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The Behavioral Effects of the "Lactone-free" Hot Water Extract of Catnip (*Nepeta cataria*) on the Young Chick

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ABSTRACT

The "lactone-free" hot water extract of catnip (*Nepeta cataria* L.) causes a significant decrease in wakefulness and an increase in sleep behavior, particularly deep sleep.

Catnip (*Nepeta cataria* L.) potentially contains at least three active agents: 1) components of the essential oil, especially nepetalactone (Waller et al., 1969) and its close chemical relatives, epinepetalactone (Regnier et al., 1967) or dihydronepetalactone (Wolinsky and Eustace, 1972), which cause species specific behaviors in cats (Hatch, 1972; Leyhausen, 1973) and potentially act as a mild hallucinogen in humans (Jackson and Reed, 1969); 2) components of the hot water extract ("tea"), that are used as a remedy for nervous, gastric and respiratory problems in humans (Lust, 1974) and cause a significant increase in sleep behavior in chicks (Sherry and Koontz, 1979) and mice (Sherry and Hicks, 1982); 3) components of catnip roots, which act as a stimulant (Hutchens, 1973). Unfortunately, there are no reports in the literature about the chemical constituents of the hot water extract of catnip or catnip roots. The lactones (nepetalactone, etc.) apparently possess pharmacological activity and they might be present in small amounts in the hot water extract. If they are present, they might be the active agent or they might act in a additive or subtractive manner with the active agent(s). We decided to test this hypothesis by preparing a "lactone-free" sample of catnip by repeatedly extracting catnip with hexane. We then used the "lactone-free" hexane extracted catnip to prepare a hot water extract for screening for pharmacological activity. Since the young chicks respond in a sterotyped manner to other catnip preparations (Sherry and Hunter, 1979; Sherry and Koontz, 1979; Sherry et al., 1981), we decided to use the preparation as our model.

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METHODS

Male white Leghorn chickens were obtained at one day of age from the Kazmeier Hatchery (Bryan, TX) and housed in temperature-controlled brooders, with food and water available ad libitum. The "lactone-free" catnip was prepared by extracting 50 g of dried catnip (Meer Corp., North Bergen, N.J., Lot #36-82220) with 350 ml of hexane. The mixture was sonicated for 15 mins and then periodically shaken and allowed to steep in a closed container for approximately 24 hours. The mixture was then filtered under vacuum and the residue was re-extracted (2 times). On the third extraction, the residue was filtered under vacuum to dryness. This residue was used to prepare the "lactone-free" hot water extract by pouring boiling water (350 ml) over the catnip, sonicating it for 15 mins and allowing the mixture to steep for 24 hours. The mixture was filtered under vacuum and re-extracted (2 times). The extracts were combined and the water was evaporated using a Rotavapor. Weighed samples (400, 600, 800 and 1000 mg) of the "lactone-free" hot water extract were added to distilled water and the volume adjusted to 10 ml. This allowed a dose level of 0.01 ml/g of body weight. All drugs were administered intraperitoneally. The control chicks were injected with the same volume of distilled water. Groups of 12 chicks were injected at each dose level and immediately after injection, each chick was placed in a standard galvanized steel mouse cage, one animal per cage, and closely observed for two hours. During this time period, at thirty second intervals, each chick was observed and placed in one of the following categories which best described the behavior of the chick: 1) wakefulness (the chick moved about the bottom of the cage and chirped); 2) quiet wakefulness (the chick sat quietly in one position, with eyes open); 3) light sleep (the chick sat or stood quietly, without peeping, with eyes closed and head up); 4) deep sleep (the chick sat down without moving or peeping, with eyes closed and head down). Since we lack the standard neurophysiological correlates of sleep, we are using these terms for the convenience of discussion, to describe the overt behavior of the chick and not to define a specific physiological state.

All statistical evaluations utilized the Kruskall-Wallis one-way analysis of variance test and where appropriate, Nemenyi's procedure was used for the multiple comparisons tests (Kirk, 1968). All comparisons were made at the 0.05 confidence level. The total effectiveness of the extract was determined:

$$TE = 0 \cdot S_1 + 1 \cdot S_2 + 2 \cdot S_3 + 3 \cdot S_4$$

where: S_1 is the percent time the chick showed wakefulness; S_2 is the percent time the chick showed quiet wakefulness, etc., thus the total effectiveness score for an individual chick could range from 0, where the chick spent all of his time in wakefulness to 300, where the chick spent all of his time in deep sleep.

RESULTS

The average percent-time spent in each of the behavioral states is shown in Table 1. The first number in each pair is the mean percent time, the number immediately below it, its standard deviation. The overall Kruskall-Wallis score ("U") is shown at the bottom of each column, while the number immediately below it is its confidence level. The arrows indicate a significant difference when compared to the controls. A downward arrow indicates a significant decrease in the duration of the behavior, compared to the controls, while an upward arrow, indicates an increase. Clearly, the "lactone-free" hot water extract causes a significant decrease in wakefulness and a significant increase in sleep behavior, particularly deep sleep. The total effectiveness (i.e. the decrease in wakefulness coupled with an increase in sleep) of the extract is significant and tends to be dose-dependent.

Table 1. The effect of the "lactone-free" hot water extract on the behavior of the young chick. The first number in each pair is the average percent time spent in each behavioral state and the number immediately below it in parentheses, its standard deviation.

Dose level	Awake	Quiet wakefulness	Light sleep	Deep sleep	Total effectiveness
Control	65.41 (24.79)	15.77 (7.02)	18.17 (24.73)	0.63 (1.48)	54.03 (51.78)
400 mg/kg	15.54 ↓ (11.85) ↓	5.16 (4.75)	62.99 ↑ (20.93) ↑	16.31 (23.57)	180.08 ↑ (44.38) ↑
600 mg/kg	19.08 ↓ (23.44) ↓	10.25 (8.36)	35.46 (13.13)	34.31 ↑ (24.89) ↑	187.45 ↑ (78.55) ↑
800 mg/kg	14.25 ↓ (28.48) ↓	4.17 (2.21)	30.29 (16.33)	51.30 ↑ (21.10) ↑	218.62 ↑ (74.65) ↑
1000 mg/kg	5.29 ↓ (5.32) ↓	7.32 (5.53)	48.46 (9.91)	38.79 ↑ (15.46) ↑	220.90 ↑ (28.28) ↑
"U" Statistic Confidence Interval	22.62 0.0002	19.95 0.0005	27.79 0.0001	29.02 0.0001	27.10 0.001

DISCUSSION

We have recently demonstrated that the hot water extract of catnip causes a significant decrease in wakefulness and an increase in light sleep in the young chick (9 ± 5 days), but was less effective in older chicks (25 ± 7 days). The relative effectiveness of the hot water extract in the young and older chick (Sherry and Koontz, 1979) might be due to the closure of the blood-brain

barrier in the older chick. This seems to be partially confirmed by the fact that a relatively higher dose was required to be effective in the adult mouse, which has a closed blood brain barrier (Sherry and Hicks, 1982). On the other hand, the lactone-free hot water extract caused a significant decrease in wakefulness and an increase in both light and deep sleep in the young chick. This suggests that the hexane extraction potentially either removed an active agent that blocked the development of deep sleep or "enhanced" the hot water extraction of an active agent(s) that caused an increase in deep sleep.

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