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Studies on the Interactions of Copper and Cannabis

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Abstract. The action of copper ($CuSO_4$, 5 mg/kg, oral) on selected neuropharmacological actions of cannabis resin (CI, oral) was studied on albino rats and mice. Copper potentiated the barbiturate hypnosis-potentiating activity of CI in albino rats and mice and had no effect on hypothermic activity in albino rats.

Single doses of copper partially inhibited tolerance to barbiturate hypnosis-potentiation activity and markedly delayed the development of tolerance to hypothermic activity of CI. Oral as well as i.c.v. copper ($CuSO_4$, 0.1 µg) in single dose antagonised the tolerance to hypothermic activity of cannabis or THC for one to two weeks. Copper-CI interaction could be antagonised by penicillamine. Zinc ($ZnSO_4$, 5 mg/kg, oral) had an action similar to that of copper in antagonising the development of tolerance to the hypothermic activity of CI, but magnesium ($MgSO_4$, 5 mg/kg, i.p.) was devoid of any such action.

Studies indicate that, although copper has no significant neuropharmacological action, it interacts with CI activity, especially in tolerant rats, in effects on hypothermia. The site of action of copper is possibly the hypothalamus, where it inhibits the processes of tolerance development to CI on the noradrenergic neurone.

Key words: Cannabis – Copper – Interactions – Hypothermia

Various metal preparations have been used for medicinal purposes in the Ayurvedic system of medicine in India for more than 2500 years. Since the time of Susruta (1000 B.C.), copper preparations, especially as copper oxide, have been used for oral medications (Gaur and Gupta, 1970; Sen et al., 1922). The potentiating effect of copper on human responses to cannabis has been known to Indian people for centuries. People of the different parts of India, who have experience with cannabis, believe that, if during the process of preparation of cannabis drinks, copper coins are rubbed in or if cannabis drinks are kept stored for some hours in a copper vessel, the intoxicating effect of cannabis is potentiated (Chopra and Chopra, 1957; Dube, 1972). Our questioning of cannabis users in different parts of the country has indicated that cannabis users, with personal experience regarding the potentiating effect of copper on cannabis action, believe this. The present report incorporates the results of copper-cannabis interaction in experimental animals. These interaction studies have been conducted using several pharmacological actions of cannabis as parameters, including the phenomenon of tolerance. In order to determine whether the effect of copper is peculiar to the metal or is also present in other bivalent metals, we also conducted a preliminary comparative study with zinc and magnesium.

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MATERIALS AND METHODS

Resin was extracted from the flowering tops of *Cannabis indica* with petroleum ether (60–80° C) and then suspended in 2% Tween-80 for experimentation. The delta-9-tetrahydrocannabinol (THC) content of the resin was biologically assayed by the four-point assay method, using pure THC and taking hypothermic activity in albino rats as the parameter. The THC content of the resin was estimated to be 17%.

Experiments were conducted on adult albino rats (125–200 g) and albino mice (15–20 g) of both sexes at an ambient temperature of 25 ± 1° C. All the experiments were conducted 'blind' to avoid subjective errors. Cannabis resin was administered orally. Our earlier studies (Singh and Das, 1975) showed that 50 mg/kg cannabis produces moderate hypothermia that peaks at 4 h. Therefore, in all the experiments on body temperature, and as well as in other experiments, the dosage of cannabis was 50 mg/kg and the temperature was recorded after 4 h. An equivalent volume of 2% Tween-80

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was administered to control animals. Copper was used as copper sulphate (BDH, Analar) dissolved in glass-distilled water. Preliminary experiments with 1, 5, 10, and 20 mg/kg of oral copper sulphate on the pentobarbitone hypnosis-potentiating activity of cannabis showed that 1 mg/kg was ineffective and that there were no significant differences among the effects of 5, 10, and 20 mg/kg. In order to check the toxicity of copper sulphate, rats were administered 5 mg/kg of oral copper sulphate each day for 15 days, there were no deaths. Copper sulphate was given in the dose of 5 mg/kg orally only once in most of the experiments, but in some experiments it was given twice. In some experiments it has been given intracerebroventricularly (i.c.v.) in the dose of 0.1 µg. To study copper-cannabis interactions, both these drugs were given simultaneously. The following experiments were conducted.

A. Effect of Copper on the Pharmacological Actions of Cannabis

1. Hypnotic Potentiation. Pentobarbitone sodium was administered in dosages of 25 and 40 mg/kg i.p. in albino rats and mice, respectively, 2 h after cannabis treatment. Sleeping time was recorded as the time between loss and recovery of the righting reflex.

2. Hypothermic Activity. The rectal temperatures of albino rats were recorded using a Telethermometer with the probe inserted 2 cm into the rectum. The temperature was recorded before and 2, 4, 6, and 24 h after cannabis treatment.

B. Interaction of Copper and Cannabis in Cannabis-Tolerant Rats

1. Effect on Pentobarbitone-Induced Hypnosis in Cannabis-Tolerant Rats. The method of studying the effect of cannabis on pentobarbitone-induced hypnosis was the same as that mentioned above. Twenty rats were allowed to develop tolerance to potentiation of pentobarbitone hypnosis by daily administration of 50 mg/kg of cannabis for 7 days. On the 8th day, half the rats were given cannabis alone and the other half were given cannabis along with copper sulphate.

2. Effect on Rectal Temperature. The method of studying the effect of cannabis on the body temperature of albino rats was the same as that described above. The following experiments were conducted:

a) Cannabis was administered daily for 28 days.
b) Cannabis was administered daily for 28 days, but on the first day cannabis was administered along with copper sulphate.
c) Cannabis was administered daily for 28 days, but on the 8th day cannabis was given along with copper, whereas on the 22nd day copper sulphate was given 2 h before cannabis.
d) Lateral cerebral ventricle was cannulated in a group of rats as described by Feldberg and Lotti (1967). These rats were given oral cannabis daily for 21 days and its effect on body temperature was recorded. On the 8th day 0.1 µg of copper sulphate was administered i.c.v.

3. Effect of Penicillamine on Hypothermic Activity of Cannabis. The method of studying hypothermic activity was the same as that described above. Three groups of 5 rats were used:

a) Cannabis only
b) Penicillamine 100 mg/kg i.p. only
c) Cannabis given orally and penicillamine 100 mg/kg i.p. given half an hour before temperature record.

4. Effect of Penicillamine on Copper-THC Interaction. In order to study the effect of i.c.v. copper on the hypothermic activity of THC, the lateral ventricles of a group of 5 rats were cannulated. These rats were given 10 mg/kg THC orally each day for 14 days except on the 10th day. On the 10th day 0.1 µg of copper sulphate was given i.c.v. To determine the effect of penicillamine on copper-THC interaction, 100 mg/kg of penicillamine was given i.p. on the 12th day.

C. Interaction of Zinc or Magnesium With Cannabis on Body Temperature

In these experiments zinc sulphate and magnesium sulphate 5 mg/kg were given orally and i.p., respectively. The following experiments were conducted.

1. Effect of Zinc and Magnesium on Cannabis Hypothermia. Zn or Mg was administered simultaneously with cannabis in nontolerant rats.

2. Effect of Zinc or Magnesium on Cannabis-Tolerant Rats. Cannabis administered daily for 22 days, but on the 10th and 17th days cannabis was given along with either zinc or magnesium sulphate.

RESULTS

Copper alone (5 mg/kg, orally) had no effect on any of the experimental parameters.

A. Effect of Copper on the Pharmacological Actions of Cannabis

1. Hypnotic Potentiation. Copper enhanced the pentobarbitone hypnosis-potentiating activity of cannabis (20 and 50 mg/kg) in albino rats and mice. The potenof cannabis activity by copper was, however, statistically significant only in albino rats that received 20 mg/kg of cannabis (Fig. 1).

2. Hypothermic Activity. Copper did not significantly modify the onset, peak, or duration of the hypothermic activity of cannabis (50 mg/kg). The peak fall in body temperature at 4 h in groups of rats (10 each) with cannabis alone and in combination with copper was 1.18 ± 0.12 and 1.30 ± 0.15 °C, respectively.

B. Interaction of Copper and Cannabis in Cannabis-Tolerant Rats

1. Effect on Pentobarbitone-Induced Hypnosis. The pentobarbitone sleeping time in the control rats was 51.3 ± 3.2 min. The sleeping time of fifteen nontolerant rats treated with cannabis and pentobarbitone was 117.5 ± 6 min, while in cannabis-tolerant rats (7 days cannabis treatment) the sleeping time after cannabis and pentobarbitone was 58.8 ± 3.6 min. When the tolerant rats were given cannabis, pentobarbitone, and copper, however, the sleeping time was 85.8 ± 12 min. Thus cannabis tolerance to the barbiturate hypnosis-potentiating activity was partially but significantly ($P < 0.05$) inhibited by copper treatment.

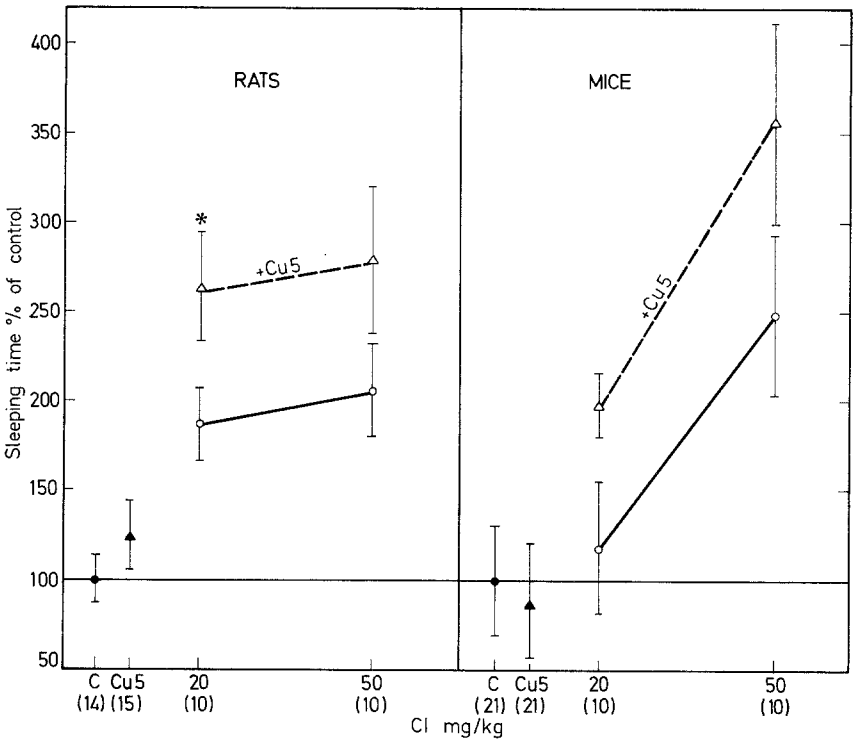

Fig. 1
Effect of copper sulphate 5 mg/kg (Cu 5) on cannabis resin (Cl) (20 and 50 mg/kg) induced pentobarbitone hypnosis potentiation in albino rats and mice. *Vertical lines:* S.E. Sleeping time of pentobarbitone-treated control (C) animals expressed as 100. *Parentheses:* number of animals used. *Stars:* significance (< 0.05) in relation to cannabis (20 mg/kg)

2. Effect of Copper on the Hypothermic Activity of Cannabis in Chronically Cannabis-Treated Rats. The effect of oral copper on the hypothermic activity of cannabis in 28-day cannabis-treated rats is summarised in Table 1. Following the daily administration of cannabis there was a rapid development of tolerance to the hypothermic activity of cannabis, which disappeared on the 5th day. From the 7th day onwards there was a slight rise in body temperature that gradually increased, but remained nearly stable from the 15th to 28th days. When copper was given along with cannabis on the first day, the hypothermic activity of cannabis disappeared on the 15th day and a slight hyperthermia was maintained until the 28th day. In the third group of rats cannabis was given daily for 7 days and tolerance was allowed to develop. On the 8th day, when copper was given along with cannabis, there was again significant hypothermia. On the subsequent days the hypothermic activity of cannabis gradually subsided and slight hyperthermia was seen on the 21st day. On the 22nd day, when cannabis was again given 2 h after copper administration, there was significant hypothermia, which gradually subsided on the subsequent days and disappeared on the 28th day. The study revealed the following:

Table 1. Effect of copper on chronic administration of cannabis (Cl) on body temperature in albino rats

Day	Change in rectal temp. °C at 4 h after Cl		
	Control ($N = 10$)	Cu given on 8th and 22nd day ($N = 6$)	Cu given on 1st day ($N = 6$)
			+ Cu
1	-0.88 ± 0.26	-0.88 ± 0.18	-0.88 ± 0.19
3	-0.58 ± 0.10	-0.67 ± 0.12	-0.67 ± 0.18
5	$+0.08 \pm 0.29$	-0.04 ± 0.15	-0.33 ± 0.22
7	$+0.29 \pm 0.23$	$+0.38 \pm 0.22$	-0.30 ± 0.19
		+ Cu	
8	$+0.30 \pm 0.18$	-0.60 ± 0.14**	–
10	$+0.33 \pm 0.19$	-0.29 ± 0.18*	-0.30 ± 0.12*
12	$+0.42 \pm 0.10$	-0.25 ± 0.13**	-0.19 ± 0.06**
15	$+0.58 \pm 0.12$	-0.20 ± 0.15**	$+0.19 \pm 0.12$*
19	$+0.50 \pm 0.23$	$+0.08 \pm 0.15$	$+0.38 \pm 0.16$
21	$+0.55 \pm 0.14$	$+0.35 \pm 0.17$	$+0.50 \pm 0.21$
		+ Cu	
22	$+0.55 \pm 0.18$	-0.65 ± 0.17**	–
24	$+0.69 \pm 0.12$	-0.35 ± 0.14**	$+0.38 \pm 0.16$
28	$+0.58 \pm 0.21$	-0.10 ± 0.07*	$+0.50 \pm 0.10$

* $P < 0.05$; ** $P < 0.01$; P in relation to control

a. The administration of a single dose of copper on the first day delays the development of tolerance to the hypothermic activity in rats chronically treated with cannabis.

b. In cannabis-tolerant rats the administration of a single dose of copper breaks the tolerance, and it takes another week to redevelop tolerance.

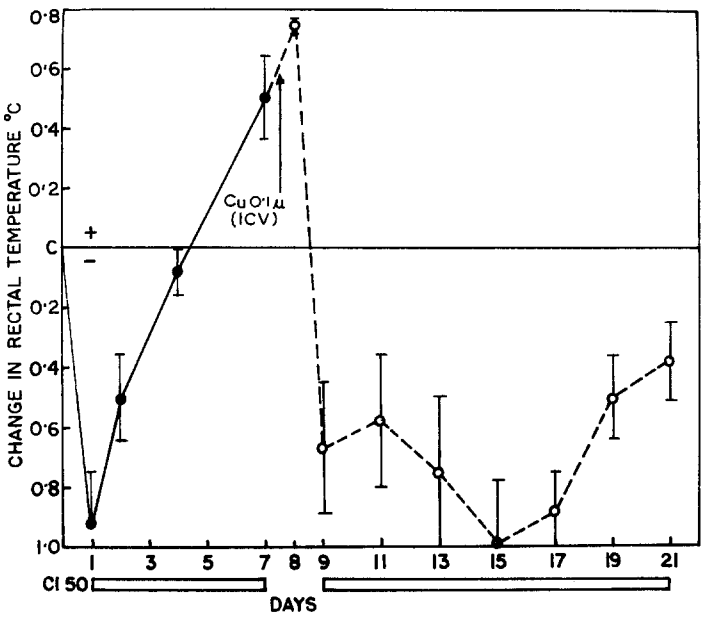

Fig. 2
Effect of daily administration of cannabis resin (CI) (50 mg/kg) on body temperature of albino rats except on 8th day, when copper sulphate (Cu 0.1 µg) was injected i.c.v. Treatment from 9th to 21st day is significant (< 0.001) against 7th day cannabis treatment

c. The effect of copper was found to be the same whether given in combination with cannabis resin or separately.

3. Effect of i.c.v. Copper on Cannabis-Tolerant Rats. The effect of i.c.v. copper on cannabis-tolerant rats is illustrated in Figure 2. Administration of i.c.v. copper produced a significant rise in body temperature ($+0.74 \pm 0.03°$ C). The hyperthermic effect disappeared after 24 h. Rats developed tolerance to the hypothermic activity of cannabis very rapidly and there was a hyperthermic response to cannabis on the 7th day. Following a single administration of i.c.v. copper there was a complete breakdown of tolerance and the hypothermic activity of cannabis was manifested for the following 13 days. During these 13 days, the hypothermic effect of cannabis was not significantly different from that of the first day for 11 days. Thus this study shows that i.c.v. copper can break down tolerance to the hypothermic activity of cannabis and prolong the effect.

4. Effect of Penicillamine on Hypothermic Activity of Cannabis. In order to elucidate whether chelation of endogenous copper affects the hypothermic activity of cannabis, interaction of cannabis with a copper chelating agent penicillamine was studied. Penicillamine alone produced significant hypothermia ($-0.88 \pm 0.10°$ C). But the hypothermic activity of cannabis-penicillamine treated rats ($-0.88 \pm 0.07°$ C) was not significantly different from that of cannabis (50 mg/kg) alone (-0.93 ± 0.05).

5. Interaction of i.c.v. Copper, Penicillamine, and THC on the Body Temperature of Albino Rats. The results are illustrated in Figure 3. Rats developed tolerance to the hypothermic activity of THC very rapidly, similar to that of cannabis resin. Administration of i.c.v. copper broke down THC tolerance, similar to that of cannabis resin. But subsequently, when THC was given with penicillamine, the hypothermic activity of THC disappeared and there was slight hyperthermia. On the following days, however, THC again produced significant hypothermia. These results show that penicillamine antagonises the activity of copper in breaking the tolerance to cannabis.

C. Interaction of Zinc or Magnesium With Cannabis on the Body Temperature of Albino Rats

1. Effect of Zinc or Magnesium on the Hypothermic Activity of Cannabis in Albino Rats. Zinc sulphate or magnesium sulphate did not produce any change in body temperature. Like copper sulphate, both these salts failed to significantly modify the hypothermic activity of cannabis.

2. Effect of Zinc or Magnesium on the Hypothermic Activity of Cannabis in Tolerant Rats. The effect of zinc sulphate or magnesium sulphate on the body temperature of cannabis-tolerant rats are given in Table 2. In the cannabis-tolerant rats the drug produced slight hyperthermia on the 9th day. On the 10th day, when cannabis was administered with $ZnSO_4$, there was significant hypothermia similar to that seen on the first day of cannabis administration. On the following days the hypothermic activity of the cannabis gradually subsided and disappeared on the 15th day. There was again slight hyperthermia on the 16th day. When cannabis was given along with Zn on the 17th day,

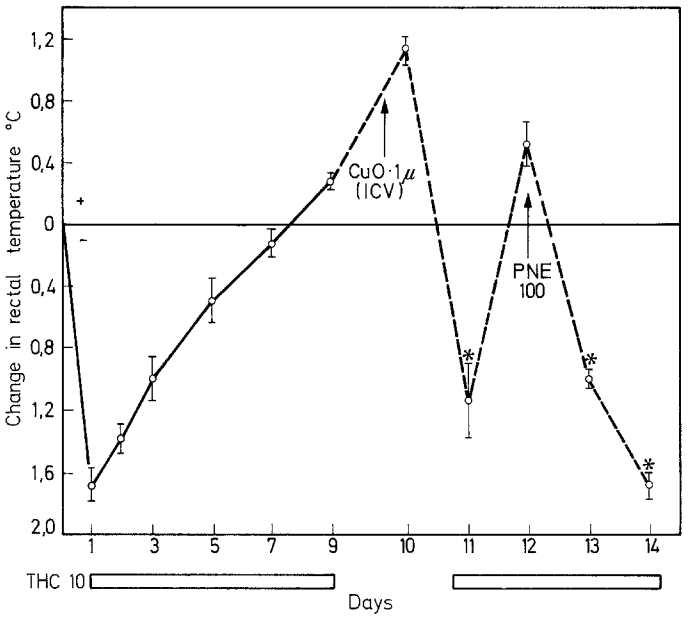

Fig. 3
Effect of daily administration of delta-9-THC (10 mg/kg) on body temperature of albino rats except on 10th day, when copper sulphate (Cu 0.1 μg) was injected i.c.v. On the 12th day penicillamine (PNE) (100 mg/kg) was also administered i.p. *Stars:* significance (< 0.001) against 9th day THC treatment

there was significant hypothermia, and on subsequent days the hypothermic activity of cannabis gradually subsided in a manner similar to that seen earlier.

In the second group of animals tolerance developed to the hypothermic activity of cannabis as usual, with slight hyperthermia on the 9th day. On the 10th day, when cannabis was given with Mg, there was a slight increase in the hyperthermic activity of cannabis, which was, however, not seen on subsequent days. From the 11th to 16th days cannabis manifested the usual hyperthermic response. On the 17th day, when cannabis was again given along with Mg, there was a slight increase in hyperthermic response. On the following days the normal cannabis activity was maintained.

A comparison of the effects of Cu, Zn, and Mg (Fig. 4) in cannabis-tolerant rats shows similarities between Cu and Zn, with Mg being different. Copper and zinc both converted the hyperthermic effect of cannabis into hypothermic response in tolerant rats. The effects of a single-dose administration of either of these metals lasted for several days. In the case of copper, however, it took 11 days for the effect to disappear, while in the case of Zn it took 6 days to disappear. Magnesium had no effect on the hypothermic activity of cannabis in tolerant rats, but it slightly increased the hyperthermic activity of cannabis on the day of its administration.

Table 2. Effect of zinc and magnesium on short-term administration of cannabis (CI) on body temperature in albino rats

Day	Change in rectal temp. °C at 4 h after CI	
	Zn given on 10th and 17th day ($N = 5$)	Mg given on 10th and 17th day ($N = 5$)
1	-1.13 ± 0.23	-0.96 ± 0.12
3	-0.81 ± 0.12	-0.69 ± 0.16
5	-0.13 ± 0.16	-0.19 ± 0.12
7	$+0.25 \pm 0.10$	$+0.13 \pm 0.07$
9	$+0.44 \pm 0.06$	$+0.38 \pm 0.07$
	+ Zn	+ Mg
10	-0.94 ± 0.21 **	$+0.56 \pm 0.06$
11	-0.75 ± 0.10 **	$+0.38 \pm 0.07$
13	-0.50 ± 0.10 **	$+0.38 \pm 0.07$
15	-0.06 ± 0.12 *	$+0.44 \pm 0.06$
16	$+0.38 \pm 0.07$	$+0.44 \pm 0.06$
	+ Zn	+ Mg
17	-0.88 ± 0.16 **	$+0.58 \pm 0.08$
18	-0.56 ± 0.12 **	$+0.50 \pm 0$
20	-0.19 ± 0.12 *	$+0.42 \pm 0.08$
22	$+0.25 \pm 0.08$	$+0.50 \pm 0.06$

* $P < 0.01$; ** $P < 0.001$; P in relation to 9th day CI treatment

DISCUSSION

In the present study selected biological actions of orally administered copper sulphate were studied in albino rats and mice with special reference to those parameters used for cannabis activity. In albino rats, 5 mg/kg of copper sulphate given orally each day for even 15 days was not found to be toxic. In most of the experiments, however, copper was administered as copper sulphate only once. Copper per se had no effect on any of the experimental parameters. So it appears that copper alone has no neuropharmacological activity, possibly because of the fact that a sufficient amount of copper is

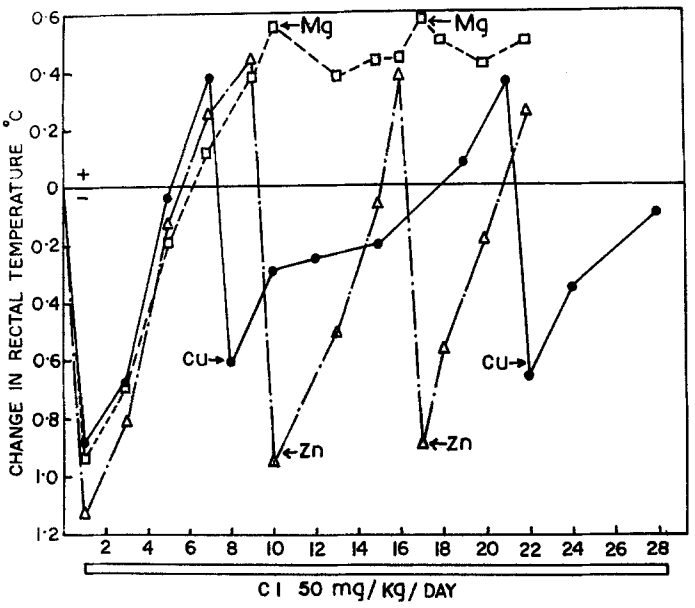

Fig. 4
The effect of daily administration of cannabis resin (CI) on body temperature of albino rats. *Solid dots:* rats given copper sulphate (Cu) (5 mg/kg) orally on 8th and 22nd day. *Triangles:* rats given zinc sulphate (Zn) (5 mg/kg) orally on 10th and 17th days. *Squares:* rats given magnesium sulphate (Mg) (5 mg/kg) i.p. on 10th and 17th days

present in the body for optimal enzymatic reactions whenever copper is needed.

Although copper did not have any neuropharmacological activity, it did interact with cannabis. In this study the parameters of neuropharmacological activity and the doses of cannabis resin used are the same as those reported earlier (Singh and Das, 1975; 1978). Copper potentiated the enhancement of barbiturate hypnosis by cannabis, but had little effect on the hypothermic action of cannabis.

In the next part of the study the effect of copper was studied in cannabis-tolerant rats. Because canabis is known to produce tolerance in several species of animals, including man, it was thought worthwhile to explore the possibility of cannabis-copper interaction during tolerance, especially because of the above-mentioned belief of Indian cannabis users. In an earlier study we reported that tolerance to hypothermic, analgesic, and barbiturate hypnosis-potentiating activities of cannabis develops very rapidly and is complete within 7 days, and that it takes 4 weeks or more for tolerance to disappear (Singh and Das, 1977). Copper partially antagonised tolerance to the barbiturate hypnosis-potentiating activity of cannabis in albino rats. It is interesting that, although copper per se did not affect barbiturate hypnosis, it did enhance the barbiturate-potentiating action of cannabis and also partially antagonised tolerance development.

The most interesting copper-cannabis interaction was seen in tolerant rats when body temperature was taken as a parameter. There were three interesting results: (1) although copper did not modify the hypothermic activity of cannabis in nontolerant rats, a single dose of copper on the first day significantly delayed the development of tolerance by at least a week; (2) in cannabis-tolerant rats a single dose of copper completely broke the tolerance, and it took another week for tolerance to redevelop; and (3) cannabis-copper interaction was not due to any physiochemical complex formation, because of the reproducibility of interaction even when these were given separately and also because of the long-lasting effect of copper (several days). In order to study the anatomical site of action of copper, i.e., whether it is peripheral or central, copper was given i.c.v. in cannabis-tolerant rats. Qualitatively similar results were observed with i.c.v. administration, indicating that the site of action of copper was probably in the paraventricular zone of hypothalamus. The results show that following oral administration of copper, a significant amount of copper is absorbed, and despite protein binding of copper in the blood, effective amounts of copper are taken up by the hypothalamus. The disposal of copper seems to be a slow process that takes at least a week. But following i.c.v. administration more copper becomes localised and the disappearance of copper is extremely slow, taking a period of more than 2 weeks. The results of the interaction between i.c.v. copper and oral cannabis were confirmed with i.c.v. copper and THC, which indicated interaction between copper and THC.

The copper-cannabis interaction raised the possibility that endogenous copper may be necessary for the hypothermic activity of cannabis. In order to test this possibility, the effect of the copper chelating agent penicillamine was studied on the hypothermic activity of cannabis in nontolerant rats. Penicillamine did not affect the hypothermic action of cannabis, indicating that at least free copper ions are not essential for the

hypothermic response of cannabis. In order to study the role of copper in cannabis-tolerant rats, pencillamine was again used. In THC-tolerant rats the administration of i.c.v. copper reproduced the hypothermic action of THC. The administration of oral penicillamine reversed the hypothermic effect of THC to hyperthermic response that is normally seen in tolerant rats. On the subsequent day, however, THC again produced hypothermia. These results indicate that penicillamine antagonised the copper-THC interaction in tolerant rats. It may be concluded that the free form of the copper and not the bound copper was responsible for antagonising the development of tolerance to cannabis.

In order to find out whether the effect of copper was specific or present in other divalent metallic ions, the effects of copper were compared with those of zinc and magnesium. The effect of zinc on cannabis tolerance to hypothermic activity was found to be qualitatively similar to that of copper. But in equivalent dosages zinc was less potent, especially in its duration of action. Magnesium had no effect in cannabis-tolerant rats. On the other hand, on the day magnesium was administered there was a slight increase in the hyperthermic activity of cannabis. Thus it seems that the action of copper is similar to that of zinc but not to that of magnesium.

Copper has no place as a medicinal agent for internal use in modern medicine, but copper is known to be an important trace metal, the deficiency of which is probably extremely rare. In a study of the presence of different metals in various regions of rat brain, Rajan et al. (1976) have shown that relatively high concentrations of copper and zinc are present in the hypothalamus, especially in the myelin and synaptosomes, suggesting a specific role for these metals. Copper plays an important role in several metal-activated enzymes or metallo-enzymes. It has been shown that copper and zinc catalyse decarboxylation (Steinberger and Westheimer, 1949; Lehninger, 1950). Dopamine-beta-hydroxylase (DBH) is a copper-containing enzyme (Molinoff et al., 1975), and copper probably also acts as a catalyst (Goldstein et al., 1968). The recent studies of Molinoff and Orcutt (1973) and Molinoff et al. (1975) show that DBH is inhibited by heterogenous inhibitors present in the biological system, including the adrenergic neurons. Copper binds the DBH inhibitors, thus allowing full expression of DBH activity. The inhibition of DBH activity by disulfiram and tropolone derivatives is related to copper chelation. Among the other enzymes associated with the noradrenergic system, the tyrosine hydroxylase activity partially depends on magnesium (Lovenberg et al., 1975) and phenylethanolamine-N-methyl-transferase is inhibited by copper (Kitabchi and Williams, 1969), but the role of copper in MAO activity is controversial (Costa and Neff, 1970).

It has been shown that the hypothermic activity of cannabis is mediated through the noradrenergic system (Singh and Das, 1976). Subsequently, was shown that the hyperthermic response of cannabis in tolerant rats is also mediated through the noradrenergic system in albino rats (Singh and Das, unpublished). It was proposed that cannabis initially produces hypothermia because of a marked release of noradrenaline, but with the development of tolerance, the release of noradrenaline subsides, resulting in a rise in body temperature in albino rats. The absence of any significant effect of copper per se indicates that in normal animals an adequate amount of copper is available for optimal function. The failure of copper to interact with single administration of cannabis, hypothermia being the parameter, also suggests that copper does not facilitate release of NA induced by cannabis. But copper facilitates cannabis/THC activity, and especially their effects on the body temperature of tolerant rats. In cannabis-tolerant rats it is possible that there is either an enzyme blockade at the level of dopa-decarboxylase or DBH or an increase in the concentration of DBH inhibitors. In either of these circumstances exogenous copper might restore enzyme activity and thus cannabis activity. The studies also show that the action of zinc, but not that of magnesium, is similar to that of copper. The availability of relatively high amounts of copper and zinc in the hypothalamus, and especially in the synaptosomes, strongly suggests that the action of these metals on cannabis activity on body temperature in tolerant rats is related to the noradrenergic system.

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REFERENCES

Chopra, I. C., Chopra, R. N.: The use of cannabis drugs in India. Bull. Narc. **9**, 4–29 (1957)

Costa, E., Neff, N. H.: Estimation of turnover rates to study the metabolic regulation of the steady state level of neuronal monoamines. In: Handbook of neurochemistry, vol. IV, A. Lajtha, ed., p. 45. London: Plenum Press 1970

Dube, K. C.: Manner of intake, different preparations, frequency, dose, etc. I.C.M.R. seminar on long-term effect of cannabis use in India, New Delhi, December 1972

Feldberg, W., Lotti, V. J.: Temperature responses to monoamines and an inhibitor of MAO injected into the cerebral ventricles of rats. Br. J. Pharmacol. **31**, 152–161 (1967)

Gaur, D. S., Gupta, L. P.: A study on drug evaluation in ancient India. In: Advances in research in Indian Medicine, K. N. Udupa, G. N. Chaturvedi, and S. N. Chaturvedi, eds., p. 357. Varanasi: B.H.U. Press 1970

Goldstein, M., Joh, T. H., Garvey, T. Q.: Biochemistry 7 [pp. 2724–2730, 1968]. Quoted in: Molinoff, P. B., Axelrod, J.: Biochemistry of catecholamines. Annu. Rev. Biochem. **40**, 465–500 (1968)

Kitabchi, A. E., Williams, R. H.: Phenylethanolamine-N-methyl transferase in human adrenal gland. Biochim. Biophys. Acta **178**, 181–184 (1969)

Lehninger, A. L.: Role of metal ions in enzyme systems. Physiol. Rev. **30**, 393–429 (1950)

Lovenberg, W., Bruckwick, E., Hanbauer, I.: Protein phosphorylation and regulation of catecholamine synthesis. In: Chemical tool in catecholamine research II, O. Almgren, A. Carlsson, and J. Angel, eds., p. 37. Amsterdam: North Holland Publishing Co. 1975

Molinoff, P. B., Nelson, D. L., Orcutt, J. C., Harden, T. K.: The regulation of dopamine-β-hydroxylase activity. In: Chemical tool in catecholamine research II, O. Almgren, A. Carlsson, and J. Angel, eds., p. 81. Amsterdam: North Holland Publishing Co. 1975

Molinoff, P. B., Orcutt, J. C.: Dopamine betahydroxylase and the regulation of catecholamine biosynthesis. Frontiers in catecholamine research, p. 195. London: Pergamon Press 1973

Rajan, K. S., Colburn, R. W., Davis, J. M.: Distribution of metal ions in the subcellular fractions of several rat brain areas. Life Sci. **18**, 423–432 (1976)

Sen, K. B., Sen, K. A., Sen, K. P.: Copper In: Datta's Materia medica of Hindus. Calcutta: Adi-Ayurveda Machine Press 1922

Singh, P. P., Das, P. K.: A neuro-psychopharmacological study of *Canabis indica*. Ind. J. Pharmacol. **7**, 51–57 (1975)

Singh, P. P., Das, P. K.: Role of catecholamines in the hypothermic activity of cannabis in albino rats. Psychopharmacology (Berl.) **50**, 199–204 (1976)

Singh, P. P., Das, P. K.: Tolerance to cannabis in albino rats. Ind. J. Exp. Biol. **15**, 280–284 (1977)

Singh, P. P., Das, P. K.: Effect of *Cannabis indica* on locomotor activity. Ind. J. Ex. Biol. (in press, 1978)

Steinberger, R., Westheimer, F. H.: Metal-ion catalyzed decarboxylation dimethyloxaloacetic acid. J. Am. Chem. Soc. **71**, 4158 (1949)

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