extract

Dried fruit of *Piper longum* L. was taken. These parts were washed. For extract preparation, 10g of dried plant parts will be dissolved in100ml of distilled water and left for 12hrs, filtered and diluted with distilled water for required dose. After 12 hrs the solution will be filtered. The filterate is now ready for dilution and required dose preparation (Shubhangi *et.al*,2018 and Choudhary *et.al*;1990).

Experimental animal

Male Albino rats (180-200) gm of body weight of proven fertility were selected for the experiment. The rat ware divided into three separate groups (one for control and two for experimental). Each group containing 07 animals. The experimental group of rats were administered orally with suspension of test plant extracts (alcoholic and aqueous extract) at a dose of 100mg /kg body weight for 21 days. The control group was fed with distilled water for the same period of treatment (choudhary et.al,1990).

Fertility Performance Test

Fertility performance of individual rat was done from day 16^{th} to 21^{st} of treatment. Each male rat was caged separately with 2 healthy, adult and fertile female rat. Presence of sperms in vaginal smear indicated that the females had mated to the particular male and the day of mating was considered to be the day 1^{st} of pregnancy.

Laprotomy was done on 8^{th} day of pregnancy to examine the record of Corpora lutea and Implantation sites. Litters was examined and litters size was recorded at term.

Biochemical Parameters

Male rats were sacrificed on 22nd day and different tissues were collected and weighed on Torsion balance for parameters study. Serial sections of testis were prepared for microscopic observations. (Choudhary et.al,1990). Fructose estimation in coagulating gland (CG) was evaluated by colometry (Mann, 1981; Choudhary et.al,1990).

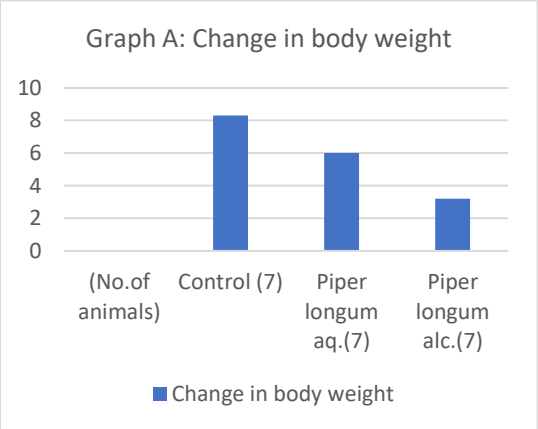
Acid phosphatase activity in ventral prostate (VP) was evaluated by method adopted Sigma Technical Bulletin no. 104. Protein estimation in seminal fluid was estimated by colorimetry (Lowry et.al,).

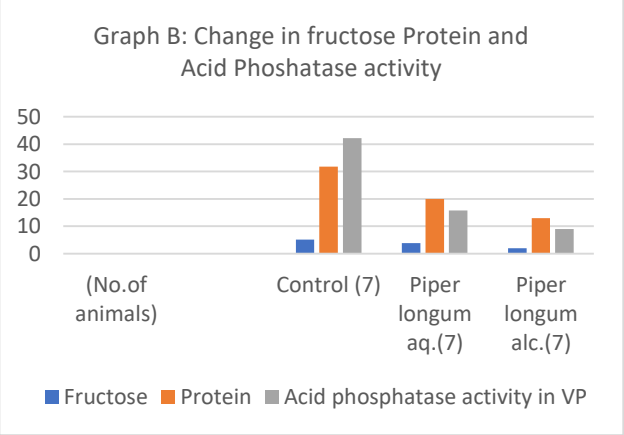
Result

The result of the present study is as follows

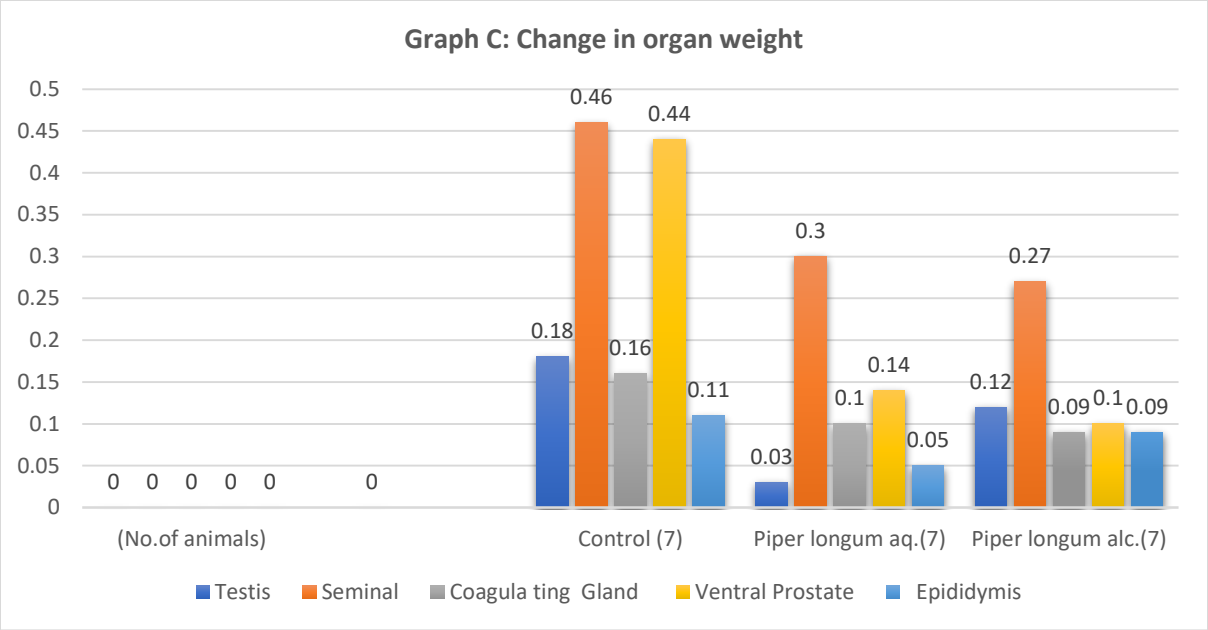
Treatment (No.of animals)	Change in body weight g	Weight of Organ					Fructose (CG) mg/100mg of tissue	Protein (SV) mg/100mg of tissue	Acid phosphatase activity in VP mg/hr/100 mg of tissue
		Testis	Seminal Vesicle	Coagulating Gland	Ventral Prostate	Epididymis			
Control (7)	8.3±2.26	0.18±0.12	0.46±0.04	0.16±0.01	0.44±0.06	0.11±0.05	0.51±0.02	31.83±0.91	42.23 ±0.45
Piper longum aq.(7)	6±1.87	0.03±0.01	0.30±0.01	0.1±0.00	0.14±0.02	0.05±0.01	0.38±0.08	20±3.53	15.72±1.58
Piper longum alc.(7)	3.2±0.58	0.12±0.03	0.27±0.02	0.09±0.01	0.10±0.01	0.09±0.02	0.20±0.03	13±2	8.98±0.46

Table 1: Effect of plant extracts (Alcoholic & Aqueous) on reproductive organs weight and different biochemical parameters.





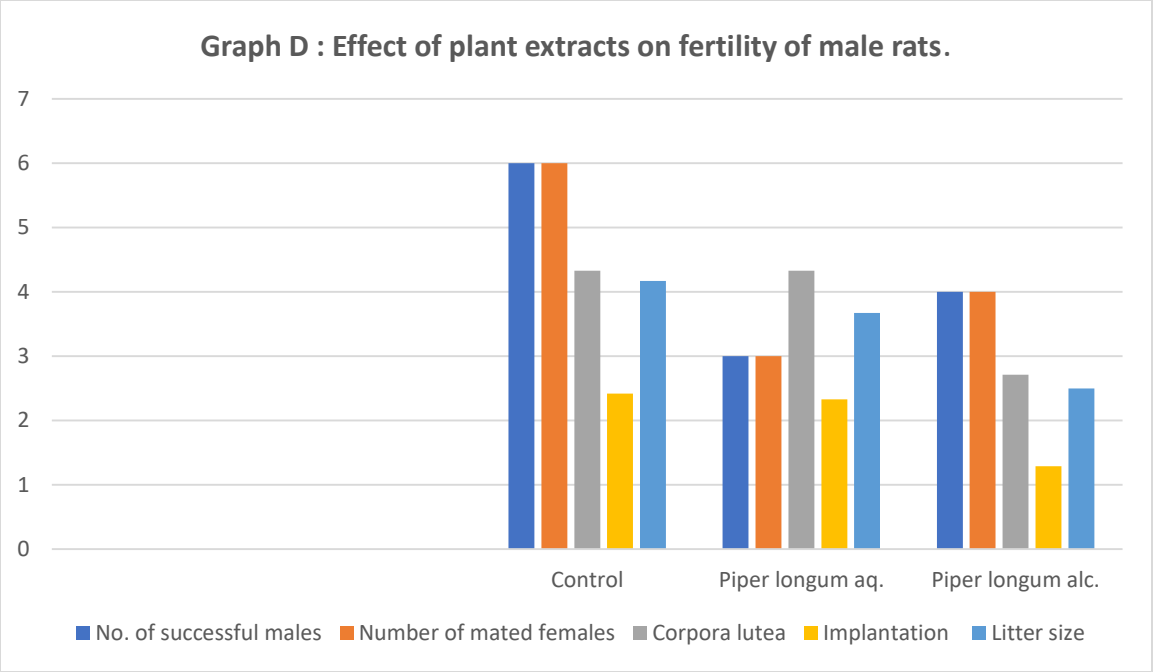
Graph A and B represent Change ion body weight and Fructose and Protein level in reproductive organ tissue respectively in control and experimental group of rat.



Graph C represent Change ion reproductive organ weight in control and experimental group of rat.

Effect of plant extracts on fertility of male rats. (Number of breeder male used is 6)					
Plants	No. of successful males	Number of mated females	Corpora lutea sites	Implantation sites	Litter No (In Inch)
Control	6	6	4.33±0.28	2.42±0.29	4.17±0.48
Piper longum aq.	3	3	4.33±0.67	2.33±0.71	3.67±1.86
Piper longum alc.	4	4	2.71±0.4	1.29±0.3	2.5±0.6

Table 2: Shows effect of plant extracts on fertility of male rats



Graph D: Graphs shows effect of plant extracts on fertility of male rats.

Discussion

From the obtained result it is clear that both extract (aqueous and alcoholic)of *Piper longum* affect the fertility and related parameters in rat. The body weight and reproductive organ weight reduced after administration of *Piper* extract (alcoholic and aqueous). The body weight reduced 27% in experimental rat with aqueous extract of *Piper longum* and the body weight reduced 61% in the experimental rat with alcoholic extract of *Piper*. The Weight of testis seminal vesicles coagulating gland ventral prostate and epididymis weight significantly reduced (t value 4.38, 5.267 & 3.805) after administration of both types of extract of *Piper longum* (table1 & Graph C). The Piper extract also interferes with the biochemical parameters in reproductive organs. it significantly reduced CG fructose and S.V protein (t value is 9.11304 and 9.5249) in experimental group rat (table 1 & graph A & B). It also alters acid phosphatase enzyme activity in ventral prostate. The *Piper* extracts reduced fertility performance in experimental group rat. It also has been seen that the reduced C.L cite, implantation cite as well litter size (table2 and graph D) in female rat after successful mate with experimental male rat. It may also indicate the poor fertility performance of male rat.

Work of Lakhmi *et al*. revealed that *P. longum's* hexane fraction has strong antiimplantation activity that results in animal mortality. According to Garg in 1981, *P. longum* roots combined with *Embelia ribes* seeds completely inhibited reproduction in female albino rats. The possibility of combining *P. longum* with other plant products needs to be further investigated in order to create a female contraceptive that can be used for both males and females without interfering with the action of ovarian hormones on the uterus, as reported in the Ayurvedic Garbhanivarana Aushadham (Munshi *et al*., 1972, Munshi & Ljungkvist 1972). Hasnath *et al*. studied the spermicidal action of hexane extract from the fruits of *Piper longum* Linn. The sperm immobilisation studies showed that 20 mg/mL of hexane extract was able to immobilise sperms completely within 20 s. The sperm revival test demonstrated that the effects were spermicidal since the effect of sperm immobilisation was permanent. Additionally, the treatment group's sperm viability was significantly lower than the control group's. These sperms' hypo-osmotic swelling was dramatically decreased, suggesting that the hexane extract may likely harm the sperm plasma membrane. Therefore,

our investigation demonstrated that P. longum's hexane extract has potential spermicidal contraceptive action Abu *et al*. 2104.

In the present study there was marked reduction in level of fructose in coagulating gland in treated mice compared to controls suggest that the suggest that the *P. longum* treatment has an adverse effect on the secretory function of the gland. Further. marked reductions in weight of epididymis and seminal vesicle in rat treated with *P. longum* for 21 days as noted in the present study indicate the possibility of testosterone depletion in circulation in treated rat.

The amount of protein in seminal vesicle was significantly reduced in experimental group compare to control group. The acid phosphatase (AP) activity in ventral prostate also altered due to treatment of *P longum*. In control group rat the activity of AP was measured 42.23 ±0.45 mg/hr/100mg of tissue. It became 15.72±1.58 mg/hr/100mg of tissue in group treated with aqueous extract of *P longum* and become 8.98±0.46 mg/hr/100mg of tissue in group treated with alcoholic extract of *P longum*. High-level of this type of enzymatic activity in the endometrium is responsible for conception. Absence or reduction in the level of the same in the endometrium is one of the causes of conception control (Das, 1983). The "Rhythm Method" idea and the "qualitative curve of phosphatase upon the human endometrium" play a part in fertility control. As the enzymic AP level in the endometrium is advantageous for conception and reaches its highest between the 16th and 18th day of the menstrual cycle, conception typically occurs between the 9th and 20th day of the menstrual cycle, which is designated "Baby Days." The AP is at a low level or nearly nonexistent from days 1 through 8 and 21 through 28 of the aforementioned cycle. At this point, it is no longer appropriate to implant, and "No Baby Days" is suggested. As a result, it can be said that AP's enzymatic effect contributes to both conception and contraception. (Ponnampalam *et al*. 2006).

Conclusion

On the basis of obtained result in present study and discussion it can be conclude that the aqueous and alcoholic extract of *Piper longum* has an adverse effect on primary as well as secondary reproductive organs in rat. The *Piper longum* may use as contraceptive agent.

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