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SEDATIVE ACTIVITY OF CANNABIS IN RELATION TO ITS Δ′-*trans*-TETRAHYDROCANNABINOL AND CANNABIDIOL CONTENT

JOAN T. PICKENS

Department of Pharmacognosy, The School of Pharmacy, University of London, 29–39, Brunswick Square, London WC1N 1AX

1 The oral sedative potencies of cannabis herb, crude ethanolic and petroleum-ether fractions, were assayed against Δ′-*trans*-tetrahydrocannabinol (THC) administered orally to mice, by measuring spontaneous motor activity over 30 min periods, at selected times, up to 6 h.

2 The THC contents of the extracts were determined chemically by gas-liquid chromatography analysis and the B/C ratio (biological activity divided by chemical activity) calculated for each. The B/C values for cannabis herb, which contained THC but no CBD, was 4.47 and for ethanolic and petroleum-ether extracts, 5.26 and 4.39, respectively.

3 The sedative potency expressed as SDA_{50}, the dose required to give 50% effect over 6 h, was 1.06 (0.98 to 1.15) mg/kg for THC; 4.72 (4.22 to 5.27) mg/kg for cannabidiol and 1.26 (1.22 to 1.80) mg/kg for chlorpromazine.

4 An infusion of cannabis herb made with boiling water was shown to have sedative activity of very low potency.

5 When the cannabinoids were completely extracted from a sample of herb with petroleum-ether the aqueous and ethanolic extracts of the marc had some sedative activity; but the 70% ethanolic fraction had none.

6 The sedative activity of THC, cannabis herb and a water soluble fraction is blocked by aspirin, a cyclo-oxygenase inhibitor, and restored by prostaglandin E_2 (PGE_2).

7 The sedative effect of chlorpromazine is not blocked by aspirin.

Introduction

Oral cannabis preparations and Δ′-*trans*-tetrahydrocannabinol (THC) have a sedative and tranquillising effect in man accompanied by diminished anxiety at doses much lower than those which produce psychoactivity (Hollister, Richards & Gillespie, 1968; Noyes, Brunk, Baram & Canter, 1976; Graham, Davies, Seaton & Weatherstone, 1976). Extracts of cannabis and THC also have a sedative effect in mice, measured as a decrease in spontaneous motor activity (Carlini, Karniol, Renault & Schuster, 1974). I have used Tuck No. 1 strain albino female mice in these experiments and measured sedation as a reduction in spontaneous motor activity. The method is based on that of several other workers (Siegel, 1946; Riley & Spinks, 1958; Harris, 1964; Somers, 1960; Svensson & Thieme, 1969). I have used THC as a standard and I have chosen the oral route since previous work has shown that THC and cannabis are active at very much lower doses after oral administration than when given intraperitoneally (Fairbairn & Pickens, 1979; 1980; 1981). I have attempted to discover whether the sedative action of 'whole' herb and extracts is related to their THC contents measured by gas-liquid chromatography (g.l.c.) analysis, by the method of Fairbairn & Liebmann (1973), as previous work has shown that the herb and extracts are at least two or four times more active than one would expect from their THC content (Carlini *et al.*, 1974; Fairbairn & Pickens, 1981). The effect of aspirin on sedative action and its reversal by prostaglandin E_2 (PGE_2) has also been determined since previous work showed that the cataleptic effects of herb and extracts were antagonized by aspirin and restored by PGE_2 (Fairbairn & Pickens, 1979; 1981). In addition I have determined the sedative activity of cannabidiol (CBD). Finally, as cannabis tea made by infusing powdered herb with boiling water is also sedative in man, I have attempted to find the proportion of sedative action residing in the water soluble fraction, by testing an infusion made by pouring boiling water on cannabis herb. I have also tested an aqueous extract from a sample of herb from which the can-

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nabinoids were completely extracted by petroleum-ether.

Methods

Preparation of material for oral administration

Δ'-trans-*Tetrahydrocannabinol and cannabidiol* were suspended in 2.5% solution of Tween 80 as previously described (Fairbairn & Pickens, 1979).

Cannabis Herbal cannabis was prepared by careful drying of plant material and then separation from the thick stems by rubbing or by sifting. The plants were grown from seed in our own experimental gardens and extracts were prepared from two varieties. One variety, UNC 335, was from a THC-rich plant and the other, UNC 354, was from a CBD-rich strain (Fairbairn & Liebmann, 1974). All experiments were performed using non-decarboxylated powder. For administration, the powdered herb was suspended in mucilage of tragacanth B.P.

Ethanolic and petroleum-ether (40°–60°) extracts were prepared as described in a previous paper (Fairbairn & Pickens, 1981).

Water extracts of 'whole' herb were prepared by adding 500 ml of boiling water to 100 g dried cannabis. The infusions were allowed to stand until cool and then filtered.

Water extracts of cannabinoid-free herb were prepared from both THC-rich and CBD-rich strains by percolating 50 g of finely powdered herb with petroleum-ether (40°–60°) until all traces of cannabinoids had been removed, as described in our previous paper (Fairbairn & Pickens, 1981). Hot water extracts were prepared by adding 500 ml of boiling water to 100 g of the cannabinoid-free dried marc, allowing to cool and filtering. Doses of filtrate were expressed in mg of 'whole' cannabis herb/kg. Some of the freshly prepared filtrates were tested in mice, the remainder was freeze-dried and stored at −20°C in the dark, and the re-dissolved powders were tested in mice for sedative activity. Cold water extracts were prepared by percolating cold chloroform-water through the petroleum-ether-exhausted marc. The extract was reduced to small bulk by evaporation *in vacuo* and dried in a P_2O_5 vacuum desiccator to constant weight. This exhausted marc yielded about 11% water soluble material which was redissolved in water and tested in mice. Doses of this extract have been expressed in terms of the dried water soluble residue.

Chemical estimation of tetrahydrocannabinol All suspensions given to the mice were analysed for THC and CBD content by g.l.c. analysis by the method of Fairbairn & Liebmann (1973). The coefficient of variation in these assays is 1.4% for THC and 2.55% for samples of herbal cannabis.

Measurement of sedative activity

LAC A Tuck No. 1 strain albino female mice weighing 18 to 23 g were used throughout. The mice were housed and fed as described in previous papers (Fairbairn & Pickens, 1979; 1980; 1981). Sedative activity was measured as a reduction in spontaneous motor activity of control mice (see later). The activity was measured with an 'activity-monitor' containing two sensitive transducers which recorded the combined movements of groups of 7 mice and was set to print out the score every 2.5 min. In these experiments the mice were housed at 30 to 32°C and groups of 7 transferred to a perspex mouse box (305 × 160 × 130 cm) with a metal lid, just before the start of the count. The activity was measured at selected times after oral administration at t_0 min of standard compounds or extracts. Seven consecutive scores were obtained at each selected time. The 'activity monitor' transducers are sufficiently sensitive to respond to any loud background noise and vibration and so we used a second monitor which was run at the same time as the sedative assays and recorded background noise only. This was usually zero as the apparatus was housed in a glass-fronted cabinet. When the background noise was excessive the experiments were stopped as the results were meaningless. No mouse was left in the activity cage for more than 10 consecutive readings (i.e. 25 min) as scores after this were not reliable. No group of mice was re-tested without a minimum interval of 2 h, otherwise the group would give too high a sedative score. The same group of mice was used to give the scores at $t_0 + 2$, $t_0 + 4$ and $t_0 + 6$ h. A second group was used for $t_0 + 0.5$ h and a third group for $t_0 + 1$ h. One control group was run each day at the beginning, middle and end of the test groups, with a 2 h interval between each. Mice receiving test extracts were killed at the end of the day. The untreated control mice were returned to stock for 7 days and then re-used. The dose-response relationships for herb and extracts were established for three or four dose levels using 7 mice per group. All calculations are based on cumulative moving means of the activity scores carried out on different days as described in a previous paper (Fairbairn & Pickens, 1981).

Control mice were untreated or dosed with vehicle only, but otherwise were handled as test groups. When cannabis herb was tested, control mice were given mucilage of tragacanth B.P. by oral administration. When extracts were tested, control mice were given 2.5% w/v Tween 80 and when aqueous

extracts and infusions were tested, controls were given water. It was not possible to combine all the control scores between March 1976 and April 1980 because the readings are only constant for one position of the transducers. If the transducers are moved by 1 mm the readings can alter from 1400 to 2500. So the cumulative linear moving mean has been combined for eight to ten consecutive experiments only. An example of scores from control mice will make clear what this involves and is described in the results.

Prostaglandin inhibition

In these experiments all mice were pretreated with aspirin, 10 mg/kg orally, suspended in mucilage of tragacanth, followed 3 min later by the cataleptic compounds. The sedative effect was measured at t_0 + 2 h. Further groups of mice were given 10 mg/kg aspirin orally at t_0 min, cataleptic samples at t_0 + 3 min, and intraperitoneal PGE_2 (3.3 μg/kg) at t_0 + 100 min. The sedative effects were measured at t_0 + 2 h as before.

Calculation of sedative potency

This has been expressed in two ways: (a) the sedative dose fifty (SD_{50}) which is the dose in mg/kg required for 50% reduction in activity scores for control mice and calculated with 95% confidence limits by Finney's probit analysis (Finney, 1964), from the linear graph relating probit percentage sedation (ordinate scale) against log dose (abscissa scale). (b) Calculation of areas or SDA_{50} which is the dose in mg/kg required for 50% reduction in the area beneath the graph relating sedation (expressed as Y_2%) on the ordinate scale against time to 6 h on the abscissa scale. The method of calculating Y_2 is described in detail in an earlier paper (Fairbairn & Pickens, 1981).

Biological/chemical ratios (B/C)

The B/C ratio for each sample is the THC equivalent by biological assay (B) divided by the THC content by chemical analysis (C) and also described in the earlier paper (Fairbairn & Pickens, 1981).

Drugs and apparatus

THC and CBD were prepared as stock solutions containing 10 mg/ml in 2.5% w/v Tween 80. All dilutions were made with distilled water. The following drugs were used: THC and CBD (Makor Chemical Co., Limited., Jerusalem), aspirin (BDH Ltd.), chlorpromazine (May and Baker Ltd). The apparatus for measuring spontaneous motor activity called an 'activity monitor' was supplied by Bioscience, Harbour Estate, Sheerness, Kent, ME12 1RZ.

Results

Controls

Cumulative linear moving means for control responses have been calculated for 10 experiments carried out between November 1979 and April 1980 (Table 1). The activity scores per group of 7 untreated control mice (experiments 1–7 in Table 1) moves from 2626 ± 133 (7) in the first experiment to 2500 ± 38 (275). There was no significant difference between the control mice given mucilage of tragacanth, Tween 80, water or left untreated and all 4 have been combined to give a cumulative linear moving mean for the controls. The final value, 2550 ± 31 (354), has been used to calculate SD_{50} and SDA_{50} values for the standard compounds and extracts. Figure 1 shows that the activity of the controls does not vary within the test time period and Table 1 shows that the control scores do not differ between t_0 and t_0 + 6 h.

Evaluation of the bioassay estimation of sedation

The activity of THC 3 mg/kg orally and an infusion made with boiling water from a THC-rich cannabis plant is illustrated in Figure 1. In these experiments the initial readings probably represent exploratory activity by the mice when put into a new cage (Svensson & Thieme, 1969). However, I have included all the readings in the estimates of sedation.

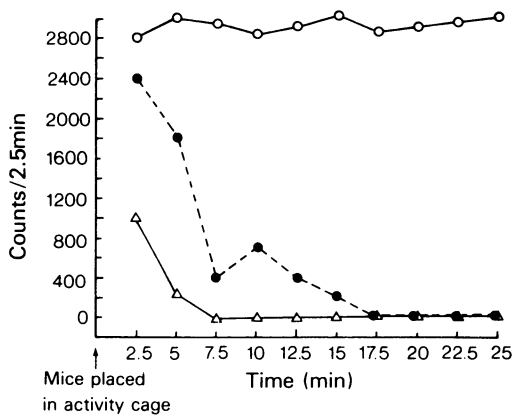

Figure 1 Motor activity of groups of 7 albino, female, Tuck No. 1 strain mice after oral administration of Δ'-*trans*-tetrahydrocannabinol (THC) 3 mg/kg and an infusion prepared by pouring boiling water over dried cannabis herb. Mice were put in the activity cage at zero time and counts recorded at the end of 2.5, 5, 7.5 minutes, etc; (○) control mice received no treatment; (△) mice given THC 30 min before zero time; (●) mice given the filtered infusion 30 min before zero time.

Figure 2 shows the dose-response relationship for THC at t_0 + 4 h for five doses of THC. A satisfactory dose-response relationship held for this dose-response line and at all the times tested. SD_{50} values calculated from such dose-response lines are given in Table 2. There was no significant deviation from parallelism for the dose-response lines of THC, CBD, chlorpromazine, and herb and extracts and comparisons between them are therefore valid (Figure 2).

Sedative activities

Δ′-trans-*Tetrahydrocannabinol* (THC) was administered orally in doses from 0.06 mg/kg to 3 mg/kg; doses above 3 mg/kg gave 100% effect and were not used in the calculations of potency. The onset of the sedation was rapid and low values for SD_{50} were obtained from t_0 + 30 min to t_0 + 6 h (Table 2), when the experiments were terminated. Peak activity occurred at t_0 + 4 h (Figure 2) and the SDA_{50} value for potency over 6 h was 1.06 (0.98–1.15) mg/kg (Figure 3).

Cannabidiol (CBD) was active in doses from 1 mg/kg to 20 mg/kg (Figure 2 and Table 2). The onset of sedation was rapid and the SD_{50} value at t_0 + 30 min

Table 1 The cumulative linear moving mean of activity scores recorded every 2.5 min for untreated control groups of 7 female albino Tuck No. 1 strain mice at 30–32°C in the sedative test.

Expt.	*Date*	*Mean activity score /group of 7 mice* ± s.e. mean •	*Set*	*Cumulative moving mean Activity score* ± s.e. moving mean	s.e. moving mean *as a %*
1a	21.11.79	2626 ± 133 (7)	1 to 1	2626 ± 133 (7)	± 5.06
b		2030 ± 163 (8)	1 to 2	2308 ± 129 (15)	± 5.59
c		2263 ± 115 (18)	1 to 3	2284 ± 83.4 (33)	± 3.65
d		3032 ± 45 (6)	1 to 4	2398 ± 83.3 (39)	± 3.47
2a	1.2.80	2734 ± 127 (10)	1 to 5	2467 ± 72.8 (49)	± 2.95
b		2703 ± 140 (11)	1 to 6	2510 ± 64.9 (60)	± 2.58
c		2377 ± 195 (7)	1 to 7	2497 ± 60.9 (67)	± 2.44
d		2050 ± 223 (9)	1 to 8	2443 ± 60.9 (76)	± 2.50
3a	26.2.80	1940 ± 254 (9)	1 to 9	2390 ± 61.9 (85)	± 2.59
b		2399 ± 162 (9)	1 to 10	2391 ± 57.7 (94)	± 2.41
c		2485 ± 84 (13)	1 to 11	2402 ± 51.6 (107)	± 2.15
d		2579 ± 85 (10)	1 to 12	2418 ± 47.8 (117)	± 1.98
4a	11.3.80	2061 ± 167 (6)	1 to 13	2400 ± 46.5 (123)	± 1.94
b		2991 ± 56 (9)	1 to 14	2400 ± 45.4 (132)	± 1.86
c		1900 ± 157 (7)	1 to 15	2413 ± 44.8 (139)	± 1.85
d		2823 ± 118 (7)	1 to 16	2433 ± 43.5 (146)	± 1.79
5a	13.3.80	2929 ± 69 (7)	1 to 17	2455 ± 42.4 (153)	± 1.73
b		2911 ± 60 (12)	1 to 18	2488 ± 40.6 (165)	± 1.63
c		3080 ± 79 (15)	1 to 19	2538 ± 39.6 (180)	± 1.56
d		2509 ± 212 (17)	1 to 20	2535 ± 40.1 (197)	± 1.58
6a	14.3.80	2447 ± 184 (7)	1 to 21	2533 ± 39.1 (204)	± 1.54
b		2427 ± 145 (7)	1 to 22	2529 ± 38.1 (211)	± 1.50
c		3072 ± 133 (7)	1 to 23	2546 ± 37.6 (218)	± 1.48
d		2700 ± 190 (7)	1 to 24	2551 ± 36.9 (225)	± 1.45
7a	17.4	1580 ± 147 (11)	1 to 25	2506 ± 38.1 (236)	± 1.52
b		2527 ± 101 (9)	1 to 26	2506 ± 36.8 (245)	± 1.46
c		1952 ± 270 (7)	1 to 27	2491 ± 36.8 (252)	± 1.48
d		2593 ± 180 (23)	1 to 28	2500 ± 36.77 (275)	± 1.47
8a	15.4.80	3155 ± 242 (9)	1 to 29	2520 ± 36.9 (284)	± 1.48
b		2528 ± 92 (22)	1 to 30	2521 ± 34.8 (306)	± 1.38
c		2655 ± 124 (19)	1 to 31	2529 ± 33.5 (325)	± 1.32
d		2750 ± 116 (5)	1 to 32	2532 ± 33.0 (330)	± 1.30
9	18.3.80	2824 ± 80 (7)	1 to 33	2540 ± 32.3 (339)	± 1.27
10	12.3.80	2768 ± 70 (15)	1 to 34	2550 ± 31.2 (354)	± 1.22

No of scores given in parentheses. Untreated control mice were tested at t_0 (a); t_0 + 2 h (b); t_0 + 4 h (c) and t_0 + 6 h (d). When cannabis herb was tested, control mice were given mucilage of tragacanth B.P. (experiment 8); when extracts were tested, control mice were given 2.5% w/v Tween 80, 0.2 ml/20 g, (experiment 9) and when aqueous extracts and infusions were tested control mice were given water by oral administration (experiment 10).

equated with the SD_{50} value at t_0 + 6 h (Table 2). Peak activity occurred at t_0 + 1 h and the SDA_{50} value for potency over 6 h was 4.72 (4.22–5.27) mg/kg (Figure 3).

Chlorpromazine was active between 0.25 mg/kg and 4.0 mg/kg. Sedative activity was marked at t_0 + 30 min and lasted until t_0 + 6 h (Table 2), with peak activity at t_0 + 4 h (Figure 1 and Table 2). The SDA_{50} value for potency over 6 h was 1.26 (1.22–1.80) mg/kg (Figure 3).

Herbal cannabis when assayed against THC was 4.47 times more active than would be expected from its THC content (Table 2). Individual B/C ratios at t_0 + 0.5, t_0 + t_0 + 1, t_0 + 2, t_0 + 4 and t_0 + 6 h were respectively 0.47, 0.48, 10.78, 1.93 and 20.06. These results indicate that the THC equivalent by bioassay exceeds the THC content by chemical analysis (3.04%) at all times except possibly t_0 + 0.5 h and t_0 + 1 h.

Ethanolic and petroleum-ether extracts when assayed against THC were 5.26 (sample 5) and 4.39 (sample 6) times more active than would be expected from their THC content (Table 2).

Water infusion of whole herb showed sedative activity when very large doses of infusion were given. Figure 1 illustrates the 82% sedative effect of 8 g dry cannabis/kg at t_0 + 30 min. The SD_{50} value for this infusion, from a THC-rich plant was 3300 mg dried herb/kg at t_0 + 30 min. A similar infusion from a CBD-rich plant was also active but less potent.

Water infusions of cannabinoid-free herb showed sedative activity up to t_0 + 6 h; SDA_{50} values were 2200 mg/kg and 6400 mg/kg respectively for infusions from a THC-rich and CBD-rich herb.

Aqueous and ethanolic extracts of cannabinoid-free herb The aqueous and ethanolic extracts of the freeze-dried powders 28-84A and 28-84 B, prepared from cannabinoid-free herbs had low sedative potencies; the ethanolic extracts were less active than the aqueous extracts and despite repeated experiments, eight in all, no sedative activity was found in the 70% ethanolic extracts.

Effect of aspirin

Aspirin, a prostaglandin synthesis inhibitor, had no sedative effect in doses of 10 to 400 mg/kg but 10 mg/kg of aspirin reversed the sedative responses to 3 mg/kg of THC, 40 mg/kg of herb and 1000 mg/kg of residue 28–84 A (Table 3). The sedative effect of chlorpromazine was not inhibited by aspirin. Prosta-

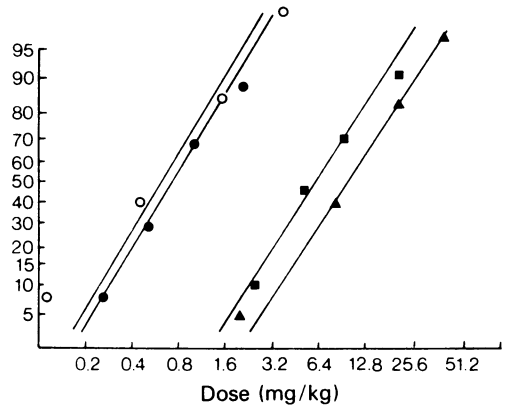

Figure 2 Relative potencies of Δ'-*trans*-tetrahydrocannabinol (THC) (○), cannabidiol (CBD) (■), cannabis herb (▲) and chlorpromazine (●) at t_0 + 4 h after oral administration to female, albino, Tuck No. 1 strain mice at 30–32°C in the sedative test. Ordinate scale: probit percentage sedation; abscissa scale: log dose mg/kg. Seven mice were injected at each dose. Potency as SD_{50} for THC is 0.61 (0.58–0.63)mg/kg; for CBD is 6.31 (5.47–7.28) mg/kg; for herb is 10.5 (10.1–10.9) and for chlorpromazine 0.70 (0.74–0.77). The potency of the herb which contained 3.04% THC is greater than would be expected due to the presence of a synergist, B/C = 5.86.

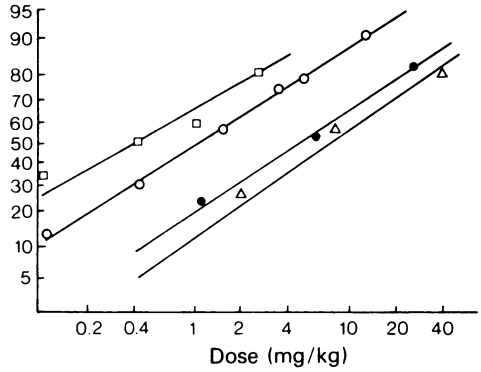

Figure 3 Relative overall sedative potencies of Δ'-*trans*-tetrahydrocannabinol (THC) (○), cannabidiol (CBD) (●), cannabis herb (△) and a petroleum-ether extract (□) from t_0 to t_0 + 6 h after oral administration at t_0 to female, albino, Tuck No. 1 strain mice in the activity cage. Ordinate scale: probit Y_2% where Y_{22} is calculated as described in Fairbairn & Pickens, 1981. Abscissa scale: log dose mg/ or ml/kg. Potency as SDA_{50} for THC = 1.06 (0.98–1.15) mg/kg; for CBD = 4.72 (4.22–5.27) mg/kg; for herb = 7.76 (7.37–8.17) mg/kg and for the petroleum-ether extract (0.43/0.38–0.48) ml/kg. The potency of the herb which contained 3.04% THC and no CBD is greater than would be expected with B/C = 4.47. The potency of the petroleum extract which contained 0.56 mg THC/ml was also greater than would be expected with B/C = 4.39.

glandin E_2 (3.3 μg/kg i.p.) restored the sedative responses to THC, cannabis herb and residue 28–84 A; however, this small dose of PGE_2 had a sedative effect of its own (Table 3).

Discussion

The biological assay

Estimates of sedative potency have been calculated on the cumulative moving means of the sedative scores for ten control groups measured on different days between November 1979 and March 1980. Thus estimates of potency are based on much larger sample sizes than would have been possible if comparisons between THC as standard, and extracts, as test preparations, had been limited to data collected on the same day, or days. Additionally the cost of the assay and the number of mice is reduced as some of the preceding data are used in the estimate of potency. Table 1 shows that the standard error of the moving mean reduces as more results are added to previous data. Thus on day 1 the standard error of the mean

Table 2 Potency, expressed as SD_{50} (the dose in mg/kg required to give 50% sedation at different times after oral administration at t_0 hours) and as SDA_{50} (the dose required to give a 50% effect over 6 h) of Δ'-*trans*-tetrahydrocannabinol (THC), cannabidiol (CBD), chlorpromazine (Cpz), cannabis herb and extracts to female, albino, Tuck No. 1 strain mice, at 30–32°C in the sedative test

No.	*Sample* (THC by g.l.c.)	*Time* (h)	SD_{50} (mg or ml/kg)	SDA_{50} (mg or ml/kg)	*THC by bioassay* (% or mg/ml)	*B/C ratio*
1	*THC*	t_0 + 0.5	0.90 (0.84–0.96)			
		t_0 + 1	0.80 (0.76–0.86)			
		t_0 + 2	0.99 (0.90–1.08)			
		t_0 + 4	0.61 (0.38–0.63)			
		t_0 + 6	6.83 (6.13–7.62)	1.06 (0.98–1.15)		
2	CBD	t_0 + 0.5	4.70 (4.38–5.04)			
		t_0 + 1	2.50 (2.10–2.90)			
		t_0 + 2	2.80 (2.61–3.00)			
		t_0 + 4	6.31 (5.47–7.29)			
		t_0 + 6	4.11 (3.75–4.51)	4.72 (4.22–5.27)		
3	Cpz	t_0 + 0.5	2.33 (2.12–2.55)			
		t_0 + 1	2.00 (1.61–2.47)			
		t_0 + 2	1.44 (1.38–1.51)			
		t_0 + 4	0.70 (0.74–0.77)			
		t_0 + 6	1.21 (1.07–1.67)	1.26 (1.22–1.80)		
4	Herb 28–59A (3.04%)	t_0 + 0.5	62.6 (60.0–65.4)			
		t_0 + 1	54.9 (43.5–70.1)			
		t_0 + 2	3.02 (2.69–3.39)			
		t_0 + 4	10.4 (10.0–10.9)			
		t_0 + 6	11.2 (10.7–11.8)	7.76 (7.37–8.17)	13.59	4.47
5	Ethanolic extract 28–159 (0.159 mg/ml)	t_0 + 0.5	8.90 (8.19–9.66)			
		t_0 + 1	15.4 (12.6–18.9)			
		2	1.98 (1.88–2.08)			
		t_0 + 4	0.18 (0.16–0.20)			
		t_0 + 6	0.70 (0.67–0.73)	1.27 (1.18–1.35)	0.836	5.26
6	Petroleum spirit extract 28–163 A (0.56 mg/ml)	t_0 + 0.5	18.6 (16.0–21.7)			
		t_0 + 1	1.72 (1.46–2.04)			
		2	0.13 (0.12–0.14)			
		t_0 + 4	0.11 (0.10–0.12)			
		t_0 + 6	7.30 (5.95–8.95)	0.43 (0.38–0.48)	2.46	4.39

All samples were given orally at t_0 h and the motor activity measured at the times shown. 95% confidence limits are given in parentheses. Herb = aqueous suspension of cannabis in mucilage of tragacanth B.P. The extracts were given in 2.5% Tween 80. Doses of THC and herb are in mg/kg and extracts in ml/kg. The THC content of the herb and extracts by g.l.c. analysis is given in parentheses (column 1). All samples were from non-decarboxylated herb. THC by bioassay has been calculated from SDA_{50} values (column 5).

was ± 133 and this reduced progressively through ± 129, ± 83, ± 72, to a final standard error of ± 31.2 by the 34th set of readings. Fairbairn & Pickens (1981) suggested that the 'difference between days' in their cataleptic work was due to the small sample for a day's work compared to the standard deviation and the sedative results described in this paper suggest that this principle applies to the sedative data too. The argument is further strengthened by showing that the 354 scores from the 34 control experiments form part of a normal distribution.

Variation of relative potencies with time

The peak sedative activities of THC, CBD, chlorpromazine, herb and extracts do not coincide so that potencies of these, relative one to another, will vary according to the time at which the readings are made; thus one overall figure for potency (SDA_{50}) has been calculated which is the activity over the 6 h period by the 'area method' described previously (Fairbairn & Pickens, 1981). The comparisons of sedative potency are valid as the overall dose-response relationships do not differ significantly from parallelism (Figure 3).

Sedative potency of 'whole' extracts compared to Δ'-trans-tetrahydrocannabinol (THC)

The potency of the herb, ethanolic and petroleum-ether extracts of the herb with no CBD content, cannot be accounted for in terms of their THC content alone. The results agree with those from our cataleptic work with THC, herb and extracts (Fairbairn & Pickens, 1981) and with those of Carlini *et al.*, (1974) and indicate that THC accounts for only one quarter the activity of cannabis herb and resin.

Sedative potency of cannabinoid-free extracts compared to Δ'-trans-tetrahydrocannabinol (THC)

The aqueous and ethanolic extracts prepared from herb samples from which the cannabinoids had been completely extracted had very low sedative potencies equivalent to 0.89% and 0.23% of THC for the acqueous extracts from a THC-rich and CBD-rich plant respectively; and 0.18% and 0.05% for the ethanolic extracts respectively. The water/ethanol soluble non-cannabinoid components of herbal cannabis therefore account for only a very small

Table 3 The effect of prostaglandin E_2 on the reversal by aspirin of the sedative effects of Δ'-*trans*-tetrahydrocannabinol (THC), cannabidiol (CBD), chlorpromazine (Cpz), cannabis herb and extracts in female, albino, Tuck No. 1 strain mice at 30–32°C

No.	*Treatment*[a]	*Sedative scores (n)*	*% sedation*
101	No treatment	2513 ± 32.8 (363)	–
102	Aspirin (10)	2490 ± 133 (21)	NS
103	PGE_2	1804 ± 145 (21)	28.2
104	THC (3)	502 ± 100 (7)	80.0
105	THC (3) + aspirin (10)	2485 ± 84 (7)	NS
106	THC (3) + aspirin (10) + PGE_2	352 ± 98 (7)	86.0
107	CBD (6.25)	534 ± 86 (7)	78.7
108	CBD (6.25) + aspirin (10)	2612 ± 129 (7)	NS
109	CBD (+ aspirin (10) + PGE_2	499 ± 71 (7)	80.1
110	Cpz (4)	25 ± 50 (7)	99.0
111	Cpz (4) + aspirin (10)	37 ± 64 (7)	98.5
112	Herb (40)	829 ± 87 (7)	67.0
113	Herb (40) + aspirin (10)	2691 ± 97 (7)	NS
114	Herb (40) + aspirin (10)	1002 ± 102 (7)	60.0
115	28–84A (268)	477 ± 90 (7)	81.0
116	28–84A (268) + aspirin (10)	2312 ± 121 (7)	NS
117	28–84A (268) + aspirin (10) + PGE_2	512 ± 204 (7)	79.6

n = Number of mice. Score are given ± s.e. mean.

[a] Aspirin, 10 mg/kg orally, given at t_0 min, THC, CBD, Cpz, herb and extract given at t_0 + 3 min. PGE_2 given at t_0 + 100 min and sedation measured at t_0 + 120 min. Doses in mg/kg are given in parentheses. PGE_2 was given at 3.3 μg/kg i.p.

NS = Not statistically significantly different from control score ($P>0.05$).

proportion of the sedative effects of whole herb and extracts.

Effect of aspirin

In cataleptic experiments it was shown that the responses to THC, herb and extracts involved a PGE_2 mechanism (Fairbairn & Pickens, 1979; 1980; 1981). It has now been shown that the sedative effects of THC, cannabis herb and extracts can be abolished by aspirin and restored by a very small dose of PGE_2. However, this small dose of PGE_2 had a sedative effect of its own. So it is not, in this sedative assay, unequivocal evidence that the sedative effects of cannabinoids, herb and extracts depend exclusively upon the availability of PGE_2. It may be that PGE_2, THC, CBD, herb and extracts compete for the same central receptors and this would be in agreement with the work of Jackson and colleagues (1976) who have suggested that THC, PGE_1, PGE_2 and $PGF_{2\alpha}$ compete for identical intraperitoneal receptors (Jackson, Malor, Chesher, Starmer, Welburn & Bailey, 1976).

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