Effect of *Cannabis Sativa* on Spermatogenesis of Male Albino Rats

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Abstract-Herbaceous plants have been used as food and medicine since time unknown. Excess of everything is harmful. Many plants may also be harmful if taken for long time and may impair function of reproductive organs i.e. testis and ovary. *Cannabis sativa* Linn. (Hemp plant), commonly known as "Bhang" or "Marijuana", now cultivated all over India, found wild in Himalayan region. Its preparations mainly 'cannabinoids' used as narcotic and psychotropic (medicinal) drugs. In the present communication, the effect of *C. sativa* (leaf powder) on spermatogenesis is reported. The leaf powder as aqueous suspension at doses of 50, 100 and 200 mg/kg/day were fed to three groups (dose wise) with a control group (vehicle treated) of male albino rats for 60 days. On day 61st, all the rats were sacrificed. The reproductive organs were taken out from body and processed for histological examination. Both initial and final body weight were recorded. The weight of organs were also taken before autopsy. The weight of reproductive organs were significantly reduced at higher doses. The spermatogenesis was arrested in testes. The seminiferous tubules were disfigured and reduced in size. Their lumen filled with cellular debris. The Leydig's cells were atrophied. The epididymes and vasa deferentia were devoid of spermatozoa. It is concluded that *Cannabis sativa* is harmful to male reproductive status of animal and human beings.

Keywords: *Cannabis sativa*,Medicinal plants, Reproductive organs, Anti spermatogenesis, Herbal drugs, Fertility and Sterility.

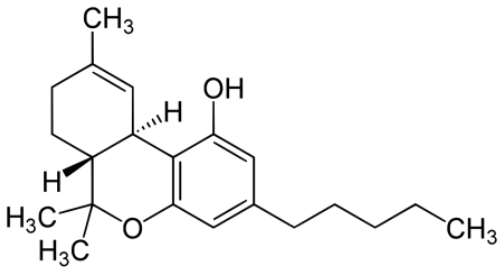
Introduction

Successful reproduction is essential for continuity of species including human beings. Herbaceous plants have been used as food and medicine since time immemorial. Excess of everything is harmful. Many plants may also be harmful if taken for long period of time and may impair function of of reproductive organs i.e., testis and ovary. *Cannabis sativa* Linn (Hemp plant), commonly known as "Bhang" or "Marijuana", now cultivated all over India, found in Himalayan region also. The plant is nitrophilic, blooming well in nitrogenouswastes
of soil near human habitation. Its preparations mainly "Cannabinoids" are used as narcotic and psychotropic (medicinal) drugs. In traditional medicine of India, particularly *Cannabis sativa* has been used as hallucinogenic, hypnotic, sedative, analgesic and anti-inflammatoryagent (Bonini*et al.*, 2018).

Many plants have been reported to impair reproductive function including libido and fecundity of animals if feeding them for long period i.e., chronically administration. Notable research work was reported by Nelson and Patanelli (1965), Pakrashi and Pakrashi (1977), Das (1980), Joshi *et al.* (1981), Dixit and Joshi (1982) and Khanna*et al.* (1986). in this regard. Effect of long term feeding of *Cannabis sativa* was also reported earlier by Dixit *et al.* (1974, 1978) and Singh and Singh (2017). Due to its psychoactive properties, it is commonly used as a drug of abuse and said to affect reproductive function. Considering the above point, the present study was done by authors and same is reported in this communication.

Phytochemistry

The main psychoactive constituent of *Cannabis sativa* is Tetra hydrocannabinol (THC). The plant is known to contain more compounds. They are commonly called as *Cannabinoids*. The constituents have been studied by various techniques *viz.*, paper chromatography, thin layer chromatography, gas-liquid chromatography, spectro-photometry etc. According to Sharma *et al.* (1972), the following are present – Tetrahydrocannabinol (T.H.C.) whose chemical structure is given. It is most active cannabinoid. Other compounds are Cannabinol (C.B.N.). It is pharmacologically not active. Cannabidiol (C.B.D.) is also not very active. Podder and Ghosh (1974) have also reported same compounds in *Cannabis* samples from other states – U.P., M.P., Orissa, West Bengal. Krishnamurthy and Kaushal (1974) have reported compounds T.H.C. C.B.N. and C.B.D. in the samples of *Cannabis sativa* collected from Northern Indian regions and concluded that T.H.C. was in rich amount and found most active pharmacologically.



Tetrahydrocannabinol (THC)

Material and Methods

Experimental Animals

The experiment was done on swiss male albino rats (*Rattusrattusnorvegicus*). Healthy, adult, colony-breed Swiss albino rats, four-five months old weighing between 150- 200 gms were selected and acclimatized to the laboratory condition for seven days prior to commencement of experiment. The rats were kept in polypropylene cages (60 cm × 45 cm × 45 cm) under normal conditions of photoperiod and room temperature. Four groups of male rats were made, each containing 05 male rats. First group served as control (05 rats) vehicle treated, while three groups (05 rats in each group) were treated as drug treated group (dose wise). The vehicle was gum acacia powder dissolved in water (20%) by weight and volume (w/v). All the rats were fed twice a day with balance laboratory diet (Hindustan lever limited, Mumbai). Tap water was provided them *ad libitum*. All the rats were maintained as per U.G.C. guidelines and supervised by the members of Animal Ethical Committee appointed by the then principal of the college.

Plant Material

The leaves of *Cannabis sativa* were collected from surrounding areas of Haridwar and Dehradun, Uttarakhand. The plant was identified at Botanical Survey of India (Northern circle) Dehradun, India during June and July months. The leaves were dried in shade and after that kept in oven at 30°C for two days. Then, these were grinded mechanically to a fine powder, filtered through muslin cloth and stored in sealed glass bottles and labelled. This dried powder fed to experimental male rats.

Doses and Administration

Male rats of groups II, III and IV were orally fed with three doses 50, 100 and 200 mg/kg leaf powder respectively for 60 days. Each dose was dissolved in distilled water until it changes into a homogenous mixture. The volume was adjusted in such a way that 01 ml of this solution containing 50 mg powder. The rest of doses 100 and 200 mg were prepared and administered to rats of group III and IV respectively for 60 days.

The administration of doses were done orally with specially designed knobbed feeding needle fitted into a syringe. The vehicle was administered in similar way to male rats of control group i.e. group I. Record of body weight and organ weight.The initial and final body weight of the control and treated male rats were taken dose wise. The weight of reproductive organs i.e. testes, epididymes etc. noted (after dissected rats) in a semi-micro balance. The weights were recorded in tabular form of both control and treated rats. The data were statistically analysed using student 't' test. The values were expressed as mean ± standard error (S.E.). The significance of difference of weights between control and treated rats was taken $P < 0.05$ as significant.

Histological Studies

For histological studies, the testes, epidermes and vasa deferentia were taken out from body after dissection of all groups of male rats on day 61st. These organs were fixed in Bouin's fixative. After removing the fixative, these organs were dehydrated in graded series of ethanol, clearedin xylene and embedded in paraffin wax. The organs embedded in paraffin wax were sectioned at 05µ (micron) using "Rotary Microtome" and mounted on glass slides. The mounted section of organs were stained with dyes haemotoxylene and eosine. Stained slides of testes etc. were examined under the microscope and photographed.

Results and Observations

Oral administration of aqueous suspension of leaf powder of *Cannabis sativa* in treated male rats revealed the effect on reproductive organs.

Effect on body and organ weight Tabledisplays the changes in the body and reproductive organs weight. The male rats of control group did not show any change (reduction) in body weight. It was maintained throughout the experimental period i.e. 60 days. Treated male rats administered with leaf powder aqueous suspension of *Cannabis sativa* showed some reduction in the body weight at higher doses i.e. 100 and 200 mg/kg doses for 60 days. The higher doses i.e. 100 and 200 mg/kg doses for 60 days caused significant reduction in the weight of reproductive organs i.e. testes and epididymes in comparison to control male rats.

Histopathological changes

The administration of leaf powder as aqueous suspension through oral route for 60 days caused pathological changes in reproductive organs i.e. testes, epididymes and vasa deferentia were observed and compared with controls. The higher doses i.e. 100 and 200 mg/kg caused most deleterious (harmful) effects.

Effect on testes

Control-The testes of control male rats showed the normal histological features. The seminiferous tubules were rounded in shape, wide and lined with germinal epithelium which appeared with normal germinal cells and large sized sertoli cells. All stages of spermatogenesis were clearly indicated on seminiferous tubules. The spermatogonia with nuclei of moderate size. Spermatocytes and spermatids are present in normal position. The tubular lumens filled with spermatozoa. The Leydig's cells were present in space between seminiferous tubules known as interstitium. Vascularity appeared normal (Figure-1).

Treated-The administration of *Cannabis sativa* leaf powder at 50 mg/kg dose for 60 days caused insignificant histological changes in the testes. Various spermatogenic cell types in the seminiferous tubules were normal as in control rat testes. The administration of 100 mg/kg and 200 mg/kg doses for 60 days caused degenerative changes. The spermatogenesis was arrested at spermatid stage in majority of seminiferous tubules. Germinal epithelium appeared normal. The degeneration in the testes consisted of damaged spermatocytes, spermatids and spermatozoa. Deshaped seminiferous tubules were also noted. The interstitium (space between two seminiferous tubules) contained atrophied Leydig's cells. Vascularity was also increased (Figure-2).

Effect on Epididymes

Control- The epididymes of the control male rats after 60 days showed a normal histological structure. The epithelium showed columnar epithelial cells with basal nuclei. The lumen of the ductules were wide and filled with numerous spermatozoa. The well organisedstereocilia were present at the border of epididymalductules. The intertubular connective tissues with vascularity were normal. The spermatozoa are matured and stored in epididymes. These were present in epididymes (Figure-3).

Treated- The administration of of*Cannabissativa* leaf powder as aqueous suspension at 50 mg/kg dose for 60 days caused no changes in histological structure of epididymes. The columnar epithelial cells appeared normal with basal nuclei. The epididymal tubular lumen was without spermatozoa. The stereocilia were normal. The higher doses i.e. 100 and 200 mg/kg administered for 60 days caused nuclear dysplasia in columnar cells of epithelium of the ductules of epididymes. The lumen of epididymalductules were devoid of spermatozoa. There were no spermatozoa at all. The stereocilia were distorted at certain points and adhered to each other. Intertubular spaces were increased (Figure-4).

Effect on vas deferens

Control-The vas deferens of control male rats after 60 days showed normal histological picture of its organisation. The cellular layers i.e., external longitudinal muscles and internal circular muscles were normal. Lamniapropria and innermost layer, the mucosal lining which surround the lumen appeared normal with stereocilia and folds. The lumen was full of spermatozoa. The vas deferens is a passage for spermatozoa (Figure-5).

Treated – The administration of *Cannabis sativa* leaf powder as aqueous suspension to male rats for 60 days did not cause any untoward effect on vasa deferentia at 50 mg/kg dose. The structural details resembled to control rat's vas deferens. The higher doses i.e. 100 and 200 mg/kg administration for 60 days caused notable changes in this part. Histological structure was not much changed except the innermost layer i.e. mucosal lining which showed distortion of stereocilia and cellular organization. The lumen appeared empty. There were no spermatozoa (Figure-6).

Discussion

Cannabis sativa leaf powder as aqueous suspension was administered orally at doses of 50, 100 and 200 mg/kg for 60 days to male albino rats. The purpose behind this study was to assess the effect of long term feeding of *Canabissativa* on spermatogenesis that occur in male reproductive organs i.e. testes, epididymes and vasadeferentia. The result showed marked effect on male reproductive organs with total arrest of spermatogenesis. Varying degree of damage caused in different testicular elements i.e. testes and androgenic male hormone producing cells, the Leydig's cells were mostly atrophied. The weight of the testes and epididymes was also reduced at higher doses i.e. 100 and 200 mg/kg doses. The effect appeared to be anti-androgenic.

In the male albino rat, the testes are scrotal and the complete spermatogenic cycle requires about 65 days (Jackson, 1966). During the first 45 days, the differentiation and maturation of spermatozoa occur in the testes. During the last 20 days, the spermatozoa are transported through the epididymes to the vas deferens for ejaculation. The sperms are stored in the caudaepididymes for further maturation from the testes.The follicle stimulating hormone (F.S.H.) secreted from pituitary gland, is directly related to the weight of testes. Heavier will be the testes, more will be secretion of F.S.H. It is confirmed that both non-steroidal (plant product etc.) and steroidal agents inhibit pituitary gonadotropin either acting directly on pituitary or through the hypophyseal axis. The reduced secretion of F.S.H. causes significant decrease in the weight of testes and accessory reproductive organs of male rats (Dorfman*et al.,* 1963). Paul *et al.*, (1953) had demonstrated the reduction of weight of testes and accessory reproductive organs in absence of spermatids and

spermatozoa. The change in weight of testes also corresponds to the presence or absence of post-meiotic cells in the testes. The physiology of reproductive organs (also known as genital organs) are androgen dependent (Nelson and Patanelli, 1965).

In the present study, the chronic administration of *Cannabis sativa* caused arrest of spermatogenesis in male albino rats. It also caused reduction of weight of testes etc. Similar findings by chronically administration of flower extract of *Malvaviscusconzattii* in male albino mice were made by Joshi *et al.* (1981). The anti-spermatogenic effect of the extract of *Aristolochiaindica* in male mice was reported by Pakrashi and Pakrashi (1977). A marked reduction in population of spermatozoa was observed when aqueous suspension of seed powder of *Carica papaya* was administered to adult male rats (Das, 1980). The leaf extract of *Vincarosea* caused significant histological changes in the testes, epididymes and also decreased weight of reproductive organs (Chinoy and GeethaRanga, 1983). Similar effects of long-term feeding of Tulsi leaves, *Ocimum sanctum* caused reduction of testicular weight and decrease in sperm population (Khanna*et al.*, 1986). The long and short term administration of flower extract of *Hibiscus rosasinensis* (Kholkute and Udupa, 1974) resulted in the significant decrease in the weight of male reproductive organs i.e. testes, epididymes and seminal vesicles in male albino rats under experiment. Singh and Singh (2017) reported that *Solanumxanthocarpum* (seeds) caused similar effects in male Guinea pigs also.

Dixit *et al.* (1974) reported that the testicular function was arrested with the chronically administered *Cannabis* extract to male mice. Dixit *et al.* (1978) also reported the adverse effect of *Cannabis* extract on testicular function of Toad, *Bufoandersonii*Boulenger. The above-mentioned studies of *Cannabis sativa* leaf on arrest of male reproductive functions strongly support our studies as reported in this communication.

Conclusion

Cannabis sativa Linn. is a herbaceous plant commonly known as "Bhang", "Hemp" and "Marijuana". It has been used as a psychotropic drug since time immemorial. It has therapeutic effect on many psychosomatic disorders. Its preparations are narcotic also. The most effective phytochemical constituent is "Tetrahydrocannabinol" (T.H.C.). It is said that long term use of the *Cannabis* cause sexual weakness. According to present study, the *Cannabis* leaf powder when fed to male albino rats for 60 days, the doses 100 and 200 mg/kg caused anti-spermatogenic effects.

Acknowledgement

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Table-1 Effect of *Cannabis sativa* leaf powder on Body Weight (gm) and Genital organ Weight (mg) of male rats administered at different doses for 60 days. 05 rats were used in each group (control and treated). Values are mean ± S.E.

Treated	Doses (mg/kg)	Body weight (gm)		Genital organ wt. (mg)	
		Initial	Final	Testes	Epididymes
Control	–	156.10 ± 19.24	172.15 ± 13.20	698.12 ± 10.65	198.35 ± 23.07
Aqueous suspension	50	156.20 ± 17.15	157.15 ± 12.18	696.44 ± 7.73	191.10 ± 17.43
	100	156.10 ± 23.20	157.10 ± 13.12	690.46 ± 13.12	190.25 ± 13.25
	200	154.15 ± 16.43	155.25 ± 27.10	295.17 ± 11.10*	117.16 ± 12.75*

*P < 0.05

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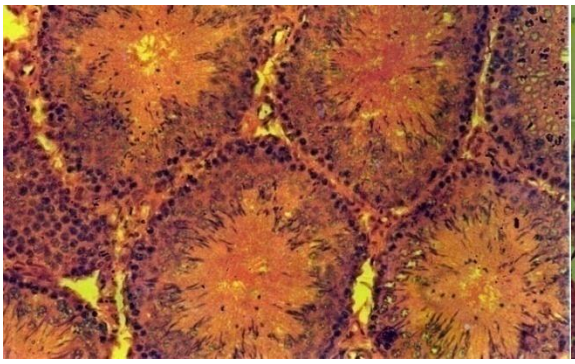

Figure-1 T.S. of testis of control male rats. Note all spermatogenic elements including organized germinal epithelium, spermatocytes, spermatids and spermatozoa in seminiferous tubules and Leydig's cells in the interstitium X 400.

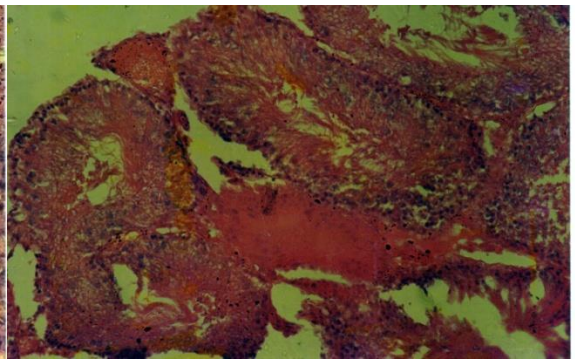

Figure-2 T.S. of testis of treated male rat with *C. sativa* leaf powder suspension at 200 mg/kg dose for 60 days. Note arrest of spermatogenic activity in damaged seminiferous tubules and absence of Leydig's cells. X 400

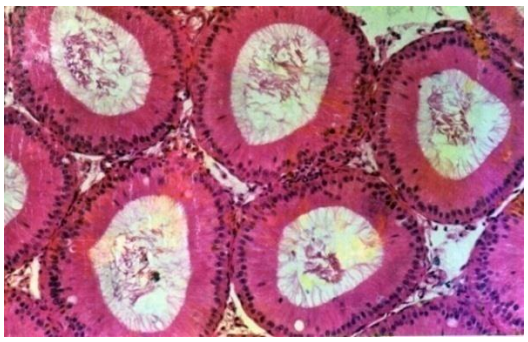

Fig. 3 : T.S. of epididymis of control male rat. Note the normal histology with organized epididymal epithelium and spermatozoa in the epididymalductules and normal stereocilia. X 400.

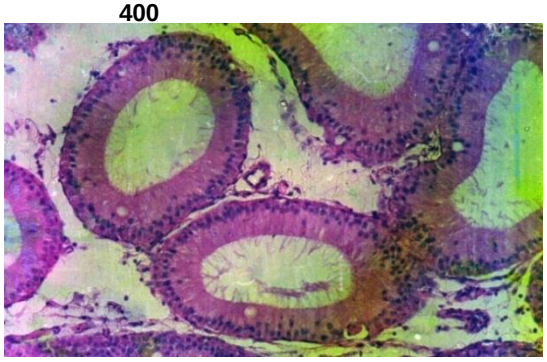

Fig. 4 : T.S. of epididymis of treated male rat with *C. sativa* leaf powder at 200 mg/kg dose for 60 days. Note the vacuoles in epididymal epithelial cells, distorted stereocilia and empty

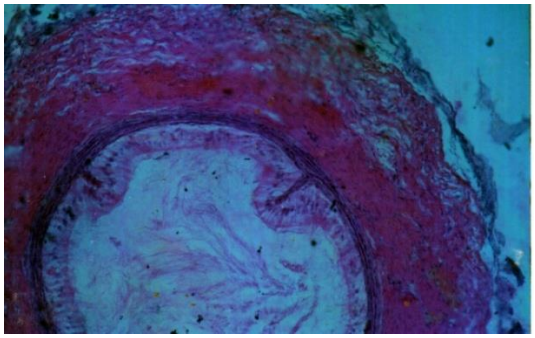

Figure-5 T.S. of vas deferens of control male rat. Note the normal histoarchitexture including mucosal lining with epithelial cells, folds and stereocilia. Plenty of spermatozoa in the lumen. X 400

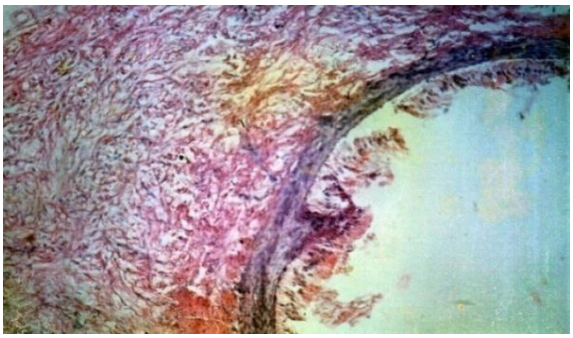

Fig. 6 : T.S. of vas deferens of treated male rat with *C. sativa* leaf powder at 200 mg/kg dose for 60 days. Note the absence of spermatozoa in the lumen, distorted luminal epithelium and mucosal layer and loss of stereocilia. X 400

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