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EFFECT OF CANNABIS EXTRACT ON THE UTERINE MONOAMINE OXIDASE ACTIVITY OF NORMAL AND ESTRADIOL TREATED RATS

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Abstract—Studies have been made on the effect of repeated administration of cannabis extract on monoamine oxidase (MAO) activity of uterine tissues of prepubertal rats, treated with or without estradiol benzoate at two time intervals (14 and 40 hr). Results indicated that the MAO activity of uterine tissues is increased significantly by cannabis extract both in normal as well as in estradiol treated rats. Estradiol, on the other hand, decreased the enzyme activity significantly by itself. The increase in MAO activity following cannabis extract administration is however seen to be more prominent in 14 hr than in the 40 hr hormone-treated groups.

Although the work from several laboratories [1–5] has established that cannabis extract or delta-9-tetrahydrocannabinol (Δ^9-THC) affects various hormonal levels connected with reproduction, the site of action of the drug on the neuroendocrinal axis connected with gonadal function, is less well-known. A recent report from this laboratory [6] indicates that monoamine oxidase (MAO, EC 1.4.3.4) activity is significantly increased in the hypothalamus and brain tissues of rats treated with cannabis extract or Δ^9-THC. In view of the observation made by Kamberi and Kobayashi [7] that MAO activity in hypothalamus and other gonadal tissues changes in a cyclical manner during the estrous cycle in rats, it was of interest to investigate further about the characteristic changes in the MAO activity in uterine tissues of rats due to *in vivo* administration of cannabis extract.

MATERIALS AND METHODS

Animals and treatment. Eleven days old female rats of Charles Foster strain were used in this study. The rats used were from an average litter size of 4 or 5 per mother and were maintained under standard conditions of light for 14 hr/day (5·00 to 19·00 hr). The animals were divided into six groups, each group having at least ten rats.

(I) Control groups treated with saline–6% Tween–80 vehicle using the same volume as in treated animals.

(II) Animals treated daily with cannabis extract starting on day 11 after birth to 21 days of age.

(III) Animals treated with a single dose of estradiol 14 hr prior to sacrifice.

(IV) Animals treated with cannabis extract as in Group II plus treatment with estradiol 14 hr before sacrifice.

(V) Animals treated with estradiol 40 hr prior to decapitation.

(VI) Animals treated with cannabis extract as in Group II plus treatment with estradiol 40 hr prior to sacrifice.

Injection was given between 10 a.m. to 11 a.m. daily and all the rats were sacrificed by decapitation on 22 days after birth, about 24 hr after the last injection of cannabis. Estradiol was administered at two time intervals *viz.*, 14 hr (at 20.00 hr) and 40 hr (at 18.00 hr previous day) before decapitation. The uterine tissues were rapidly removed, trimmed, weighed and homogenized in ice cold saline using Potter–Elvehjem homogenizer. The homogenate was centrifuged at a low speed of 3,000 *g* for 30 min in an International Refrigerated Centrifuge and the supernatant was used as the enzyme source.

MAO activity was measured according to the method of Green and Haughton [8] using only Kynuramine dihydroxy bromide (Sigma) as substrate. Activity was measured by observing the change in O.D. at 365 mμ. Protein was estimated according to the method of Lowry *et al.* [9].

Dosage of Δ^9-THC. Standard samples of cannabis obtained from United Nation Narcotics Laboratory, Geneva was semi-purified in the laboratory [10] and used for the experiments. The cannabis extract sample containing 7·1% Δ^9-THC, 0·8% cannabidiol, 2·5% cannabinol and other undetermined cannabinoids, was administered at a dose of 10 mg Δ^9-THC/kg body weight subcutaneously. Dosages were prepared by diluting the extract to a concentration of 1 mg Δ^9-THC/ml with normal saline containing 6% Tween–80 and the suspension was administered in a volume of 0·1 ml per 10 g body weight to each animals for 11 consecutive days, beginning when the rats were 11 days old till they reached 21 days of age. Control animals received equivalent volumes of saline Tween–80 vehicle for a similar period.

Dosage of Estradiol. Estradiol benzoate (Ovocycline, Ciba) in olive oil suspension was administered subcutaneously at a single dose of 1 μg/rat either 14 hr or 40 hr prior to sacrifice of animals.

RESULTS AND DISCUSSION

Results shown in Table 1 and Fig. 1, signify cannabis extract to have an increasing effect on the uter-

Table 1. Effect of cannabis extract on uterine monoamine oxidase activity

Groups	Sp. act. (Mean ± SEM)	p-Value
Control	43·76 ± 4·26	<0·001*
Cannabis	56·42 ± 6·61	
Estradiol^{14}	16·60 ± 1·59	<0·001*
Cannabis + Estradiol^{14}	31·20 ± 0·68	
Estradiol^{40}	16·72 ± 1·59	<0·001*
Cannabis ± Estradiol^{40}	21·80 ± 1·21	

* Highly significant. p-values have been calculated from the data obtained using ten individual rats in each case. Superscript in the group column indicates hours after estradiol treatment. Specific activity expressed as change in O.D. at 365 mμ/mg protein/hr.

ine MAO activity in prepubertal rats. This effect of cannabis extract is evident even in the case of estradiol-treated rat uteri. On the other hand, the present work as well as reports from previous investigators [11, 12] suggest that estradiol by itself reduces the uterine enzyme activity significantly. It may thus be stated that, in this case, cannabis extract counteracts the effect of estradiol on the uterus to some extent. This effect of cannabis extract however, is not so prominent after 40 hr of estradiol treatment. Our recent work [13, 14] showing the inhibitory effect of cannabis extract on the estradiol-induced glycogen and water accumulation in prepubertal rat uteri also supports this conclusion. Cannabis extract appears to have rather a progesteronic type of effect in the present case [11, 12]. The work gets further support from the recent reports of Reese [15], who showed that Δ^9-THC decreases the estradiol level in man and also from that of Dingell *et al.* [16] who showed that the drug actually inhibits the conjugation of estradiol. Our previous work which showed that cannabis extract prolongs the met/di-estrus phase of cyclic female rats also support this data [1].

The work of Nir *et al.* [3] showing that Δ^9-THC blocks ovulation in adult rats and also that of Freudenthal *et al.* [17] showing a high accumulation of THC in corpora lutea after intravenous administration indicate the drug to have a direct effect on the ovary. The effect of Δ^9-THC on pituitary hormones on the other hand, has also been reported by ourselves in other communications [4, 5]. Further work may clarify the antiestrogenic mode of action of cannabis extract and Δ^9-THC in mammalian reproductive system.

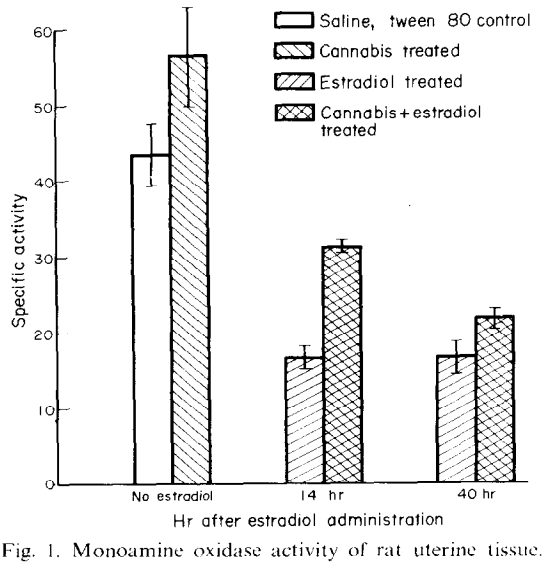

Fig. 1. Monoamine oxidase activity of rat uterine tissue.

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