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Effects of crude aqueous marijuana extract on length of oestrous cycle and ovulation

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ABSTRACT: The effects of crude marijuana extract on the length of oestrous cycle and production of ova were studied in female Wistar rats. Adult regularly cycling rats (120g – 160g) received oral doses of 20mg/kg/d and 75mg/kg/d crude aqueous marijuana extract for a period of 18 days. Vaginal smears of the animals were observed daily and the number of ova shed at the end of the treatment period evaluated. Lengthening of the dioestrous and pro-oestrous phases was observed while the oestrous phase was shortened. There was a significant reduction in the number of ova shed by the crude marijuana – treated groups ($P < 0.05$). While it is obvious from these findings that marijuana produces anti-fertility effects, the exact mechanism by which is does so remains to be elucidated.

Key Words: Oestrous cycle; Ovulation; Neuroactive drugs; Marijuana; Reproductive functions.

Introduction

Previous studies had demonstrated the effects of some neuroactive drugs on the reproductive functions of female animals (1,2). Laboratory studies aimed at investigating the effects of cannabis on certain reproductive parameters had led to the discovery that marijuana disturbs both oestrous cyclicity and ovulation in female animals (3). However, the reports of these investigations appear conflicting with respect to the effects of marijuana on the length of oestrous cycle and the degree to which ovulation is inhibited. The present investigations were therefore designed to study the effects of crude marijuana extract (CME) on the length of oestrous cycle and production of ova in albino rats.

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Materials and Methods

Adult female Wister rats (120- 160g) were obtained from the department of Physiology, University of Ibadan. Animals were randomly sorted into groups and their vaginal smear taken on a daily basis. Only those with regular cycle as previously described for rats (4) were included for the purpose of the present investigations. All animals were exposed to 12 hours light, 12 hours dark photo-period, temperature and cross-ventilation were maintained. Pellet rat chow was freely administered and water was provided *ad libitum*.

Dry leaves of Cannabis saliva was sourced locally and authenticated in the Department of Biological Sciences of the University of Ilorin. The leaves were pulverized into a fine powder and weighed. 20g of this powder was dissolved in 100ml of distilled water and the resultant solution made up to 300ml.

Oral doses of 20mg/kg/d and 75mg/kg/d crude marijuana extract were administered to animals in groups A (n = 6) and B (N = 6) respectively. Group C (n = 7) was administered distilled water and constituted control. Vaginal smears were obtained on a daily basis to monitor the regularity and length of the phases of oestrous cycle throughout the 18-day treatment period. All animals were sacrificed by means of ether anaesthesia; median laparotomy was performed and the fimbriated lateral end of uterine tube identified and excised. This was opened up and visualized under the light microscope to evaluate the number of ova shed into it.

Statistics: Data obtained for the animal were analysed statistically for test of significance using the ANOVA.

Results

Oestrous cycle length in the CME-treated groups was prolonged. The cycle proceeded for an average of 144 hrs compared to an average length of 96 hrs maintained by the vehicle treated animals. Administration of 20mg/kg/d and 75mg/kg/d CME to rats resulted in prolonged dioestrous (108 ± 3hrs and 110 ± 4 hrs respectively) and pro-oestrous (27 ± 1 hrs and 25 ± 2 hrs respectively) phases; the oestrous phase was shortened (13 ± 1 hrs and 12 ± 2 hrs respectively) as shown in Table 1. These differences were statistically significantly ($P < 0.05$). The metestrous phase was completely phased out in all the CME – treated groups.

Table 2 showed the number of ova shed by the CME – treated animals. Ovulation was delayed and the number of ova released was reduced. Animals in group A shed an average of 4 (± 0.49) ova while those in B released an average of 4 (± 0.09) ova. No anovulation was observed in all the CME-treated groups.

Table 1: Effect of crude marijuana extract on length of oestrous cycle phases.

Treatment group	**Length of time of oestrous cycle phases**		
	Dioestrous	**Pro-oestrous**	**Oestrous**
Vehicle-treated group	56 ± 2	12 ± 3	26 ± 1
20mg/kg/d	108 ± 3	27 ± 1	13 ± 1
75mg/kg/d	110 ± 4	25 ± 2	12 ± 3

Mean ± SEM
$P < 0.05$

Table 2: Effect of crude marijuana extract on number of ova produced

Treatment groups	Number of ova shed
Vehicle-treated group	9 ± 1.14
20mg/kg/d	4 ± 0.49
75mg/kg/d	4 ± 0.09

Mean ± SEM
$P < 0.05$.

Discussion

The present studies investigated the effects of crude marijuana extract (CME) on length of oestrous cycle and production of ova in Wister rats. Previous investigations by Kostellow *et al* (1980) demonstrated that cannabis had no significant effect on the length of oestrous cycle in A/J mice (3); this finding appears to contradict the reports of Asch *et al* (1981) and Lares *et al* (1981) which indicated that marijuana increased oestrous/menstrual cycle length in rats and primates (1,5). Our findings in the present studies are similar to the latter.

CME lengthened oestrous cycle period by an average of 48 hrs in excess of the usual average of 96 hrs documented for regularly cycling rats (Olowookorun, 1986). Lares *et al* (1981) administered resin and smoke condensates of Cannabis sativa and reported similar findings in rats (5).

Previous studies on the effect of marijuana on ovulation had demonstrated its anti-ovulatory effect on animals. However, what remains to be clarified in this regard is the degree of inhibition of ovulation by this drug. Wenger *et al* (1988) reported partial inhibition of ovulation by marijuana in rats (6), contrary to earlier reports of complete anovulation in primates exposed to Cannabis (7,8). Our findings in the present studies indicate that marijuana produces partial inhibition of ovulation in Wister rats as reported by Wenger *et al* (1988). We are of the opinion that the species of animals used for these investigations partly accounted for the contradicting reports; a particular specie may present with a specific neuroendocriminological make-up which may modify the response of the animals to marijuana.

It is now well known that marijuana (delta 9 – tetrahydrocannabinol) produces its anti-fertility effect by a central mechanism (9, 10, 11, 12, 13). The main hypothesis favours a direct suppressive action of this drug on the hypothalamus (14), such that synthesis of luteinizing hormones releasing hormone (LHRH) is inhibited or suppressed. Consequently, lowering of gonadotrophin synthesis, as previously reported (8, 11) as well as inhibition of ova production and disruption of oestrous cycle is therefore inevitable.

It is however premature to conclude that the lengthening of oestrous cycle period and partial inhibition of ova production by marijuana, as reported in our studies, are direct effects of the hypothesized inhibitory function of cannabis on the hypothalamic LHRH-producing neurons only. Detailed mechanism by which marijuana produces its anti-fertility effects awaits further investigations.

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