MECHANISM OF ACTION OF CHRONICALLY ADMINISTERED CANNABIS EXTRACT ON THE FEMALE GENITAL TRACT OF GERBILS *MERIONES HURRIANAE*

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Summary: Daily administration of cannabis extract (2.5 *mg*/day for a period of 60 days) caused degenerative changes in the ovarian tissue. Luteinization was inhbited. Corpus-luteum degeneration was conspicuous. Distinct effects were produced upon the uterine biochemistry, consisting of decreased RNA, protein, sialic acid and glycogen concentration of the uterus. Vaginal RNA and protein contents were low. An anti-estrogenic action of cannabis extract in female gerbils is suggested.

Key words: Cannabis extract ovarian atrophy inhibition of RNA & protein synthesis anti-estrogenicity

Cannabis is one of the oldest intoxicants in use. $\triangle^9$-Tetrahydro-cannabinol ($\triangle^9$-THC). has been shown to be the active component of marihuana (4 and 6). The $\triangle^9$-THC content of marihuana varies depending upon the source of the cannabis plant. Good quality marihuana contains about 1.5% of THC. Little attention has been paid to the examination of the action of this compound on the reproductive system of mammals.

In our previous studies we found that chronic administration of cannabis extract is anti-estrogenic in female mice and rats (2). The present study is a further confirmation of the results in the desert gerbills.

MATERIALS AND METHODS

As $\triangle^9$-tetrahydrocannabinol ($\triangle^9$-THC), the most potent naturally occuring cannabinol lacks specific effect on reproductive system, a total plant extract rather than THC or a purified active principle was preferred. Therefore, we extracted the authenticated samples of *cannabis sativa* harvested in Rajasthan (India), with 98% alcohol in a Soxhlet apparatus for 4 hours. The extract was evaporated to dryness and the residue weighed and redissolved in a mixture of alcohol, tween 80 and distilled water (10 : 1 : 89), to make concentration of 10 *mg/ml.*

Twenty young adult female gerbils with a regular oestrous cycle (4-6 days) were injected with cannabis extract intraperitoneally (2.5 *mg*/day for a period of 60 days). An equal number of controls received the vehicle alone. The animals were given standard rat feed (Hindustan Lever Private Ltd.) and water *ad libitum.* All animals were sacrificed 24 hour after administration of final dose of cannabis-extract and various organs were removed and weighed.

Ovaries, right uterine horn and a portion of vaginae were fixed in Bouin's fluid for histological preparations whereas, the left uterine horn and vagina were frozen and total RNA, protein, sialic acid and adrenal ascorbic acid determined later (5,7,8,9)

RESULTS

Organ weights: Relative and absolute ovarian weight of cannabis extract treated female gerbils was decreased markedly, indicating wide spread damage. The weights of uterus, vagina and submaxillary glands were reduced (Table I). Adrenal and thyroid gland weights did not change.

Histology: Cannabis extract administration for a period of 60 days resulted in wide spread degenerative changes in the ovarian tissue. Primordial ova were reduced in number. Atresia of large ovarian follicles was conspicuous. Luteinization was inhibited. Lutein cells were vacuolated and undergoing cytolysis. Their nuclei were shrunken (mean nuclear diameter of lutein cells, cannabis extract : $10.3 \pm 0.7\ \mu$; control : $13.3 \pm 0.8\ \mu$; $P < 0.01$). Histologically the corpus luteum showed signs of degeneration, luteal tissue showing infiltration with fibroblasts. The average mean diameters of thirty corpora lutea was, cannabis treatment: 0.303 mm; vehicle treated control : 0.529 mm.

Uterus: In control female myometrium consists of 60% of the uterine volume. After cannabis extract administration myometrial volume decreased in proportion to uterine weight. Decrease in stromal connective tissue also paralleled the changes in uterine weight. Uterine glands were regressed and without secretion. Stromal oedema was conspicuous.

Vagina: In cannabis extract treated gerbils, the vaginal epithelium appeared to be atrophic. Cannabis extract administration interrupted the estrous cycle after 3 weeks. Cells found in the vaginal smear during the cessation of cycling appeared to be that of dioestrous.

Biochemical changes: Uterine glycogen and sialic acid contents were low after cannabis extract administration (Table I). Total RNA and protein contents were decreased significantly in uterus and vagina after cannabis extract administration, suggesting an inhibition of synthesis. A slight depletion in the adrenal ascorbic acid concentration was noticed (Table I).

DISCUSSION

When female gerbils with regular oestrous cycle were given a daily injection of cannabis extract (2.5 *mg*/day for a period of 60 days), cyclic activity as judged by vaginal smears, ceased after 3 weeks. Smears of the dioestrous type persisted as long as the medication was continued. The vaginal cytology gave an appearance similar to that of late dioestrous type (1),

TABLE I : Changes in organ weights and in the concentration of RNA, protein, sialic acid, glycogen and adrenal ascorbic acid of female gerbils after Cannabis extract administration*.

Treatment	No. of animals	Body wt g	Ovarian wt mg/100g body weight	Uterine wt mg/100g body wt	Thyroid wt mg/100g body wt	Adrenal mg/100g body wt	Submaxillary mg/100g body wt	RNA µg/mg tissue		Protein µg/ mg tissue		Uterine sialic acid µg/mg tissue	Uterine glycogen µg/mg tissue	Adrenal ascorbic acid mg/ 100g tissue
								Uterus	Vagina	Uterus	Vagina			
Control	10	65±3	35.3±3.2	105±11	10.2±0.5	56.3±1.9	159±22	4.91± 0.2	3.67± 0.4	315±11	338±20	1.14± 0.2	200±15	650±35
Cannabis Extract	10	65±6	11.5± 2.3[2]	66.6± 5.7[1]	11.7±0.9	63.6±3.5[1]	127±8	3.19± 0.3[1]	2.19± 0.2[1]	172± 7[2]	287± 13	0.39± 0.1[2]	145±9[1]	500±15

[1] $P < 0.02$ compared with controls

[2] $P < 0.01$ compared with controls

*Biochemical estimations : means of six determinations.

2.5 *mg* Cannabis extract daily for a period of 60 days : total dose = 150 *mg*.

Suppression of the ovarian activity was reflected in large follicular atresia and degenerating corpora lutea. Furthermore, a reduction in the size of corpora lutea might have been due to interference by cannabis extract with ovulation.

The decreased concentration of uterine RNA, protein, glycogen and sialic acid indicate the possibility that cannabis extract inhibits estrogen production (3).

ACKNOWLEDGEMENTS

We are indebted to Professor A.S. Kapoor for providing facilities and encouragement. The investigation was supported by University Grants Commission (Grant No. 5672) New Delhi.

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