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Effects of Guilingji on brain monoamines and their metabolites in mice.

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Abstract

Guillingji powder, a complex prescription of traditional Chinese medicines, has been pharmacologically shown to be a possible anti-aging agent, and also used for the treatment of male hypogonadism and female dysgenesis. Since the levels of numerous brain monoamines and their metabolites such as norepinephrine (NE), dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-HT), and 5-hydroxyindole acetic acid (5-HIAA) have been correlated with aging, we examined the effects of Guilingji powder and its extract on such monoamines and metabolites in the cerebrum and the cerebellum of mice using HPLC-ECD. The results showed that dietary intake of Guilingji powder had the effect of increasing the NE level and the DOPAC/DA ratio, and decreasing the 5-HT level and 5-HT/NE ratio in the cerebrum. In the cerebellum, the powder did not produce any changes in the levels of the compounds investigated. The extract of Guilingji, on the other hand, not only increased the NE level and the DOPAC/DA ratio but also the 5-HIAA/5-HT ratio in the cerebrum. However, the extract did not decrease the 5-HT in the cerebrum, although it decreased the 5-HT/NE. Unlike the powder, the extract had an effect in the cerebellum, which increased the DOPAC/DA ratio. These results suggest that Guilingji may have the action of modulating the levels and the metabolism of monoamines.

KEYWORDS: Guilingji, monoamines, norepinephrine, serotonin, metabolism

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Effects of Guilingji on Brain Monoamines and Their Metabolites in Mice

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Guillingji powder, a complex prescription of traditional Chinese medicines, has been pharmacologically shown to be a possible anti-aging agent, and also used for the treatment of male hypogonadism and female dysgenesis. Since the levels of numerous brain monoamines and their metabolites such as norepinephrine (NE), dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-HT), and 5-hydroxyindole acetic acid (5-HIAA) have been correlated with aging, we examined the effects of Guilingji powder and its extract on such monoamines and metabolites in the cerebrum and the cerebellum of mice using HPLC-ECD. The results showed that dietary intake of Guilingji powder had the effect of increasing the NE level and the DOPAC/DA ratio, and decreasing the 5-HT level and 5-HT/NE ratio in the cerebrum. In the cerebellum, the powder did not produce any changes in the levels of the compounds investigated. The extract of Guilingji, on the other hand, not only increased the NE level and the DOPAC/DA ratio but also the 5-HIAA/5-HT ratio in the cerebrum. However, the extract did not decrease the 5-HT in the cerebrum, although it decreased the 5-HT/NE. Unlike the powder, the extract had an effect in the cerebellum, which increased the DOPAC/DA ratio. These results suggest that Guilingji may have the action of modulating the levels and the metabolism of monoamines.

Key words : Guilingji, monoamines, norepinephrine, serotonin, metabolism

Guilingji, a complex prescription of 19 different traditional Chinese medicines, has been clinically used as a possible anti-aging agent and also used for the treatment of male hypogonadism and female dysgenesis for about 400 years (1). Phamacological studies have shown that dietary intake of Guilingji could increase the level of ascorbic acid in adrenal cortex, and also protect the gland from exhaustion induced by adiministration of hydrocortisone (2). In addition, Guilingji has been shown to prolong the mean life and to increase the survival rate of mice (3). Recently, using electron spin resonance spectrometer our laboratory has demonstrated an *in vitro* scavenging action of Guilingji on free radicals such as 1, 1-diphenyl-2-picrylhydrazyl, superoxide anion (DMPO-O_2^-), and hydroxyl radicals generated by $FeSO_4$ and H_2O_2 system (4). We also found that Guilingji has the effects of increasing the weights of the thymus and the spleen, and decreasing the thiobarbituric acid-reactive substances (TBARS) in the seminal vesical in mice (5). Our further

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study showed that pretreatment of Guilingji extract decreases the levels of TBARS in the left cortex, midbrain and olfactory lobe, as well as increases the superoxide dismutase activity in the midbrain and the hypothalamus in the brain of rats with $FeCl_3$-induced tramatic epilepsy model (6). In the present study, we further investigated using high performance liquid chromatography with electrochemical detector the effects of Guilingji powder and its extract on the levels of the cerebral and the cerebellar monoamines and their metabolites [*i.e.*, norepinephrine (NE), dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA)] in order to get a better understanding of its action from the neurochemical aspect.

Materials and Methods

Dietary preparation. The commercially available Guilingji (Shanxi Pharmaceutical Company of Traditional Chinese Medicines, Shanxi, P.R. China) is a powder mixture of the following 19 traditional Chinese medicines: Ginseng radix, Cornu cervi pontatrichum, Fructus lycii, Caryophylli flos, Radix achyranthis bidentatae, Herba cynomorii, Fructus psoraleae, Semen cuscutae, Cortex eucommiae, Herba cistanchis, Glycyrrhizae radix, Herba cynomorii, Fructus amomi, Hippocampus kelloggi, Rhiuoma rehmannial, Chuanshanjia, Shiyan, Daqingyan, and Quenao. The extract was obtained by the general procedure of water and ethanol extraction, but with a little modification. Briefy, Guilingji powder was soaked with water for 4 h and then filtered. The filtrate, as would the subsequent filtrates, was kept in 4 °C temperature while the residue was soaked with ethanol and water (1: 1) for 4 h. After filtration, the residue was left overnight soaking in a 99 % ethanol solution. After another filtration, it was extracted with ethanol and water (1: 1) using Sohxlet extractor. The 4 filtrates were merged, filtered, then evaporated with a vacuum thin layer evaporator. The extract was dried down with nitrogen gas and diluted with water before use.

Animals. Seventy, 8 weeks-old, male ddY mice weighing 23–26 g were used. The mice were divided into 2 groups, each having a control and an experimental subgroup. In group 1, the control (n = 15) was given a normal powder forage whereas the experimental subgroup (n = 20) was given a powder forage containing Guilingji powder for 10 days (5 mg/10 g body weight for the first 4 days and 8 mg/10 g body weight for the ramaining 6 days) (1, 2). To study the effect of Guilingji extract, 20 mice (the experimental subgroup) in group 2 were given orally (*i.e.*, inserted to stomach once a day) 0.2 ml extract solution which were obtained from the Guilingji powder of about the same amount and same days as were given to the mice in group 1. As for the control, 0.2 ml saline solution was administered orally to 15 mice.

Amine analysis. On the 11th day, the mice were killed by exposing the head to microwave irradition at 3 kW for 0.2 sec using metabostat NJE 2501 (New Japan Radio Co., Ltd., Saitama). The whole brain was removed and the cerebrum and the cerebellum were separated and stored at − 80 °C temperature until amine analysis. The levels of NE, DA, DOPAC, HVA, 5-HT and 5-HIAA in cerebrum and cerebellum were measured by high performance liquid chromatography with electrochemical detector (Model ECD-100, Eicom Japan) (7). Amine levels were expressed against tissue protein levels which were determined using the BCA Protein Assay Reagent (Pierce Chemical Company, Rockford, IL, USA).

Sources of compounds. NE, HVA and 5-HIAA were obtained from Sigma Chemical Company (St. Louis, MO, USA), DA and 5-HT from Wako Pure Chemical Industries Ltd. (Osaka, Japan), and DOPAC from Aldrich Chemical Company, Inc., (Milwaukee, WI, USA). All other chemicals and reagents were of the highest grade available from commercial suppliers.

Statistical analysis. The statistical significance of the differences between the experimental and control subgroups was examined by Student's *t*-test.

Results

Effects of Guilingji on the levels of NE, DA, DOPAC, HVA, 5-HT and 5-HIAA in mice cerebrum and cerebellum are shown in Table 1 and Table 2. The mice fed on a diet supplemented with Guilingji powder had a higher cerebral NE level and a lower cerebral 5-HT level than their corresponding controls fed a normal diet. The levels of the cerebral DA, DOPAC, HVA and 5-HIAA had no significant changes. In the cerebellum, Guilingji powder supplementation did

Table 1 Effect of the powder and extract of Guilingji on the levels of NE, DA, DOPAC, HVA, 5-HT and 5-HIAA in the cerebrum of mice

Group	n	NE	DA	DOPAC	HVA	5-HT	5-HIAA
Control	15	4.86 ± 0.16	12.43 ± 0.47	2.04 ± 0.10	2.25 ± 0.11	6.37 ± 0.28	4.26 ± 0.21
Powder	20	5.73 ± 0.23**	11.01 ± 0,71	2.01 ± 0.12	2.17 ± 0.10	5.59 ± 0.23*	3.90 ± 0.15
Control	15	5.25 ± 0.22	13.17 ± 0.54	1.71 ± 0.58	1.96 ± 0.09	6.41 ± 0.27	3.31 ± 0.17
Extract	20	6.12 ± 0.21*	12.28 ± 0.41	1.87 ± 0.05	1.84 ± 0.06	6.19 ± 0.23	3.69 ± 0.14

Values are given as mean ± SEM. * $p < 0.05$ and ** $p < 0.01$.
The levels of monoamines and their metabolites were expressed as ng/mg protein.
Abbreviations: NA, norepinephrine; DA, dopamine; DOPAC, dihydroxylacetic acid; 5-HT serotonin; 5-HIAA, 5-hydroxindole acetic acid.

Table 2 Effect of the powder and extract of Guilingji on the levels of NE, DA, DOPAC, 5-HT and 5-HIAA in the cerebellum of mice

Group	n	NE	DA	DOPAC	5-HT	5-HIAA
Control	15	4.62 ± 0.16	0.33 ± 0.03	0.51 ± 0.03	3.66 ± 0.31	4.98 ± 0.40
Powder	20	5.14 ± 0.38	0.34 ± 0.03	0.50 ± 0.05	3.50 ± 0.35	3.93 ± 0.38
Control	15	5.97 ± 0.26	0.44 ± 0.03	0.50 ± 0.02	4.21 ± 0.32	4.53 ± 0.19
Extract	20	5.93 ± 0.28	0.40 ± 0.03	0.59 ± 0.03*	4.32 ± 0.30	5.64 ± 0.34*

Values are given as means ± SEM. * $p < 0.05$.
The levels of monoamines and their metabolites were expressed as ng/mg protein.
Abbreviations: See Table 1.

not produce any change in the levels of the monoamines and their metabolites investigated. In the case of Guilingji extract supplementation, the observed differences were significant increases in the cerebral NE level, and in the cerebellar DOPAC and 5-HIAA levels. No values for the levels of HVA in the cerebellum are reported as they were lower than the detectable limit (Table 2).

In the cerebrum, the effects of both Guilingji powder and extract on DOPAC/DA, HVA/DA, 5-HIAA/5-HT, and 5-HT/NE ratios were similar as shown in Fig. 1. The ratios of DOPAC/DA in mice treated with Guilingji powder or extract were significantly higher than their corresponding controls (Fig. 1 A). The 5-HIAA/5-HT ratios of both experimental subgroups showed tendency of increase, but only in the case of extract treatment, the difference was significant (Fig. 1, C). The 5-HT/NE ratio was decreased after supplementation of Guilinji powder or extract (Fig. 1, D). However, neither the powder nor the extract had an effect on the HVA/DA ratio (Fig. 1, B). In the cerebellum, the only ratio that was changed was the DOPAC/DA ratio which was increased after an extract treatment (Fig. 2, A).

Discussion

Many reports have shown that the levels of DA and NE decrease with age in the brain of rats (8, 9), mice (10), rhesus monkey (11), and human (12). Dietary manipulation has shown that feeding young rats a diet deficient in tryptophan resulted to abnormally low level of 5-HT in brain and also delayed the tumor onset and the cessation of reproductive function (13,14). Administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a remarkably selective neurotoxin which can produce a syndrome strikingly similar to idipathic Parkinson's disease, induced marked decrement in the contents of DA and NE,

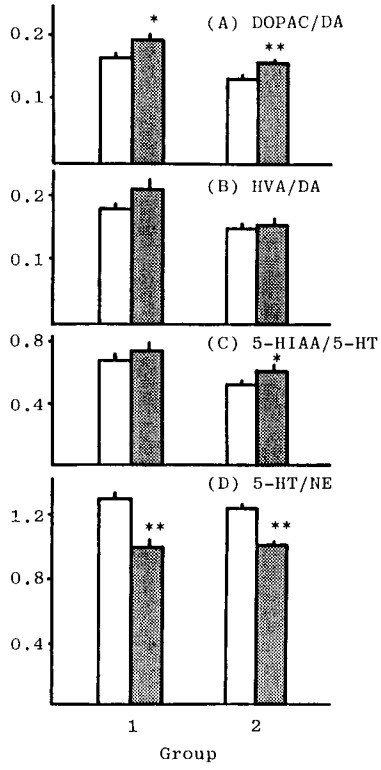

Fig. 1 Effects of Guillngji on the cerebral ratios of DOPAC/DA (A), HVA/DA (B), 5-HIAA/5-HT (C), and 5-HT/NE (D). Group 1 was given the Guilingji powder, while group 2, the extract. Dark columns are experimental groups and open columns are controls. Values are expressed as mean ± SEM. * $p < 0.05$ and ** $p < 0.01$ compared to their respective controls.

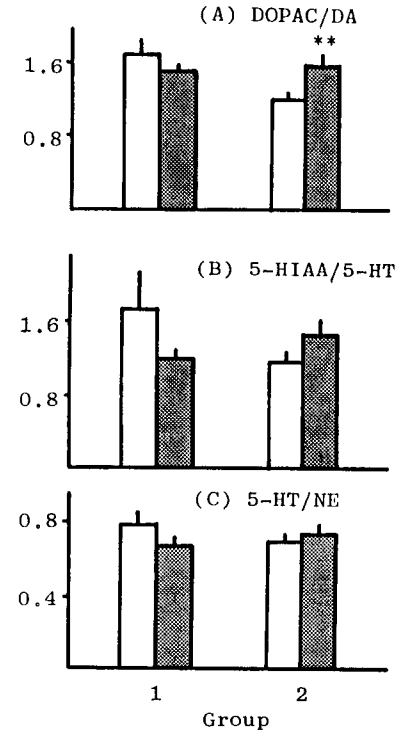

Fig. 2 Effects of Guillngji on the cerebellum ratios of DOPAC/DA (A), 5-HIAA/5-HT (B), and 5-HT/NE (C). Group 1 was given the Guilingji powder, while group 2, the extract. Dark columns are experimental groups and open columns are controls. Values are expressed as mean ± SEM. * $p < 0.05$ and ** $p < 0.01$ compared to their respective control values.

and increment in 5-HT in human, nonhuman primates, dogs and mice (15–19), and reduced the DOPAC/DA, HVA/DA and 5-HIAA/5-HT ratios as well. Conversely, treatment of pargyline, a monoamine oxidase (MAO) inhibitor which can slow the progression of idiopathic Parkinson's disease, could prevent the tremendous increase of 5-HT induced by MPTP, provide protection against the NE, DA depletion, and inhibit to a certain degree the decrease in the 5-HIAA/5-HT ratio (18, 20, 21). TJ-960, a mixture of 9 Japanese herbal drugs which is newly found to have a possible anti-aging effect, increased the NE and decreased the 5-HT levels in the hypothalamus of aged rats (22). In this study, we examined the effects of Guilingji, which has been proposed as an anti-aging agent and used clinically (23, 24), on the levels of three monoamines and their metabolites in mice brain. The significant increase in NE and decrease in 5-HT levels in the cerebrum, and the increase of the ratios of the cerebral DOPAC/DA and 5-HIAA/5-HT after both powder and extract of Guilingji administation, as well as the DOPAC/DA in cerebellum by the extract of Guilingji indicate that Guilingji may possess some actions on monoamines and their metabolites as those of TJ-960, or pargyline.

It has been reported that the ratio of 5-HT/NE in the cerebrum of 2-months-old rats was about 1.35 while that of 25-months old rats was 2.46. Therefore, it is suggested that decreasing the ratios of neurotransmitters (*e.g.*, 5-HT/NE)

may block or even reverse the aging process of animal. Conversely, increasing them would accelerate the aging process or the manifestation of age-related diseases such as senile dementia (25). Our results showed a significant lowering in the 5-HT/NE ratio after administration of Guilingji because Guilingji increased NE and decreased 5-HT by activating noradrenalinergic neurons or by inhibiting serotonergic pathway.

After administration of either Guilingji powder or extract, no significant differences were found in the levels of cerebral DA, DOPAC, HVA, and 5-HIAA between the experimental and control groups due to the homeostasis in the central nervous system (26). In addition, there were no significant increase in the ratios of DOPAC/DA and 5-HIAA/5-HT in the cerebrum and also of DOPAC/DA in the cerebellum after the extract supplementation. Central dopaminergic systems have been implicated in a variety of physiologic functions and in the etiology of neurologic and psychiatric diseases (27). The ratios of the DOPAC (or HVA) concentration against the DA concentration (or tyrosine hydroxylase activity) have been used as indices for metabolite formation. Our date which showed that Guilingji did not increase the DA, DOPAC, HVA, nor the 5-HIAA levels suggest that Guilingji, rather than interfering with the homeostasis of the brain in mice, only increases the turnover rate of both DOPAC/DA and 5-HIAA/5-HT (especially by the extrat of Guilingji), or only stimulates the metabolism of the brain monoamines.

The effect of Guilingji on the levels and ratios of monoamines and their metabolites in the cerebellum is not so significant (except the DOPAC/DA by the extract of Guilingji) as compared to those in the cerebrum. This observation is in agreement with other previous findings which showed that alterations of those neurotransmitters often occurred in striatum, hypothalamus, olfactory bulb and cortex, but not in cerebellum (25).

In the present study, only young animals were used with the treatment of Guilingji because it has been suggested, based on the treatment of testosterone to male Brown-Norway rats, which is clinically used for the treatment of male hypogonadism, that young animals might be more sensitive in the drug-induced brain monoamine metabolism, and the decreased sensitivity to drugs like testosterone might be related to decreased numbers and affinity of androgen receptors and aromatase activity in specific regions of the senescent animal brain (28).

Guilingji is commercially used as a powder mixture. To develop more effective formulations and for further studies of the chemical components of Guilingji, we made extract from the powder. From Table 1, it seems that powder Guilingji is more effective at increasing NE and decreasing 5-HT levels. However, the extract of Guilingji has more effects than the powder on DOPAC/DA and 5-HIAA/5-HT ratios in the cerebrum and DOPAC/DA ratio in the cerebellum. In the present extracting process, some components may be lost, however, the synergistic effects and counteraction of side effects may be enhanced in extract since most of the prescriptions of traditional Chinese medicines are used as decoctions.

In conclusion, acute and large dose administration of both the Guilingji powder and extract resulted in an increased NE, a decreased 5-HT, and also in a higher turnnover rate of DA and 5-HT in the brain of mice. These results suggest that one of the functions of Guilingji may be the modulating action on the metabolism of the brain monoamines. However, if we are to correlate these results with the proposed anti-aging effect, it requires more work to be undertaken in the future, such as, using the senescence accelerated mice or aged rats, administering monoamine depletors like MPTP, and also giving chronic treatment with more clinical significances.

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