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Note

Effect of Cocoa Tea (*Camellia ptilophylla*) Co-Administrated with Green Tea on Ambulatory Behaviors

Rong-rong HE,[1,2] Guo XIE,[1,3] Xin-Sheng YAO,[1,2] and Hiroshi KURIHARA[1,†]

[1]*Institute of Traditional Chinese Medicine and Natural Products, Jinan University, Guangzhou 510632, China*
[2]*School of Traditional Chinese Materia Medicine, Shengyang Pharmaceutical University, Shengyang 110016, China*
[3]*Institutes of Zhongshan, University of Electronic Science and Technology of China, Zhongshan 528402, China*

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We investigated the effects of cocoa tea and its main active compound theobromine on ambulatory activity, compared with green tea and caffeine. Although cocoa tea and theobromine themselves didn't change the ambulatory behaviors as green tea and caffeine did, combined administration with green tea or caffeine showed a synergistic action. The obtained data are perhaps contribution to the consumption of cocoa tea in the world.

Key words: cocoa tea; *Camellia ptilophylla*; theobromine; ambulatory activity

A naturally decaffeinated tea plant *Camellia ptilophylla*, which was named as cocoa tea, was discovered in 1988, and this is an endemic tree growing on cloudy and foggy highlands in the Longmen area of southern China.[1)] The leaves of cocoa tea have been consumed as a daily beverage in this region for a long time, and are known to have beneficial effects on human health and used as a traditional remedy for ailments, the enhancement of mental efficiency, and recovery from mental fatigue. Recently, it was found that theobromine, the precursor of caffeine, was accumulated in cocoa tea plants, and the reason is that these plants might be deficient in caffeine synthase, which acts on theobromine.[2)] Theobromine is an alkaloid in tea or cocoa beans and has psychoactive properties. Its effects may be to some extent similar to but much smaller than those of caffeine.[3)] It is also known that all of these methylxanthines act as antagonists of adenosine receptors, albeit with somewhat different affinities.[4)] Some results suggest that, although theobromine showed central stimulant effect, the characteristics are different from those of caffeine.[5)] However, to our knowledge, no other research has been conducted on the influence on behavior of cocoa tea that contains theobromine but little caffeine. Therefore, we evaluated the effect of cocoa tea on ambulatory activity in mice.

Leaves of cocoa tea and green tea were obtained, in April, from plants growing in a natural field at the highlands in the Longmen area of Guangdong province, southern China. Dry leaves (100 g) were extracted with 2,000 ml of hot water for 10 min at 90 °C. After filtration and evaporation, the residue was powdered under frozen-decompression conditions. The extraction ratio of cocoa tea was 21.2% and green tea was 23.7% in the same schedule. The concentrations of alkaloids and catchines in the cocoa tea and green tea were analyzed by HPLC with UV detection at 280 nm,[6)] and the results are shown in Table 1. Seven weeks old male ICR mice were purchased from the Center of Laboratory Animal Science Research of Southern Medical University (Guangdong, China). Animals were kept at constant temperature and humidity, with a 12-h light/dark cycle (lights on at 7:00 A.M.) under dim white light (about 15 lux). The care and treatment were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the United States National Institutes of Health. The data are presented as mean ± S.E.M. One-way analysis of variance (ANOVA) was applied to analyze difference in data of biochemical parameters among the experimental groups, followed by Dennett's test for pair-wise multiple comparisons. Differences were considered as statistically significant at $P < 0.05$.

The ambulatory activity of each mouse was measured by using the ambulometer, Model AMB-M1 (OHC O'hara, Tokyo). Each mouse was placed in the 30 cm of diameter and 28 cm of depth round plexiglas cage of the ambulometer, and counting of horizontal movements of

Table 1. Quantitative Analysis of Alkaloids and Catchines in Cocoa Tea and Green Tea

Components	Cocoa tea (mg/g powdered extract)	Green tea (mg/g powdered extract)
Theobromine	278.8	19.0
Caffeine	0.01	272.0
Gallocatechin gallate	199.0	5.7
Epigallocatechin	43.3	89.0
Catechin	62.8	204.1
Epicatechin	14.5	70.8
Epigallocatechin gallate	37.3	127.0
Epicatechin gallate	31.5	123.8

The concentrations of theobromine, caffeine and catchines in the cocoa tea and green tea extract were analyzed by high-performance liquid chromatography (HPLC) with UV detection at 280 nm. The analysis was performed with a Cosmosil 5PE-MS column (4.6 mm × 150 mm) at 40 °C. Compounds were eluted (eluent A: 0.05% trifluoroacetic acid in water; eluent B: 0.05% trifluoroacetic acid in acetonitrile) at a flow rate of 2 ml/min using a gradient program (eluent B content: 10% for 5 min, 21% for 8 min, 90% for 1 min, and 90% for 6 min). The quantification of alkaloids and catchines was performed using standard calibration curves. Data are average values for three experiments.

† To whom correspondence should be addressed. Tel/Fax: +86-20-85221559; E-mail: Hiroshi_Kurihara@163.com

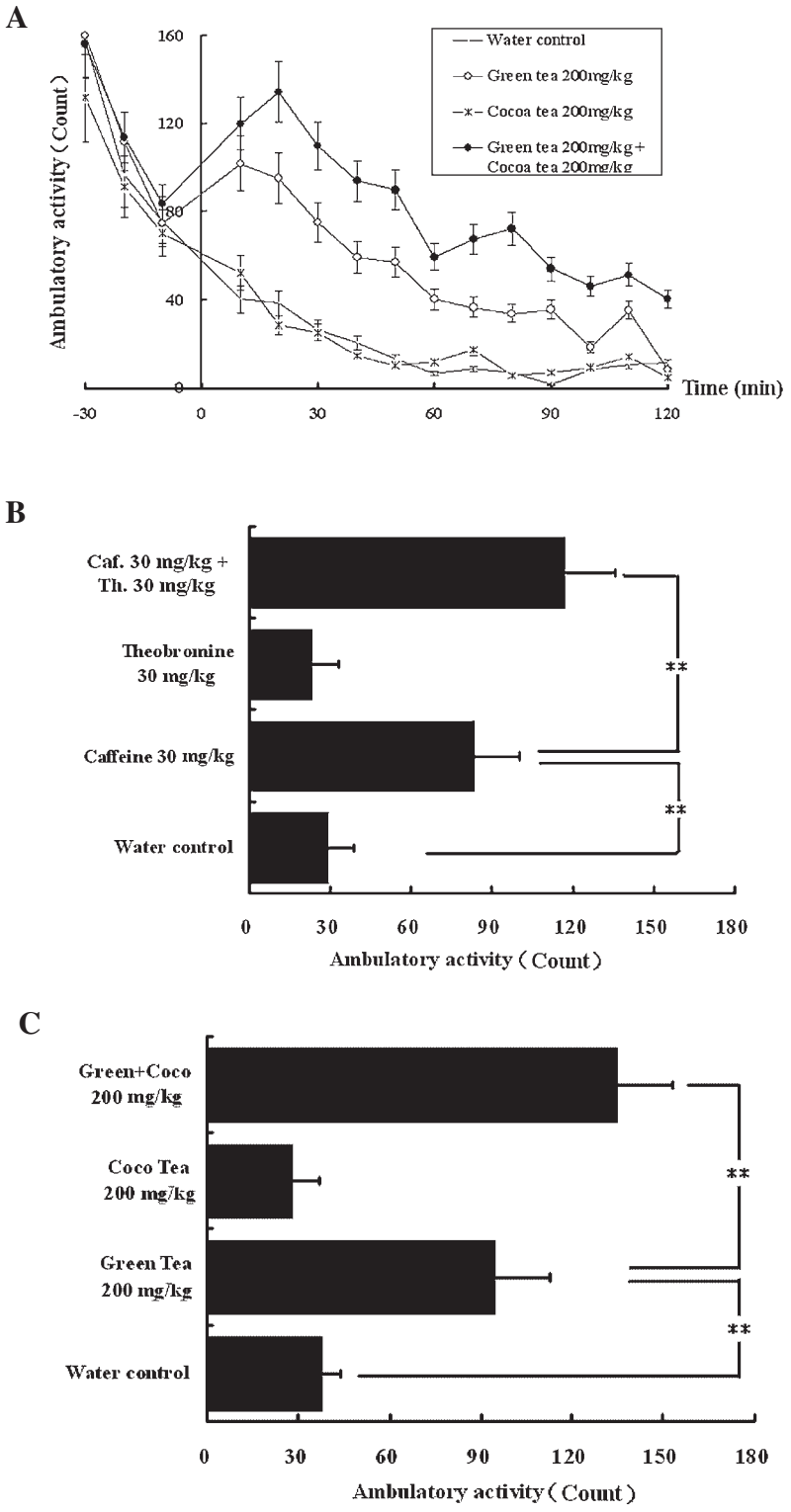

Fig. 1. Effects of Cocoa Tea and Its Active Constituent Theobromine on Ambulatory Activity.

A, Effects of administration of cocoa tea extract as well as interaction between cocoa tea and green tea on ambulatory activity over time for 2 h. B, Effects of administration of cocoa tea extract as well as interaction between cocoa tea and green tea on ambulatory activity over time during the 10 min period between 10–20 min. C, Effects of administration of theobromine as well as interaction between theobromine and caffeine on ambulatory activity during the 10 min period between 10–20 min. The results represent the mean ± S.E.M. for 10 mice. Differences were considered as statistically significant at $^{**}P < 0.01$.

the mouse inside the cage was automatically detected and counted by on-off of three external microswitches placed very close to the cage. The number of horizontal movements was printed out every 10 min for recording of locomotion activity. On the experimental day, mice were randomly assigned to each group in 10 animals and were placed in the locomotion arena. After a 30 min recovery period, a test sample was orally administered to each mouse, and then their locomotion was measured for 2 h in the locomotion arena. Samples were dissolved in water before use, and the solution was orally administered to animals at 0.1 ml/10 g of body weight.

As show in Fig. 1A, the single oral administration of 200 mg/kg of green tea extract increased the mouse's

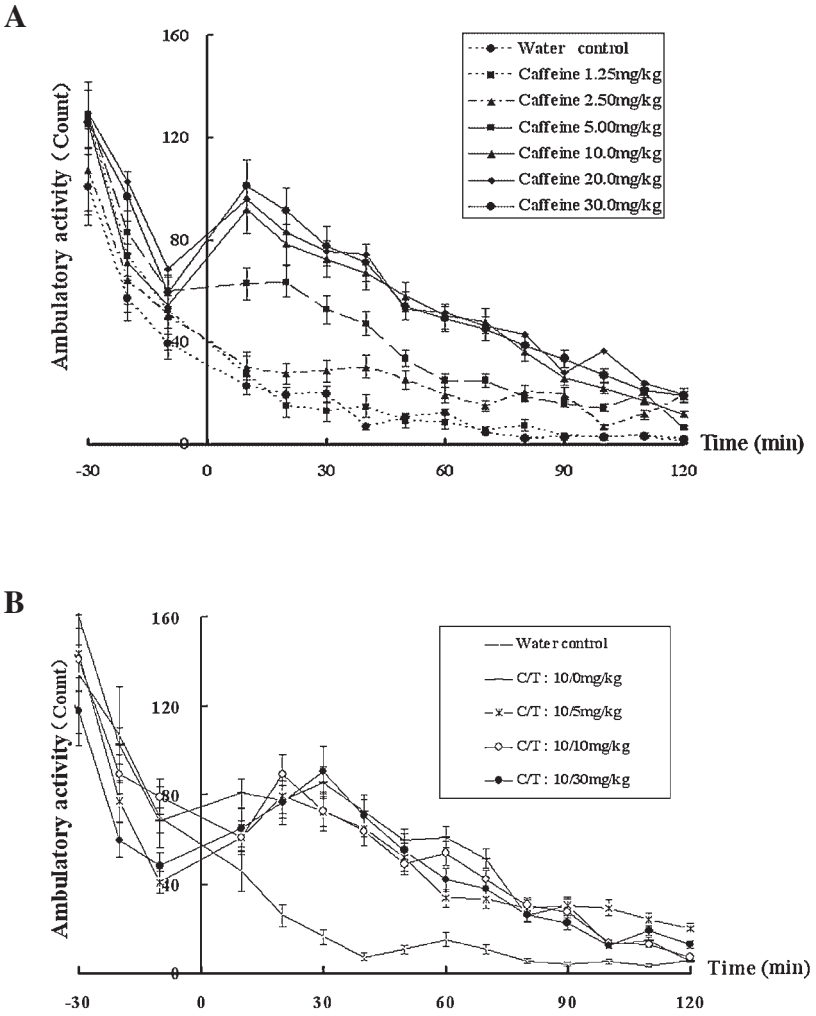

Fig. 2. Effects of Caffeine and Interaction between Caffeine and Theobromine on Ambulatory Activity.
A, Effects of coffeine treatment which led to marked increase of the ambulatory reaction within the 10 to 30 mg/kg dose range. B, Effects of co-administration with 10 mg/kg caffeine and theobromine within the 5 to 30 mg/kg dose range on ambulatory activity in mice. The results represent the mean ± S.E.M. for 10 mice.

ambulatory activity and the effect reached the maximum level at 20 min and persisted for 2 h after administration. Although cocoa tea extract did not show this activity, the combined administration of cocoa tea and green tea increased the activity compared with the activity of the green tea extract ($P < 0.01$) (Fig. 1B). The synergistic effect of cocoa tea extract is possibly due to theobromine, which behaves as its main active constituent (as seen in Fig. 1C). Although theobromine itself did not change mouse ambulation at 30 mg/kg, theobromine enhanced the ambulation activity of caffeine at 20 min after combined administration ($P < 0.01$) (Fig. 1C). Caffeine is well known as a central nervous system stimulant, and the effect could be related to blockade of adenosine receptors.[7] Furthermore, there is evidence that caffeine can directly and indirectly influence GABAergic neurotransmission, and enhance the turnover of several monoamine neurotransmitters, including 5-hydroxytryptamine, dopamine, and noradrenaline.[8] Dopamine and noradrenaline neurons are involved in arousal and ambulation activity,[9] and thus, caffeine may enhance the mouse locomotion activity. The ambulatory reaction to caffeine was in a dose-dependent manner at the dose range of 1.25 to 10 mg/kg, but in the same level at the dose range of 10 to 30 mg/kg (Fig. 2A). Therefore, 10 mg/kg was an optimum dose for studying the interaction of caffeine and theobromine by observing the ambulatory activity. However, as shown in Fig. 2B, theobromine did not make any significant change in the response at the dose range of 5–30 mg/kg to the ambulation-increasing effect of 10 mg/kg caffeine. Thus, it seems that theobromine may increase locomotion also through other mechanism, probably indirectly inducing up-regulation of adenosinergic systems.[10] Some reports indicate that passage through the blood-brain barrier is considerably more efficient for caffeine than its metabolites.[11] Recent work has also emphasized an important role of striatal adenosine receptors as a target of action for physiologically relevant concentrations of caffeine.[12] Generally, all methylxanthines act as antagonists at adenosine receptors, albeit with somewhat different affinities.[11] The ability often is cited to explain their effects, but in theory stronger evidence is required. Although theobromine showed central stimulant effects, our results indicate baseline behavioral scores in the locomotion activity test between caffeine and theobromine, which suggest that there are differences between cocoa tea containing theobromine and green tea beverage in the central nervous system activity.

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