EFFECTS OF ACUTE AND LONG-TERM CANNABIS TREATMENT ON RESTRAINT-INDUCED GASTRIC ULCERATION IN RATS

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We designed the present experiments in order to investigate the effects of *Cannabis sativa* extract (CE) treatment upon restraint induced gastric ulceration in rats. The major intent of the present study was to determine the possible relationships among the schedule of CE administration, dose of CE administered and immobilization-induced gastric lesions.

Wistar rats of both sexes and genetically similar were housed and later tested in a quiet room with a controlled temperature (19–23°C) on a non-reversed 12-hr-night-cycle, with light onset at 07:30 hr. Subjects were 80 days old at the beginning of the experiments and were provided food and water "ad libitum" until the moment of immobilization. CE extract was obtained, prepared and suspended in 0.9% saline plus Tween-80 as previously described (1). The control solutions consisted both of 0.9% saline plus Tween-80 (CS) and 0.9% saline (S). The biological activity of the extract, measured by the corneal areflexia method in rabbits (Gayer's test), according to Santos et al. (2) was 0.54 mg/kg $\pm$ 0.004. All drugs were administered i.p. in an equal volume of 1.0 ml/kg.

The experiments were performed in ten replications. For the acute experiments subjects were randomly divided into 7 groups, removed from their home cages in fixed numerical order and injected both 24 hr and immediately before immobilization respectively with 5.0, 10.0, 20.0, 40.0 and 60.0 mg/kg of CE or an equal volume of 1.0 ml/kg, of CS or S. In the long-term treatments the animals were given 40 and 60 mg/kg of CE or an equal volume of 1.0 ml/kg of CS or S for 20 days and were immobilized immediately after the last administration. Some animals were also treated for 25 days with 60.0 mg/kg of CE or 1.0 ml/kg of CS and sacrificed 24 hr after the last injection, without restraint, for stomach observation. Immobilization was performed using the standard method described in detail elsewhere (3). At the end of the restraint period the rats were anesthetized with ether, decapitated and the stomachs immediately removed, cut open along the greater curvature, washed in 0.9% saline and examined for lesions. To minimize possible effects of circadian hormonal changes that could interfere with the results (4, 5), the experiments were designed in order to allow all subjects to be restrained and/or sacrificed at the same time of the day, i.e., at noon. The degree of ulceration was evaluated by the method described by Pauls et al (6). Briefly, the stomachs free of ulcers were called "clear" or "zero" and those with the most ulceration "four plus", according to the quantity of ulcers they showed. The percentage

TABLE 1. Effects of acute and long-term cannabis extract (CE) treatment upon restraint-induced ulceration in rats

Condition	Schedule of administration	Treatment	Dose (mg/kg)	n	Average degree of ulceration	% of rats with ulcers
Restrained	acute	NaCl 0.9%	—	29	1.79	82.76
		NaCl 0.9% + Tween-80	—	32	1.84	65.63
		CE	5.0	18	1.78	77.78
		CE	10.0	19	2.44	75.00
		CE	20.0	24	2.35	78.26
		CE	40.0	16	1.00	50.00*
		CE	60.0	12	0.50	41.67**
	long-term (20 days)	NaCl 0.9%	—	17	2.94	100.00
		NaCl 0.9% + Tween-80	—	15	3.00	100.00
		CE	40.0	8	3.63	100.00
		CE	60.0	19	3.32	94.74
	long-term (25 days)	NaCl 0.9% + Tween-80	—	10	0.40	20.00
		CE	60.0	10	2.10	80.00***

n=number of rats. *= $p<0.05$ and **= $p<0.01$ in relation to animals acutely treated with NaCl 0.9% (S). ***= $p<0.01$ in relation to unrestrained animals treated for 25 days with NaCl 0.9%+Tween-80(CS)

of animals showing ulcers in each group was also determined and the median dose (AD50), 95% confidence limits and slope of the dose-response curve were calculated (7). (Chi)² test was also used, and the probability of $p<0.05$ was considered to indicate a significant difference for all comparisons made.

Immobilization induced haemorrhagic ulcers, associated with numerous petechiae only in the glandular segment of the gastric mucosa. As shown in Table 1, the incidence of ulceration was not significantly different in CS and S treated rats both in acute and long-term experiments. Nevertheless both the number of ulcers and the average degree of ulceration were higher in long-term treated animals. Concerning the effects of acute CE treatment, the doses of 40 and 60 mg/kg significantly affected the lesion pattern and incidence ($p<0.05$ and $p<0.01$ respectively). Subjects of these groups showed a low lesion incidence in the form of occasional petechiae. Based on our data, the protective AD50 for acute CE treatment was 40 mg/kg (19.20–83.68) being the slope of the dose-response curve 0.114 (0.112–0.115).

Data obtained with long-term CE treatment show that 20 daily injections with doses of 40 and 60 mg/kg provided no protection. As a matter of fact, the reverse was observed since the stomachs of five and nine animals long-term treated respectively with 40 and 60 mg/kg of CE had a total of 58 ulcers. The remaining animals of these two groups had 2 or 3 lesions each, the ulcers being circular in shape and slightly less than 1 cm in length.

Table 1 also shows that the percentage of rats with ulcers was significantly higher

($p < 0.01$) in unrestrained animals treated for 25 days with 60 mg/kg of CE. The stomachs of these animals were transparent rather than opaque, thinner than normal and showed diffuse erythema, gastritis, and prominent rugae. Moreover, recently ingested food and mucosal lining were virtually absent. In contrast, the stomachs of all 10 control animals, long-term treated with CS and sacrificed without restraint were filled with food, and the mucosa were entirely normal except for some lesions with a diameter of 3 mm or less in two of these rats.

Both cannabinoids and belladonna alkaloids produce dry mouth (8) in addition bradycardia produced by vagal stimulation in the anesthetized dog may be attenuated by cannabinoids (9, 10); finally Δ^9 -THC, a constituent of CE (11), inhibited Ach-induced contraction of the vas deferens (12). These findings may be considered to support the notion that the protective effect of acute CE treatment on immobilization-induced gastric lesions was a consequence of its effect at muscarinic sites. Nevertheless, such speculation is not feasible since it has been reported that acute administration of CE, Δ^9 -THC and its isomers and derivatives induce, in laboratory animals, many effects similar to those produced by chlorpromazine and reserpine (13), and it is known that tranquilizers also prevent the development of stress ulcers (14). In the meantime, our results show that long-term CE treatment induced gastric lesions in unrestrained animals. The stress of being given daily injections also may be important in ulcer production since the percentage of rats with ulcers were higher in all long-term treated animals. In a previous work, we observed that long-term CE treatment caused not only a significant increase in plasma corticosterone but also in both wet pituitary and paired adrenal weights (15). Since Δ^9 -THC also produces strong pituitary-adrenocortical activation (16) it is reasonable to assume that the animals submitted to the long-term treatment in our experiments were under conditions of severe stress. Another possible explanation could be the development of tolerance to the protective effects of CE, which has already been observed by different authors in different behavioral tests (13).

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