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ANTIFERTILITY EFFECTS OF AQUEOUS EXTRACT OF *ANNONA SQUAMOSA* (SEEDS) IN MALE ALBINO RATS

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ABSTRACT
The present study was to evaluate the effect of *Annona squamosa* on fertility of male albino rats. 40 adult males and 60 female albino rats weighing between 150-220 gm were used for the study. The male rats were randomly divided in to 4 groups of 10 each. Group I which served as control were orally administered distilled water while group II, III and IV received 50, 100 and 200 mg/kg/day of aqueous extract of the seeds of *Annona squamosa* for 60 days and the effect of aqueous extract on reproductive organs and fertility was investigated. 5 males from each group were subjected to fertility test before they were sacrificed to harvest testes for histological and other reproductive organs for weight analysis. The administration of the aqueous extract was discontinued for another 30 days period and the remaining 5 males in each group were subjected to fertility test before they were sacrificed to obtain the testes for histological and other reproductive organs for weight analysis. The results showed normal pregnancy outcome in the females paired with the control group, reduced and zero pregnancy outcome in the females paired with the 100 and 200 mg/kg groups respectively after 60 days administration of the aqueous extract. After 30 days of discontinued administration of aqueous extract normal pregnancy outcome were recorded in control and group II, III, and IV showed 80 %, 60% and 20 % fertile mating respectively. The treatment caused decrease in weight of testes, epididymes, vas deferens, seminal vesicle and prostate glands and the histological examination of testes revealed a reduction in the size of seminiferous tubules along with thickness of germinal epithelial cells, though some of the epithelial cells and interstitial cells of leydig showed hypertrophy. Moreover, the lumen of the tubules was found to be devoid of sperms.

KEY WORDS: *Annona squamosa* seeds, seminiferous tubules, sperms, testes, male albino rats.

INTRODUCTION

Population explosion is a burning problem today in populated countries. Control is an issue of global and national public health concern. Today, different chemical, barrier (diaphragm, caps, IUDs, condoms), hormonal (progestogen only pills, combined oral pills, injectables, implants, microspheres and microcapsules, skin patches and gels, LHRH antagonists, anti-progesterone) and sterilization methods are available to women. However, most of the achievement in male contraception is confined to improving the already existing barrier and sterilization methods (WHO, 1998; Wilson *et al*., 2011). Current methods of contraception result in an unacceptable rate of unwanted pregnancies and having side effect also. Thus there is a need to replace these agents by safe and effective agents such as plant based contraceptive agents. A large number of medicinal plants growing in different parts of the world are used by the native people for their antifertility efficiency (Chaudhury, R.R.1966; Farnsworth *et al*., 1975; Pokharkar *et al*., 2010). A research work on medicinal plants as contraceptive in systematic way is lacking. The need for a cheap, safe and effective oral contraceptive is currently needed. It should also be easily available and should not have any side effect (Lohiya *et al*., 1992). As part of this research Programme, we present in this paper antifertility efficacy of seeds of the plant *Annona squamosa. A.squamosa* (Annonaceae) is a small semi deciduous much branched shrub or small tree. The unripe fruit, seed, leaves and root are reported to have medicinal properties including antifertility (Gupta and Sharma, 2006). Keeping all these reports in view, fact encouraged us to take up the seeds of *A. squamosa* for detailed investigation specially on the reproductive capabilities of male.

MATERIALS & METHODS

Experimental Animals

The experiment was done on male Swiss albino rats weighing between 150-220gms. They were acclimatized to laboratory conditions for 7days prior to commencement of experiment. The rats were kept in open air cages (60 x 45 x 45 cm) at room temperature under natural conditions of photoperiod which was around 13-14 hours at the time of experimentation. All the animals were fed twice a day with balanced laboratory diet (Hindustan Liver Limited, Mumbai) and also supplemented either with bread, spinach leaves, soaked black grams or dalia. Tap water was provided *ad libitum*. General body weight of the animals was monitored regularly during the entire tenure of experiment. Animals were maintained according to the guideline of Institutional animal ethics committee.

Plant Material

Ripe *A. squamosa* fruits were collected from Garhwal forest areas. They were identified Botanical Survey of

India and Forest Research Institute Dehradun. The seed were removed and dried in shade and after that kept in oven at 30 C temperatures for two days. Then they were grinded mechanically to a fine powder, filtered through muslin cloth and stored in sealed bottles and labeled. The dried powder of known quantity was dissolved in distilled water (W/V) for administration to rats.

Experimental design

The animals were randomly selected for investigation. 40 male rats selected for study were divided in to 4 groups of 10 animals each. After 7 days period of acclimatization, the animals in group II, III, and IV were orally administered 50 mg, 100 mg and 200 mg /kgBW/day of aqueous extract of *A. Squamosa* seeds respectively for 60 days. The animals in group I which served as control were administered same volume of distilled water daily. The volume was adjusted in such a way that 1 ml of solution corresponded to 50 mg of plant material. Similarly other doses were prepared.

At the termination of extract administration, 5 animals were randomly picked from each group and paired with the fertility proven females in a ratio of 1:2 and fertility performance test carried out. The fertility tested male rats were then sacrifice under anesthesia and their reproductive organs *viz*. testes, epididymes, seminal vesicles, vas deferens and prostate obtained and processed for histological studies while the females were allowed to litter. The remaining male albino rats were allowed to recover from extract administration for another period of 30 days. At the termination of 30 days extract withdrawal the remaining 5 male rats from each group were also paired with fertility proven females in a ratio of 1:2 and fertility performance test carried out. These rats were also sacrificed under anesthesia and their reproductive organs obtained and processed for histological studies while the females were allowed for litter.

Record of body weight and organ weight

The initial and final body weight of the control and experimental rats were taken in each experiment. The weight of reproductive organs i.e. testes, epididymes, seminal vesicles, vas deferens and prostate glands were noted in a semi- microbalance. The weights were recorded in tubular form of both control and treated animals.

Fertility Performance Test

The rats from all groups were used fertility performance test. The rats were mated with the fertile female albino rats for a period of 5 days. The first day of successful mating confirmed by presence of spermatozoa in vaginal smear was confirmed day first of pregnancy (D1). The females were allowed to complete gestation period. The mated female were laparotomized on day tenth (D10) of pregnancy and the implantation sites in each horn of uterus were observed. Litter sizes were recorded at birth and 4 days after percentage fertility was obtained by dividing the number of females delivered by the number of females mated and multiplied by 100 (Lohiya *et al*., 2005).

Percentage fertility = No of females delivered / No of females mated x 100

Reversibility Studies

For reversibility studies the rats mated again with proven fertile female rats after 60days of discontinuation of respective treatment. The genital organs *viz*. testes, epididymes, vas deferens, seminal vesicles and prostate glands were dissected out and weighted. Body weight was also taken. For histological preparation, the testes were fixed in bouin's fluid and paraffin sections of the tissues were stained with ehrlichs haematoxylene and eosin. Recovery, if any was noted in the genital organs and body weight.

Histological Studies

For histological studies testes were randomly selected from left or right sides of the rats from each group. Testes were fixed in bouins fixative. These were dehydrated in graded ethanol series. Cleared in xylene and embedded in paraffin. Then these were sectioned at 5m using rotary microtome. Then stained with haematoxylene– eosin examined and photographed (250 x).

Statistical Analysis

The data were statistically analyzed using students t-test. The values were expressed as mean ± S.D.

RESULTS

Oral administration of aquous extract of *A. squamosa* seeds in treated rats revealed the effect on genital organs of male albino rats.

Effect on Body Weight and Genital Organ Weight

The body weight reduced significantly after the administration of this plant extract at 200 mg/kg dose for 60 days. However, the reduction in body weight was not significant at other doses.

The 50mg/kg dose was ineffective to cause a change in the weight of testes, epididymes, seminal vesicle and prostate for 60 days. At 100 mg/ kg dose for 60 days testes weight was not reduced significantly but the weight of epididymes, seminal vesicle and prostate was reduced significantly. After administration of 200 mg/kg dose for 60 days, there was reduction in weight of testes, epididymes, seminal vesicle and prostate significantly (Table 1)

Effect on Fertility Performance

The male rats treated with *A. squamosa* (seeds) as aqueous extract, 60% exhibited fertile mating at 50 mg/kg dose. At 100 mg/kg dose, 40% mating was fertile. 100% inhibition of fertile mating was noted at 200 mg/kg. Thus percentage of infertile mating was increased with the increase of doses (Table 2).

Male rats treated with aqueous extract of this plant regained the fertility after 30 days of discontinuation of the administration. The 80% and 60% of the rats gained the fertility at 50 and 100 mg/kg doses respectively while at 200 mg/kg dose only 20% rats gained the fertility (Table 3).

Effect on Reversibility

The reversibility study was confined only to the highest dose (200 mg/kg) for longest duration because lower doses could not cause infertility significantly on administration of various extracts of *A. squamosa* (seeds) in male rats (Table 3).

TABLE 1: Effect of aqueous extract of *Annona squamosa* (seeds), on body weight (gm) and genital organ weight (mg) in male albino rats administered for 60 days at different doses. 5 animals were included in each group, values are mean ±S.E.

Name of Treatment	Doses (mg/kg)	Body weight (gm)		Organ weight (mg)			
		Initial	Final	Testes	Epididymes	Seminal vesicle	Prostate
Control	-	186.00±6.94	213.00±5.12	2462.28±22.34	1150.20±15.57	928.68±13.70	962.76±10.92
Aqueous extract	50	167.00±9.73	159.00±9.68	2033.61±13.21*	842.70±5.38*	809.31±8.71	715.50±10.06*
	100	158.00±6.58	154.00±7.64	1934.24±17.15	814.66±9.56	774.62±7.91	685.30±7.97
	200	168.00±11.18	148.00±10.38*	1790.80±15.31	732.60±6.81*	741.48±9.96*	599.40±8.73

* $P < 0.05$

TABLE 2: Effect of aqueous extract of *Annona squamosa* (seeds) on the fertility of male albino rats (after 60 days of continuous treatment)

Name of treatment	Dose (mg/kg)	No. of treated males	No.of females Inseminated	Fertile * Mating	Infertile** Mating
Control	-	5	5	05(100%)	00(0%)
Aqueous Extract	50	5	5	03(60%)	02(40%)
	100	5	5	02(40%)	03(60%)
	200	5	5	00(0%)	05(100%)

* Those which resulted in pregnancy
** Those which did not result in pregnancy

TABLE 3: Effect of aqueous extract of *Annona squamosa* seeds on the fertility of male albino rats (after discontinuation for 30 days after treatment)

Name of treatment	dose (mg/kg)	No. of treated males	No. of females Inseminated	Fertile * Mating	Infertile** Mating
Control	-	5	5	05(100%)	00(0%)
Aqueous Extract	50	5	5	04(80%)	01(20%)
	100	5	5	03(60%)	02(40%)
	200	5	5	01(20%)	04(80%)

* Those which resulted in pregnancy
** Those which did not result in pregnancy

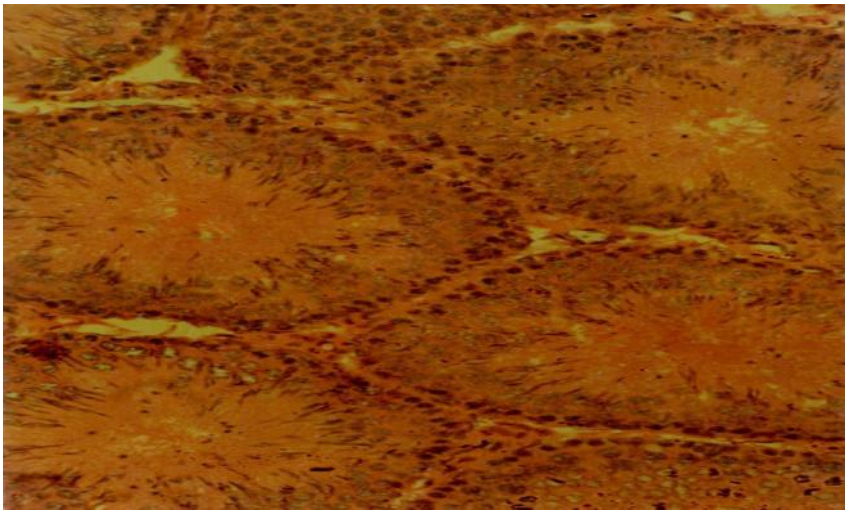

FIGURE 1: T.S. of testis of albino rat of control group.(Full spermatogenic activity in seminiferous tubules and normal Leydig's cells in interstitium). X250

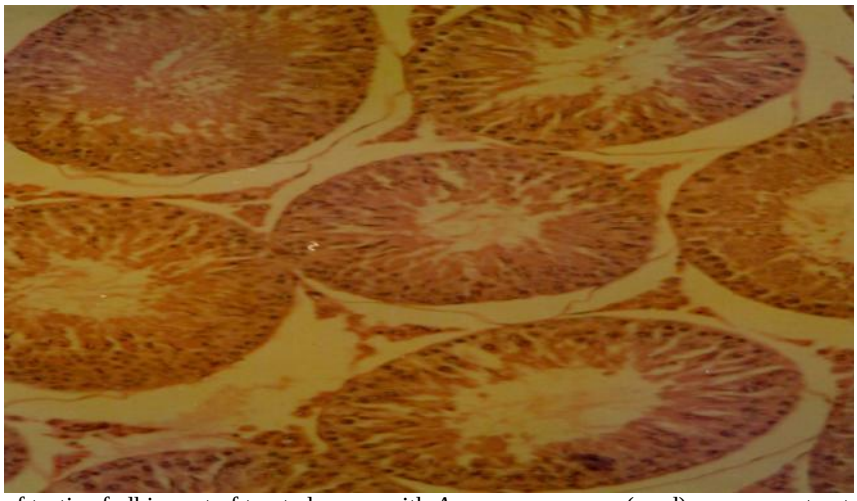

FIGURE 2: T.S. of testis of albino rat of treated group with *Annona squamosa* (seed) aqueous extract at 50 mg/kg dose for 60 days caused disfigured seminiferous tubules, arrested spermatogenesis. Oedomatous fluid in the interstitium and tubules filled with cellular debris. X250.

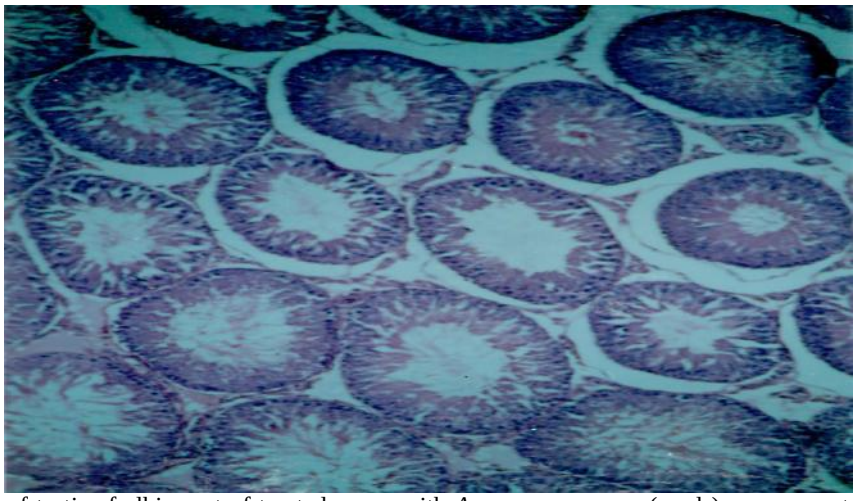

FIGURE 3: T.S. of testis of albino rat of treated group with *Annona squamosa* (seeds) aqueous extract at 100 mg/kg dose for 60 days caused degenerative changes included reduced seminiferous tubules, degenerated germinal epithelium and inhibition of spermatogenesis. Leydig's cells atrophied and interstitium with oedematous fluid. X 250.

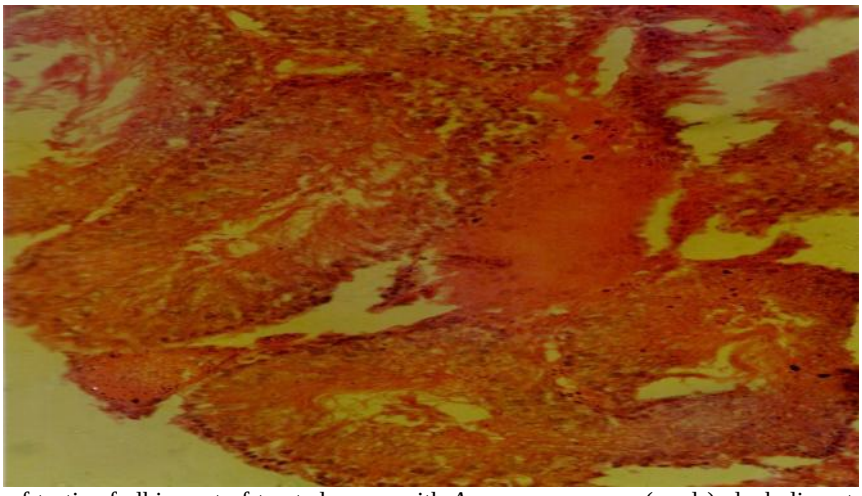

FIGURE 4: T.S. of testis of albino rat of treated group with *Annona squamosa* (seeds) alcoholic extract at 200 mg/kg dose for 60 days effect was more in 60 days treatment. Spermatogenesis arrested at spermatid stage. Tubule's lumen filled with fibrotic material and degenerated Leydig's cell.X 250.

Effect on Histology of the Testes

Feeding with *Annona squamosa* (seed) powder as an aqueous extract through oral route at 50 mg/kg for 60 days, caused no marked histological changes in the testicular elements. Cellular organization was not different from that of control testes (Fig.2).

Administration of 100 mg/kg dose of *Annona squamosa* (seeds) aqueous extract for 60 days mildly affected the histology of testes. The seminiferous tubules were normal except a few of them consisted only a few layers of germ cells. The spermatogonia and spermatocytes displayed various changes in their organization. Many spermatocyte nuclei were swollen and a few were atrophied. Sloughing of dead germ cells occurred in to the lumen of the tubule. The dissolution of the tubule membrane and leakage of germ cells was evident at certain places. Occasional immature sperms could be seen. Leydig's cells atrophied in the reduced interstitium. The vascularity was also reduced (Fig.3).

Administration of 200 mg/kg for 60 days caused severe degenerative changes in semineferous tubules. The degenerative changes included reduction in the spermatogenic cells, oedema, necrosis of the seminiferous tubules and complete inhibition of spermatogenesis. Only spermatogonia and spermatocytes were visible with pyknotic nuclei. Lumen of the seminiferous tubules was filled with oedematous fluid and cellular debris. Germinal epithelium at certain places was disintegrated (Fig.4).

DISCUSSION

The results revealed that *Annona squamosa* (Seeds) manifested antiandrogenic and antifertility effects in intact male rats. The weight of testes is known to be a good index of FSH secretion. It is confirmed that both steroidal and non-steroidal agents inhibits pituitary gonadotropins either acting directly on pituitary or through the hypothalamo- hypophyseal axis. Bustos-Obregon and Lopez (1973) observed the effect of plant alkaloids on spermatogonium of testes of albino rat. The change in the testicular weight corresponds to the presence or absence of postmeiotic cells (Nelson and Patanelli, 1965). Paul *et al.* (1953) have also demonstrated the reduction in weight of testes and accessory organs in the absence of spermatids and spermatozoa. *Annona squamosa* (seeds) as aqueous extract at high doses (100 and 200 mg/kg) for longer duration (60 days) of treatment arrested the spermatogenesis and severe damage to all the existing cell types. The Leydig's cell population was greatly reduced. Tyagi and Agarwal (1990) reported the similar results after the administration of aqueous extract of *Canscora docussata* whole plant at 25 mg/100 gm body weight to albino rats. The spermatogenesis was reversible as studied by them. The epididymal ductules were found filled with secretion, degenerated spermatozoa and cellular debris. A significant reduction of weight of the genital organs was also observed. Jain and Khan (1996) described sexual behaviour of albino rats in the presence of antifertility compounds (Petroleum ether seed extract of *Abrus precatorius*). Similar observations were made by Joshi *et al.* (1981) after chronic administration of *Malva viscus conzattii* flower extract in male mice and Verma *et al.* (1982) observed similar effects after 40 days of treatment with *Portulaca quadrifida* (Purslane) seed extract on the reproductive organs of male albino mice. Administration of this plant caused mass atrophy of the spermatogenetic elements. The germ cells were left only 1-2 cell layers. Epididymal epithelium was regressed and the lumen was devoid of spermatozoa. It also produced significant decrease in absolute weights of testes, epididymes, prostate and seminal vesicle.

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