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Antidepressant Effect of GABA-Rich *Monascus*-Fermented Product on Forced Swimming Rat Model

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ABSTRACT: γ-Aminobutyric acid (GABA) has several well-known physiological functions including antihypertension and antidepression. In this research, we focus on the antidepressant effects of oral administration of GABA-rich *Monascus*-fermented product in depression animal model (forced swimming test, FST) by Sprague−Dawley rats, and try to find its possible mechanism in the brain monoamine system. GABA and the *Monascus*-fermented product (MFP) significantly decreased the duration of immobility time in a short-term test. In a long-term test, the antidepressant-like effect of MFP was better than that of GABA at the same dosage (2.6 mg/kg), and the efficacy of MFP was similar to that of fluoxetine. Moreover, GABA might recover the level of monoamines norepinephrine, dopamine (DA), and 5-hydroxytryptamine (5-HT) in hippocampus and normalize the turnover ratio of 5-HT and DA in hippocampus and amygdala. In addition to the functions of GABA, the MFP has more potential in decreasing the turnover ratio of DA in the frontal cortex and striatum to improve depressive symptoms.

KEYWORDS: antidepression, GABA, *Monascus*, monoamine

INTRODUCTION

Major depressive disorder is a chronic, recurring, and potentially life-threatening illness that affects nearly 13−20% of the population worldwide.[1] The World Health Organization revealed that depression was the third global leading cause of disease burden in 2004, just behind lower respiratory infections and diarrheal disease and before ischemic heart disease and HIV/AIDS, and it predicts that depression will be ranked first in 2030.[2] In addition, results from the National Survey of American Life show that the lifetime prevalence of depression is greater than 10%, and on the increase, especially among the young.[3]

Up until now, the current antidepressants have exerted their effect by increasing the levels of monoamine (5-hydroxytryptamine (5-HT), norepinephrine (NE), and dopamine (DA)).[4] Although antidepressants have been used clinically for several decades, fewer than 20% of patients with depression receive psychiatric therapy, and fewer than 10% take suitable medication.[5] Furthermore, the synthetic antidepressants have disadvantages such as slow onset, relatively low response, and side effects.[6] Therefore, the discovery of new antidepressants with greater effectiveness and with little or no adverse effects is desirable, and thus it has become one of the focuses in the field of functional food research.

γ-Aminobutyric acid (GABA), a nonprotein amino acid, is widely distributed in nature. GABA has several well-known physiological functions, including antihypertensive activities.[7] In addition, GABA is a neurotransmitter in the central nervous system, and some research shows that GABA may affect monoamine levels in the brain.[8] It has also been shown to produce an antistress effect in humans[9] and related with improvement of depressive disorder.[10] Due to these physiological functions, the development of functional foods containing a high GABA concentration has been pursued actively.

In the food industry, GABA is produced largely by fermentation using bacteria, fungi or yeast. The *Monascus* species, traditional food fungi in Eastern Asia, have been studied for GABA production.[11] In addition, *Monascus* species have been shown to produce other useful secondary metabolites such as monacolin K (an inhibitor of cholesterol biosynthesis),[12] monascin and ankaflavin (yellow pigments with anti-inflammatory and antitumor potential).[13]

Since Okada et al. first showed that defatted rice germ enriched with GABA could improve symptoms such as sleeplessness, somnipathy, and depression in patients,[10] only a few studies have investigated the possible mechanism of how GABA produces these effects by oral administration. In this study, we focused on the antidepressant effects of orally administrated GABA-rich, *Monascus*-fermented product (MFP) on depression using Sprague−Dawley (SD) rats as a model system. To understand the mechanism by which GABA improves depression, we investigated changes in the brain monoamine levels induced by GABA and the MFP by using the forced swimming model.

MATERIALS AND METHODS

Preparation of the GABA-Rich *Monascus*-Fermented Product. Screening for GABA production strains on the genus of *Monascus* was carried out in a previous study.[11] For this research we selected *M. purpureus* BCRC 31499 which was purchased from the Bioresource Collection and Research Center (BCRC). The cultures were maintained on potato dextrose agar (PDA) slants at 10 °C, and transferred monthly. Seed cultures were prepared by transferring a piece of PDA agar (1 cm^2) from a slant into a 500 mL Hinton flask containing 100 mL of basal medium (5 g of rice powder, 0.1 g of K_2HPO_4, 0.1 g of $CaCl_2$, 0.05 g of $MgSO_4$ in 100 mL of distilled water). Screening for GABA-rich *Monascus*-fermented products used different concentrations of monosodium glutamate by

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HPLC analysis. Cultures were incubated at 30 °C for 72 h at 130 rpm. A 5% inoculum was then transferred to 3 L of working medium (3.5% rice bran (*Oryza sativa* ssp. *hsien*), 1.5% rice powder (*Oryza sativa* ssp. *hsien*), 2% monosodium glutamate, 0.1% K_2HPO_4, 0.1% $CaCl_2$, 0.05% $MgSO_4$) in a 6.6 L jar-fermentor (FB-6S, Firstek Co., Taipei, Taiwan). The culture conditions were 30 °C, 2 vvm (air volume/culture volume/min) for 72 h at 150 rpm, pH 6.0. The fermented product was filtered by stainless steel wire mesh (1 mm^2) and then freeze-dried immediately. After that, the product was homogenized and stored in a desiccator.

The *Monascus*-fermented powder (0.3 g) was extracted respectively with 10 mL of deionized water at 60 °C, 30 min for analysis. The analysis method was described in a previous study[14] by high performance liquid chromatography (HPLC) in triplicate. The chromatographic eluent pump (PU2089 plus, Jasco Co., Tokyo, Japan), injector (7725i, Rheodyne Co., Robert Park, CA, USA), column (C_{18}, 25 cm × 4.6 mm inner diameter, 5 μm, Discovery, Supelco, Inc., Bellefonte, PA, USA), and fluorescence detector (FL-1, Rainin Co., Tokyo, Japan) were used in GABA analysis. In MFP, the concentration of GABA (Sigma Chemical Co., St. Louis, MO, USA) was 16.4 mg/g dry powder, the citrinin, monacolin K, monascin and ankaflavin were not detected.

Animals and Diets. Male Sprague−Dawley (SD) rats weighing 130−150 g were housed in individual plastic cages and under standard experimental conditions: room temperature 23 ± 2 °C, humidity 40−60%, 12 h light/dark cycle (lights on at 9:00 a.m.). Food (standard laboratory chow diet (Ralston Purina, St. Louis, MO, USA)) and deionized water were available ad libitum during the experiment. The animals were given a week to adapt to the new environment. SD rats were weighed and randomly assigned to 7 groups of 12 animals each before the commencement of the animal experiment.

Dose and Grouping. The dosage of GABA was determined according to a previous study,[10] which showed that the intake of 26.4 mg of GABA per day was able to improve symptoms of depression in 20 female patients (60 kg). Using 26 mg of GABA as the reference dose, the daily dosage of GABA per SD rat (2.6 mg GABA/kg body weight, 26 mg/60 kg (human equivalent dose) × 6 (conversion factor of rat) = 2.6 mg/kg) was calculated in accordance with Boyd's formula of body surface area as recommended by the Food and Drug Administration.[15] Fluoxetine, a common antidepressant (Prozac, Lilly Co., Taipei, Taiwan), was used as a positive control at a dose of 20 mg/kg body weight for each SD rat.

SD rats were weighed and randomly assigned to 7 groups: (1) normal control rats (NC, normal feed and water, no exposure to the swimming test); (2) control rats (C, normal feed and water); (3) fluoxetine-treated rats (F, 20 mg/kg bw per day); (4) GABA-treated rats (G, 2.6 mg/kg bw per day); (5) rats administered nonfermented substrate (NF, 160 mg/kg bw per day including 0.2 mg of GABA from substrate); (6) rats given half the dose of GABA in the form of MFP (0.5 M, 80 mg/kg bw per day including 1.3 mg of GABA); and (7) rats given the full dose of GABA in the form of MFP (M, 160 mg/kg bw per day including 2.6 mg of GABA). Besides lack of fermentation, the NF was prepared in the same manner as the MFP. To investigate and clarify whether the antidepressant effect of MFP was better than that of NF after the fermentation process, the same feeding dose of NF (group 5) was used to compare with the MFP group (group 7).

After the prebreeding stage, all test samples were suspended in 1 mL of deionized water (containing 0.5% carboxymethylcellulose) and orally administered daily to the SD rats using a stomach tube. The food intake was recorded daily, and animals were weighed twice a week. The experiment was reviewed and approved by the Animal Care and Research Ethics Committee of the National Taiwan University.

Forced Swimming Test (FST). Previous studies had demonstrated that monoamine concentrations in rodents' brains were significantly altered after FST.[16] The procedure used was described by Porsolt et al.[17] and took some modification by others.[18] Briefly, rats were individually placed in glass cylinders (40 cm height × 20 cm diameter) containing 30 cm of water at 23−25 °C, so rats could not support themselves by touching the bottom with their feet. Two swimming sessions were conducted: an initial 15 min pretest followed 24 h later by a 6 min test. The duration of immobility was scored during the last 4 min of the 6 min test period. Test sessions were videotaped from a DV recorder (JVC GR-D230, Victor Co., Tokyo, Japan) for scoring later. Following both swimming sessions, the rats were removed from the water, and then placed into original cages after being warmed and dried. Activity was defined as the swimming, jumping, diving, or scratching of the walls. Immobility was defined when floating motionless or making only those movements necessary to keep the rat's head above the water.

Following the prebreeding stage for one week, the short-term test was conducted first, and the samples were fed by oral administration after the pretest session 30 min later. The main purpose of this test was to know whether the sample could improve the depressed emotion caused by sudden stress. After that, continued oral administration for 3 weeks for the long-term test was simulated eating health food products four-week could prevent the impact of stress on depression symptom.

Quantification of Monoamines. After 6 min swim exposure in the long-term test, rats were sacrificed by decapitation immediately. The frontal cortex, striatum, hippocampus, and amygdala were rapidly dissected from the brain, according to the Glowinski method,[19] on an ice-chilled glass plate. Four sections of brain tissue were stored at −80 °C respectively.

Concentrations of norepinephrine (NE), serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) were measured by HPLC coupled with electrochemical detector (ECD, LC-4C, BAS, West Lafayette, IN, USA) (range 5 nA, filter 0.1 Hz, AppE cell 0.750 V) with autosampler (CMA 200 refrigerated microsampler, Stockholm, Sweden). The mobile phase contained 20.5 g of NaH_2PO_4, 185 mg of EDTA, 130 mg of 1-octanesulfonic acid, sodium salt (SOS) and 200 mL of methanol per liter adjusted to pH 3.0 by H_3PO_4. The flow rate was 600 μL/min.

All tissues were homogenized in 1 mL of extract solution according to the method of Cheng et al.[20] with some modification. The extract solution was 0.1 N HCl contained 10^{-7} M ascorbic acid and 15 mg/L parglyine. Homogenates were centrifuged at 10000*g* for 20 min at 4 °C. After filtration (0.22 μm), the supernatant was injected on a column (C_{18}, 25 cm × 4.6 mm inner diameter, 5 μm, Luna, Phenomenex, Inc., Torrance, CA, USA). The chemical standards used in HPLC-ECD, including NE, 5-HT and its metabolite 5-HIAA, DA and its metabolites DOPAC, were purchased from Sigma (St. Louis, MO, USA) and were dissolved in the extract solution.

Plasma Liver, Kidney and Electrolyte Index Analysis. Plasma glutamic oxaloacetic transaminase (AST), glutamic pyruvic transaminase (ALT), blood urea nitrogen (BUN), creatinine levels and plasma electrolytes were measured in triplicate using an automatic biochemical analyzer (Beckman-700, Fullerton, CA, USA).

Statistical Analysis. Data are expressed as the mean ± standard deviation (SD). The statistical significance in the behavioral and biochemical effects were determined by one-way analysis of variance (ANOVA) using the general linear model procedure of SPSS software (SPSS Institute, Inc., Chicago, IL, USA), followed by ANOVA with the Duncan's test. Differences with $p < 0.05$ or 0.01 were considered statistically significant.

RESULTS

GABA-Rich *Monascus*-Fermented Products. Figure 1 shows that addition of 30, 60, and 90 mM monosodium glutamate caused a significant change in GABA level of MFP. Treatment with 60 mM monosodium glutamate increased the GABA level of MFP more than the other concentrations of monosodium glutamate.

Change of Body Weight and Daily Intake by SD Rats. Animal body weight was monitored twice a week as shown in

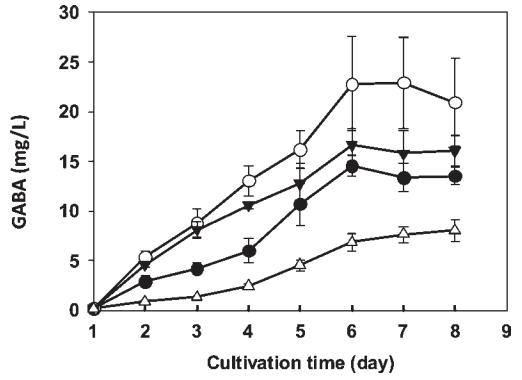

Figure 1. Effect of the concentration of monosodium glutamate on GABA production by *Monascus purpureus* BCRC 31499. The values represent the mean ± SD (n = 5). Concentration of monosodium glutamate: △, 0 mM; ●, 30 mM; ○, 60 mM; ▼, 90 mM.

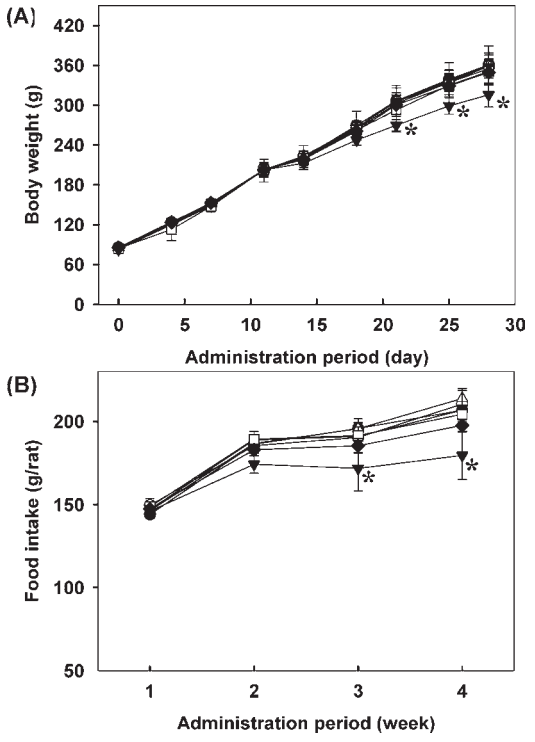

Figure 2. The changes of (A) body weight and (B) food intake in the administration period: ●, normal control; ○, control; ▼, fluoxetine, 20 mg/kg bw; △, GABA, 2.6 mg/kg bw; ■, nonfermented substrate, including 0.2 mg of GABA from substrate; □, half dosage of GABA in the form of *Monascus*-fermented product, including 1.3 mg of GABA; ♦, full dosage of GABA in the form of *Monascus*-fermented product, including 2.6 mg of GABA. Data are expressed as means ± SD, n = 12. Significantly different: (*) $P < 0.05$, compared with control (one-way ANOVA following by Duncan's test).

Figure 2A. After 1 week of adaptation (day 0 to day 7), the average body weight of the SD rats was not significantly different between groups, with identical results observed in the first week of sample feeding (day 7 to day 14). However, at 14 days, the average body weight of the antidepressant group was slightly lower than that of the other groups, and the difference became progressively more apparent during the next 2 weeks of fluoxetine administration. At 21 days, a significant difference was observed

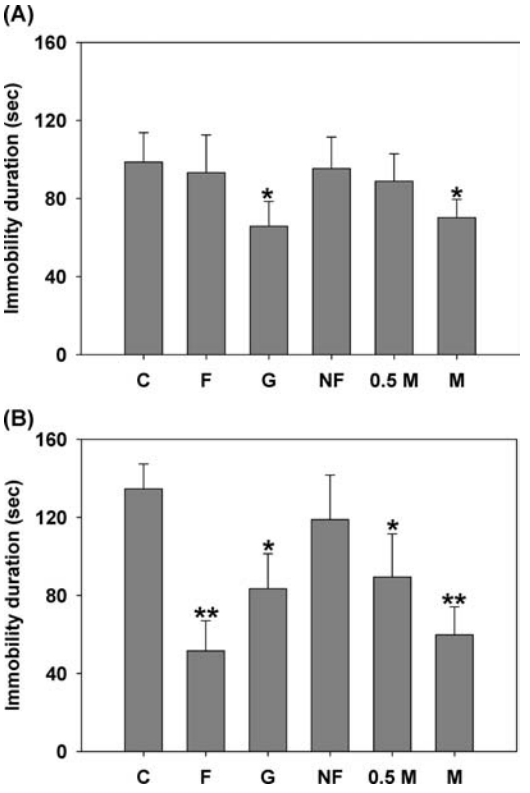

Figure 3. Effects of the *Monascus*-fermented product on the immobility time of the (A) short-term and (B) long-term test in forced swimming test: C, control; F, fluoxetine, 20 mg/kg bw; G, GABA, 2.6 mg/kg bw; NF, nonfermented substrate, including 0.2 mg of GABA from substrate; 0.5 M, half dosage of GABA in the form of *Monascus*-fermented product, including 1.3 mg of GABA; M, full dosage of GABA in the form of *Monascus*-fermented product, including 2.6 mg of GABA. Data are expressed as means ± SD, n = 8. Significantly different: (*) $P < 0.05$, (**) $P < 0.001$ compared with control (one-way ANOVA following by Duncan's test).

between the normal control and the fluoxetine-treated rats. The food intake showed a trend similar to the weight change in Figure 2B. During week 1, the average food intake was similar between the groups. Although the average food intake increased similarly over time in the other groups, it remained unchanged in the second week to the fourth week in the antidepressant group. After 3 weeks, the food intake of the fluoxetine-treated group was significantly lower than that in the others.

Effects of the *Monascus*-Fermented Product on Immobility Time in Forced Swimming Tests. Compared to the control group, the administration of GABA and the MFP (containing a dose of GABA at 2.6 mg/kg) group significantly decreased the duration of immobility time in the short term test (Figure 3A). The effects of the oral administration of GABA, 0.5 MFP and MFP diminished the duration of immobility in a dose dependent manner relative to the control group in the long term test (Figure 3B). MFP showed greater efficiency at reducing immobility time than the same dosage of GABA given to the GABA group (2.6 mg/kg). Moreover, MFP was as effective as fluoxetine at significantly reducing the immobility time ($p < 0.01$), but the duration of immobility was not significantly different between the control and NF groups.

Effects of the *Monascus*-Fermented Product on Norepinephrine, Dopamine, and Serotonin Concentrations in Different Brain Sections. The concentrations of norepinephrine (NE) in

Table 1. Effects on the Concentration of Norepinephrine in Different Brain Sections by the Administration of *Monascus*-Fermented Product[a]

	norepinephrine (ng/g brain tissue)			
groups[b]	amygdala	frontal cortex	hippocampus	striatum
NC	487 ± 18	272 ± 12	468 ± 32	1120 ± 134
C	370 ± 30#	272 ± 26	138 ± 22##	1036 ± 92
F	469 ± 40*	246 ± 29	231 ± 25*	971 ± 134
G	452 ± 54	219 ± 27	342 ± 17**	870 ± 121
NF	418 ± 30	241 ± 33	201 ± 30*	780 ± 47
0.5 M	424 ± 33	239 ± 16	218 ± 23*	804 ± 62
M	398 ± 38	288 ± 29	344 ± 25**	784 ± 46

[a] Data are expressed as means ± SD, $n = 8$. Significantly different: #$P < 0.01$, ##$P < 0.001$ compared with normal control; *$P < 0.01$, ** $P < 0.001$ compared with control (one-way ANOVA following by Duncan's test). [b] NC: normal control. C: control. F: fluoxetine, 20 mg/kg. G: GABA, 2.6 mg/kg. NF: non-fermented substrate, including 0.2 mg of GABA from substrate. 0.5 M: half dosage of GABA in the form of *Monascus*-fermented product, including 1.3 mg of GABA. M: full dosage of GABA in the form of *Monascus*-fermented product, including 2.6 mg of GABA.

Table 2. Effect on the Concentration of Dopamine in Different Brain Sections by the Administration of *Monascus*-Fermented Product[a]

	dopamine (ng/g brain tissue)			
groups[b]	amygdala	frontal cortex	hippocampus	striatum
NC	1050 ± 106	68 ± 13	88 ± 7	6065 ± 521
C	457 ± 43##	42 ± 11	54 ± 12#	4140 ± 519##
F	1183 ± 53**	51 ± 9	75 ± 14	7019 ± 482**
G	681 ± 127	69 ± 9	88 ± 12*	4383 ± 513
NF	752 ± 87*	46 ± 6	51 ± 9	4041 ± 481
0.5 M	751 ± 89*	76 ± 11*	65 ± 4	3835 ± 311
M	733 ± 129*	104 ± 14**	89 ± 14*	4807 ± 497

[a] Data are expressed as means ± SD, $n = 8$. Significantly different: #$P < 0.01$, ##$P < 0.001$ compared with normal control; *$P < 0.01$, ** $P < 0.001$ compared with control (one-way ANOVA following by Duncan's test). [b] NC: normal control. C: control. F: fluoxetine, 20 mg/kg. G: GABA, 2.6 mg/kg. NF: non-fermented substrate, including 0.2 mg of GABA from substrate. 0.5 M: half dosage of GABA in the form of *Monascus*-fermented product, including 1.3 mg of GABA. M: full dosage of GABA in the form of *Monascus*-fermented product, including 2.6 mg of GABA.

the frontal cortex, striatum, hippocampus, and amygdala are presented in Table 1. Following the FST, the concentration of NE was decreased significantly in the amygdala and hippocampus of the control group compared to that of the normal control group ($p < 0.01$). The administration of GABA, NF, 0.5 MFP, MFP, and fluoxetine reversed these reductions in the hippocampus. However, there was no significant difference in NE levels in the frontal cortex and striatum when compared to that of the control group.

The effect of dopamine levels in different brain sections is presented in Table 2. A significant increase in DA levels in the amygdala and striatum ($p < 0.001$) was observed after fluoxetine treatment. In the hippocampus, the DA level was increased in the GABA and MFP groups ($p < 0.01$). Moreover, 0.5 MFP and MFP had significantly increased DA levels in the amygdala ($p < 0.01$) and in a dose-dependent manner in the frontal cortex.

Table 3. Effect on the Concentration of Serotonin in Different Brain Sections by the Administration of *Monascus*-Fermented Product[a]

	serotonin (ng/g brain tissue)			
groups[b]	amygdala	frontal cortex	hippocampus	striatum
NC	559 ± 34	133 ± 17	377 ± 43	382 ± 7
C	434 ± 52#	106 ± 5	130 ± 15##	241 ± 16##
F	596 ± 58*	113 ± 16	204 ± 29*	331 ± 11**
G	452 ± 64	132 ± 8	217 ± 34*	253 ± 12
NF	329 ± 55	115 ± 7	151 ± 32	218 ± 29
0.5 M	445 ± 62	106 ± 7	227 ± 15*	292 ± 22*
M	436 ± 39	135 ± 11	250 ± 22**	309 ± 15**

[a] Data are expressed as means ± SD, $n = 8$. Significantly different: #$P < 0.01$, ##$P < 0.001$ compared with normal control; *$P < 0.01$, ** $P < 0.001$ compared with control (one-way ANOVA following by Duncan's test). [b] NC: normal control. C: control. F: fluoxetine, 20 mg/kg. G: GABA, 2.6 mg/kg. NF: non-fermented substrate, including 0.2 mg of GABA from substrate. 0.5 M: half dosage of GABA in the form of *Monascus*-fermented product, including 1.3 mg of GABA. M: full dosage of GABA in the form of *Monascus*-fermented product, including 2.6 mg of GABA.

Similarly, NF had significantly increased DA levels in the amygdala ($p < 0.01$).

As shown in Table 3, following FST, the levels of 5-HT in the brain were decreased, especially in the hippocampus and striatum ($p < 0.001$). The administration of fluoxetine reversed these reductions in the striatum ($p < 0.001$), hippocampus and amygdala ($p < 0.01$). Similarly, in the 0.5 MFP and MFP groups, the level of 5-HT increased significantly in a dose dependent manner in the hippocampus and striatum. In contrast, GABA reversed the reduction only in the hippocampus.

Effects of *Monascus*-Fermented Product and GABA on the Dopamine Turnover Ratio in the Brain. The turnover ratio of DA (DOPAC/DA) in different regions of the brain is shown in Figure 4. In the frontal cortex, the DOPAC/DA ratio decreased significantly in the 0.5 MFP and MFP groups in a dose dependent manner, but no difference was observed between the other groups and the control ($p < 0.05$) (Figure 4A). Similarly, a significant decrease in the DA turnover ratio was seen in the 0.5 MFP, MFP, and GABA groups in hippocampus (Figure 4B). As shown in Figure 4C, in the striatum, the DOPAC/DA ratio was decreased significantly in all experimental groups ($p < 0.05$) except in the GABA and NF groups. In addition, there was a significant decrease in the dopamine turnover ratio of amygdala among the various groups ($p < 0.05$) (Figure 4D).

Effects of *Monascus*-Fermented Product and GABA on the Serotonin Turnover Ratio in the Brain. The turnover ratio of 5-HT (i.e., 5-HIAA/5-HT) in different regions of the brain is shown in Figure 5. The results indicated that there was no difference among the various groups in the turnover ratio of serotonin in the frontal cortex ($p < 0.05$) (Figure 5A). However, in the hippocampus, there was a significant decrease in the various groups except in the NF group ($p < 0.05$) (Figure 5B). The 5-HT turnover ratio was increased in all experimental groups in the striatum after FST (Figure 5C). Compared with the control group, fluoxetine treatment at 20 mg/kg significantly decreased the 5-HIAA/5-HT ratio ($p < 0.05$) in the amygdala. GABA, 0.5 MFP, MFP and NF treatment had the same effects as fluoxetine (Figure 5D).

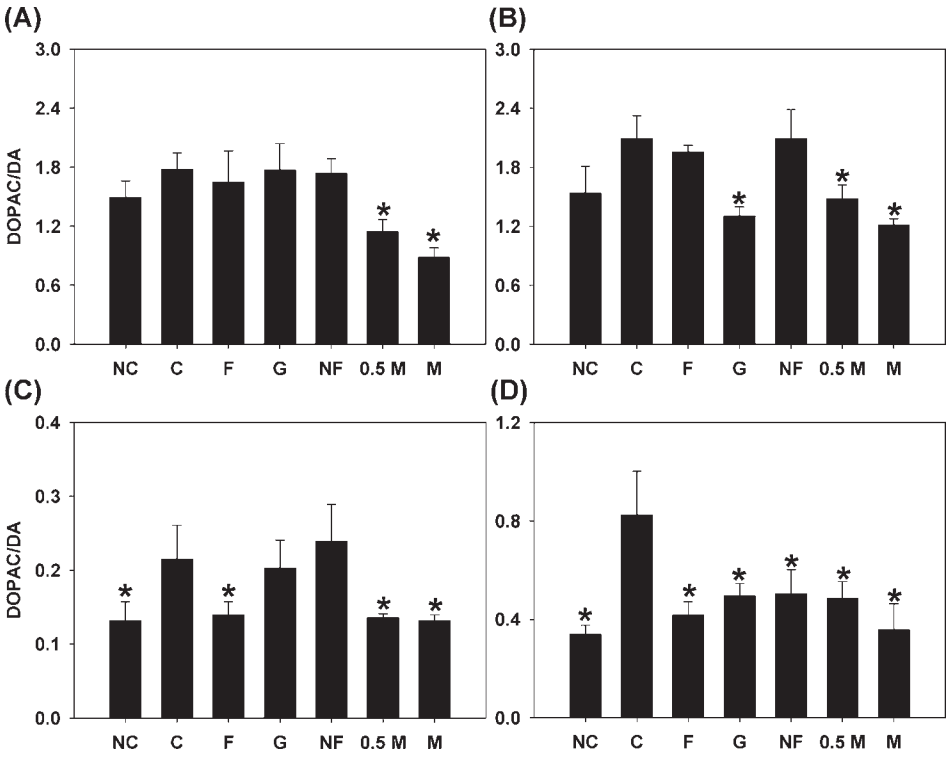

Figure 4. The effect of *Monascus*-fermented product and GABA on the dopamine turnover ratio (DOPAC/DA) in different brain sections: (A) frontal cortex, (B) hippocampus, (C) striatum and (D) amygdala. DA: dopamine. DOPAC: 3,4-dihydroxyphenylacetic acid. NC: normal control. C: control. F: fluoxetine, 20 mg/kg. G: GABA, 2.6 mg/kg. NF: nonfermented substrate, including 0.2 mg of GABA from substrate. 0.5 M: half dosage of GABA in the form of *Monascus*-fermented product, including 1.3 mg of GABA. M: full dosage of GABA in the form of *Monascus*-fermented product, including 2.6 mg of GABA. Data are expressed as means ± SD, $n = 8$. Significantly different: (*) $P < 0.05$, compared with control (one-way ANOVA following by Duncan's test).

The Liver Index, Kidney Index, and Electrolyte Balance of SD Rats. The liver index, kidney index, and plasma electrolyte balance are shown in Tables 4 and 5. AST increased by stress has been reported,[21,22] and it might decrease by secondary metabolites in *Monascus* spp. with antioxidative stress effects. In the control group, the AST (liver index) increased significantly after FST, compared with that of normal control ($p < 0.05$). These reductions were reversed by the administration of GABA. In addition, creatinine levels (kidney index) were decreased significantly in the fluoxetine, GABA, and MFP groups. Similarly, BUN was decreased in the GABA and MFP groups. However, there was no difference in the plasma electrolyte balance among the various groups during the experimental period.

DISCUSSION

The side effects of fluoxetine, the most common clinical antidepressant, include reduced appetite and weight loss. In this study, the effects on appetite and weight change were measured by monitoring the average food intake weekly and recording the body weight twice a week. Under normal circumstances, food intake increases continually as the rats grow. However, the average food intake in the fluoxetine treated group remained unchanged in the second week to fourth week. At the same time, the body weight of the fluoxetine treated group was lower than that of other groups too. Like other serotonergic agents (e.g., zimeldine), fluoxetine possesses anorectic activity. Although the precise mechanism has not been clearly established, results of animal studies indicate that the drug's appetite-inhibiting action may result from blocking serotonin reuptake and the resultant increase in serotonin availability at the neuronal synapse or decreasing the intake of sweet food, as well as a reduction in leptin levels in rats. In a previous study,[23] fluoxetine has been associated with weight loss during acute treatment (four weeks), but no controlled studies of weight change during long-term treatment (one year) have been reported. As in previous studies, these results indicate that fluoxetine produces weight decline by reducing appetite, while the oral administration of GABA, NF, and MFP has no such side effects (Figure 2).

Forced swimming test, described originally by Porsolt et al.,[17] is the most widely used animal model test for assessing the pharmacological antidepressant activity of a drug. It is also a well-established system for screening new potent antidepressant drugs in rats and mice.[24] The immobility behavior displayed in rodents when subjected to an unavoidable and inescapable stress has been hypothesized to reflect a behavioral state of despair (learned helplessness) and may therefore reflect depressive disorders in humans. There is, indeed, a significant correlation between clinical potency and the effectiveness of antidepressants in this model.[25] In a short-term FST test, treatment with GABA and MFP (at a dosage of GABA 2.6 mg/kg) reduced the immobility time significantly, but not treatment with 0.5 MFP and NF, indicating that GABA was the main active compound that improved the depressive mood, with relatively good results at a high dosage. Because of the pharmacological mechanisms of fluoxetine, the depressive mood of the patient just begins to improve 2 weeks to one month after taking the medication,[26] resulting in no immediate improvement in the short term test. On the other hand, after the administration of GABA and MFP

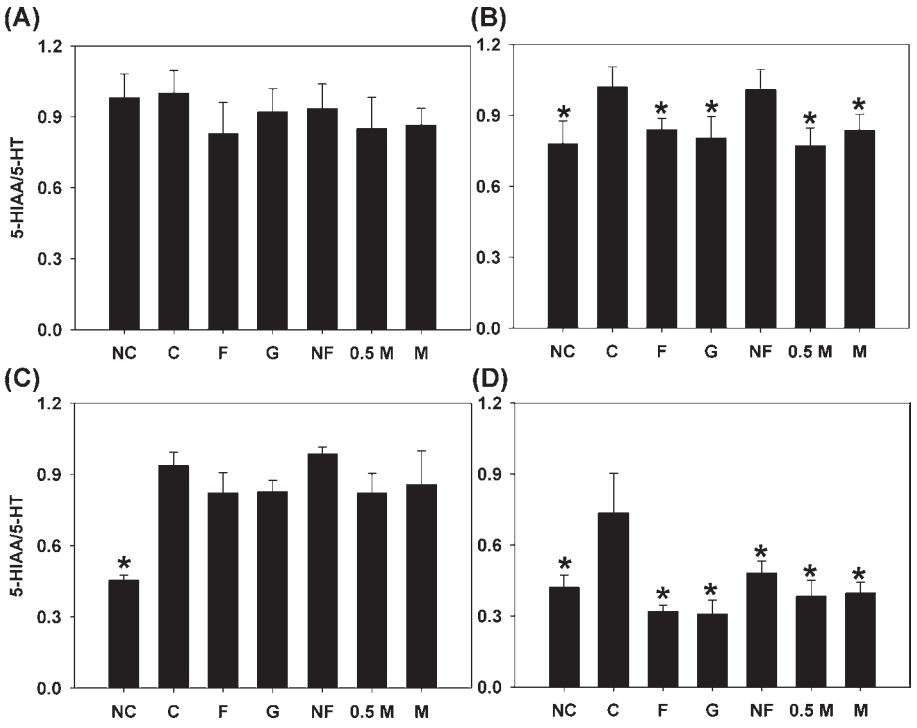

Figure 5. The effect of *Monascus*-fermented product and GABA on the serotonin turnover ratio (5-HIAA/5-HT) in different brain sections: (A) frontal cortex, (B) hippocampus, (C) striatum and (D) amygdala. 5-HT: 5-hydroxytryptamine. 5-HIAA: 5-hydroxyindoleacetic acid. NC: normal control. C: control. F: fluoxetine, 20 mg/kg. G: GABA, 2.6 mg/kg. NF: nonfermented substrate, including 0.2 mg of GABA from substrate. 0.5 M: half dosage of GABA in the form of *Monascus*-fermented product, including 1.3 mg of GABA. M: full dosage of GABA in the form of *Monascus*-fermented product, including 2.6 mg of GABA. Data are expressed as means ± SD, $n = 8$. Significantly different: (*) $P < 0.05$, compared with control (one-way ANOVA following by Duncan's test).

Table 4. Effect of *Monascus*-Fermented Product on SD Rats' Performance Serum AST, ALT, BUN and Creatinine Levels[a]

	liver		kidney	
group[b]	AST (units/L)	ALT (units/L)	BUN (mg/dL)	creatinine (mg/dL)
C	136.0 ± 15.6	73.1 ± 9.8	23.7 ± 1.7	0.44 ± 0.06
NC	113.5 ± 7.1*	69.3 ± 10.7	22.2 ± 1.8	0.44 ± 0.06
F	119.8 ± 12.6	74.3 ± 7.9	20.3 ± 2.1	0.36 ± 0.05*
G	111.3 ± 8.2*	64.9 ± 9.9	19.8 ± 2.0*	0.36 ± 0.03*
NF	125.9 ± 21.4	66.8 ± 3.7	20.8 ± 2.4	0.40 ± 0.04
0.5 M	132.3 ± 22.0	67.9 ± 7.0	21.1 ± 1.8	0.42 ± 0.07
M	130.8 ± 15.8	69.4 ± 8.0	19.7 ± 1.5*	0.38 ± 0.07*

[a] Data are expressed as means ± SD, $n = 10$. Significantly different: (*) $P < 0.05$, compared with control (one-way ANOVA following by Duncan's test). [b] AST: aspartate aminotransferase. ALT: alanine aminotranferease. BUN: blood urine nitrogen. C: control. NC: normal control. F: fluoxetine, 20 mg/kg. G: GABA, 2.6 mg/kg. NF: non-fermented substrate, including 0.2 mg of GABA from substrate. 0.5 M: half dosage of GABA in the form of *Monascus*-fermented product, including 1.3 mg of GABA. M: full dosage of GABA in the form of *Monascus*-fermented product, including 2.6 mg of GABA.

for 3 weeks, the duration of the immobility decreased in the long term test. The antidepressant-like effect of MFP is better than that of GABA at the same dosage (2.6 mg/kg), with the efficacy of MFP similar to that of fluoxetine. This indicates that MFP contains other antidepressant compounds and acts synergistically with GABA to improve the depressive symptoms.

The monoamine hypothesis was formulated about 40 years ago, and a deficiency in one or more monoamines is commonly evoked to explain the physiopathology of depression. As is well-known, depression can be alleviated by increasing the levels of the monoamine neurotransmitters 5-HT, DA, and/or NE by commercial antidepressants.[27] Furthermore, previous studies have demonstrated that monoamine concentrations in rodents' brains were significantly altered after FST. Thus, in order to confirm whether MFP similarly affects these neurotransmitters to improve the depressive symptoms, we analyzed the concentration of monoamines in rat brain tissues such as the frontal cortex, hippocampus, striatum, and amygdala, as these regions are relevant in depression mood disorder. After FST, the NE, DA, and 5-HT levels were decreased significantly in the brain. The administration of GABA reversed the decrease in NE, DA, and 5-HT levels in the hippocampus. Similar to GABA, MFP was effective at restoring DA levels in the amygdala and frontal cortex and the 5-HT level in the striatum. These results indicate that GABA is the main compound increasing the concentrations of NE, DA, and 5-HT in the hippocampus. Moreover, MFP had greater functionality in recovering DA and 5-HT levels in the amygdala, frontal cortex, and striatum than GABA.

The increase of monoamines in the brain can improve depressive symptoms. At present, however, the prescription medications used to treat depression do not supplement monoamine levels in the patient directly. Instead, they act by inhibiting monoamine reuptake. Selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine are thought to elicit clinical antidepressant effects by blocking 5-HT reuptake at the synapse, resulting in an elevation of extracellular 5-HT concentrations

Table 5. Effect of *Monascus*-Fermented Product on SD Rats' Performance Serum Electrolyte Levels[a]

	electrolyte				
group[b]	Na (mequiv/L)	K (mequiv/L)	Cl (mequiv/L)	Ca (mg/dL)	Mg (mequiv/L)
C	149.0 ± 2.0	7.4 ± 0.9	96.1 ± 1.8	13.0 ± 1.0	4.4 ± 0.6
NC	150.3 ± 1.0	6.9 ± 0.5	93.9 ± 1.0	12.9 ± 0.6	4.1 ± 0.4
F	148.0 ± 1.6	6.7 ± 0.3	94.9 ± 0.8	13.1 ± 0.5	4.2 ± 0.3
G	147.9 ± 1.6	7.1 ± 0.4	95.5 ± 1.7	12.6 ± 0.3	4.1 ± 0.4
NF	148.8 ± 2.3	6.8 ± 0.5	95.0 ± 1.9	12.8 ± 0.4	4.1 ± 0.3
0.5 M	147.5 ± 0.9	7.1 ± 0.6	95.0 ± 1.1	12.5 ± 0.7	4.1 ± 0.7
M	148.0 ± 2.6	7.3 ± 0.5	95.0 ± 0.9	12.4 ± 0.8	4.0 ± 0.8

[a] Data are expressed as means ± SD, $n = 10$. Significantly different: (*) $P < 0.05$, compared with control (one-way ANOVA following by Duncan's test). [b] C: control. NC: normal control. F: fluoxetine, 20 mg/kg. G: GABA, 2.6 mg/kg. NF: non-fermented substrate, including 0.2 mg of GABA from substrate. 0.5 M: half dosage of GABA in the form of *Monascus*-fermented product, including 1.3 mg of GABA. M: full dosage of GABA in the form of *Monascus*-fermented product, including 2.6 mg of GABA.

in the limbic regions of brain, which can act upon various postsynaptic 5-HT receptors.[28] The ratio of a neurotransmitter to its metabolites can be used as an index of neurotransmitter metabolism, with a reduction in the metabolite/neurotransmitter ratio suggesting a slowdown in the metabolism of the neurotransmitter.[29] Hence, the turnover ratio can be used to help to understand and perhaps infer the mechanism of the antidepressant or the antidepressant-like functional food as the metabolisms of the monoamines are observed. Therefore, as the 5-HIAA/5-HT ratio was reduced in the present study, it is likely that 5-HT metabolism was slowed down. Interestingly, GABA and MFP tended to normalize the turnover ratio of 5-HT in the hippocampus and amygdala, similar to the normal control group, suggesting that their mechanisms for increasing 5-HT levels in the brain are related.

On the other hand, several studies, including post-mortem investigations (particularly of subjects with severe depression), have demonstrated reduced concentrations of dopamine metabolites both in the cerebrospinal fluid and in the brain regions that mediate mood and motivation.[30] Moreover, the dopaminergic system was involved in the pathogenesis and recovery from the depression.[30] SSRIs acting on the serotonergic system might indirectly affect dopamine pathways.[31] In the present study, the enhanced DOPAC/DA ratio observed in the brain after the FST was recovered by treatment with MFP, but fluoxetine only normalized the turnover ratio of DA in the striatum and amygdala while GABA decreased the DA turnover ratio in the hippocampus and amygdala. However, MFP decreased the DA turnover ratio not only in the hippocampus and amygdala but also in the frontal cortex and striatum. Comparing the results in Table 2 and Figure 4, it appears that MFP may increase DA levels in the frontal cortex, hippocampus, and amygdala and also reduce DOPAC formation to normalize the DA turnover ratio in the brain after stress stimulation. Therefore, we speculate that the reversal of FST-induced behavioral change by MFP might result from effects on the central serotonergic and dopaminergic systems, particularly in the dopaminergic modulated ones.

In animal studies, the liver index (AST and ALT) and kidney index (BUN and creatinine) are commonly used as safety indicators of feeding dosage. Based on our present results, FST stress may cause AST to increase. The administration of GABA for 3 weeks was able to normalize AST but not ALT levels in rat serum. Similarly, it has been reported that patients who were treated with defatted rice germ enriched with GABA as a functional food showed improved depressive symptoms and that AST and ALT levels in the serum decreased 10−15% compared with those of untreated patients.[10] On the other hand, BUN and creatinine levels were decreased in the GABA and MFP groups. Moreover, GABA has several physiological functions including a diuretic effect.[32] According to information provided with the medication, fluoxetine may cause low blood sodium (less than 110 mmol/L) during the administration period. This effect may cause an imbalance in the electrolyte concentration in the blood. As shown in the present study, there was no significant difference in the level of serum electrolytes in each group, and no differences were observed in the slices of liver and kidney either (data not show). These results suggest that the dosage used for four week administration in this study might be safe in all groups and the trend of data were similar to previous research.[33] However GABA improvement of liver and kidney function needs to be further investigated in our study.

In conclusion, the present study provides the first evidence indicating that oral administration of GABA (2.6 mg/kg) can affect the brain monoamine system to improve the symptoms of depression after stress stimulation. GABA may recover NE, DA, and 5-HT levels in the hippocampus and normalize the turnover ratio of 5-HT and DA in the hippocampus and amygdala in SD rats after FST. In addition to the functions of GABA, the MFP may also decrease the turnover ratio of DA in the frontal cortex and striatum. It has more potential than GABA to have an antidepressant-like effect as fluoxetine but without the side effects. In this study, we found that MFP has better antidepressant-like effects than GABA in SD rats after FST. Use of the tail suspension test in mice and learned helplessness in rats needs to be further investigated in our study.

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