



The Role of Banana (*Musa balbisiana* Colla) Peel Floss as Functional Food Matrix to Alleviate Chronic Stress

Andreanyta Meliala^{1*}, Paramita Narwidina², Irwan Supriyanto³, Yogi Tri Sumarno⁴, Yanasta Yudo Pratama⁵, Rian Damayanti⁵

¹Department of Physiology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Jalan Farmako Sekip Utara, Sleman, Yogyakarta, Indonesia; ²Clinical Nutrition Research Group, Yogyakarta, Indonesia; ³Departement of Psychiatry, FM-PHN, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Jalan Farmako Sekip Utara, Sleman, Yogyakarta, Indonesia; ⁴Undergraduate Program in School of Medicine, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Jalan Farmako, Sekip Utara, Yogyakarta, Indonesia; ⁵Master Program of Department of Physiology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Jalan Farmako, Sekip Utara, Yogyakarta, Indonesia

Abstract

BACKGROUND: Banana peel (*Musa balbisiana* Colla) already has a diverse variety of nutritional benefits, but its perishable nature causes additional food processing, one of which is banana peel floss.

AIM: The objective of this study was to examine the antidepressant effects of banana peel floss in experimental animals subjected to chronic mild stress (CMS) for 6 weeks.

METHODS: Animals were randomly assigned into five groups. The first group was fed a control diet without CMS as a negative control. The other four groups were exposed to CMS and fed a control diet as a positive control, with three of the five groups fed a control diet supplemented with 15%, 30%, and 60% banana peel floss. The tail suspension test (TST) and the Morris water maze (MWM) were used as behavioral parameters in this study. Cortisol and serotonin levels were measured in two stages: After CMS exposure or before banana peel floss intervention, and after 4 weeks of banana peel floss intervention.

RESULTS: The results showed that the immobility time in TST and escape latency in the MWM test were significantly reduced in the groups supplemented with 15% and 30% banana peel floss, respectively. We observed a significant association between serotonin and cortisol levels and also between the duration of immobility time in TST and serotonin levels.

CONCLUSIONS: The administration of banana peel floss caused significant changes in plasma serotonin concentrations, implying that dietary fiber, tryptophan, and bioactive components in banana peel floss can reduce stress-induced depression by regulating cortisol levels and increasing serotonin levels.

Edited by: Sinisa Stojanoski

Citation: Meliala A, Narwidina P, Supriyanto I, Sumarno YT, Pratama YY, Damayanti R. The Role of Banana (*Musa balbisiana* Colla) Peel Floss as Functional Food Matrix to Alleviate Chronic Stress. Open Access Maced J Med Sci. 2021 Jun 21; 10(A):1608-1616. https://doi.org/10.3889/oamjms.2022.8432

Keywords: Banana peel; Floss; Chronic mild stress; Tryptophan; Dietary fiber; Antioxidant

***Correspondence:** Andreanyta Meliala, Department of Physiology, Universitas Gadjah Mada, Jalan Farmako, Sekip Utara, Yogyakarta 55281, Indonesia.

E-mail: a_meliala@ugm.ac.id

Received: 28-Dec-2021

Revised: 19-Jan-2022

Accepted: 28-Jan-2022

Copyright: © 2022 Andreanyta Meliala, Paramita Narwidina, Irwan Supriyanto, Yogi Tri Sumarno, Yanasta Yudo Pratama, Rian Damayanti

Funding: This research was supported under the grant of 2021 Hibah Dana Masyarakat from the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Indonesia.

Competing Interests: The authors have declared that no competing interests exist

Open Access: This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

Introduction

Stress is a common occurrence in everyday life. According to the World Health Organization (WHO), in a world of nearly 350 million people, the prevalence of stressful events is high. On a global scale, stress is the third leading cause of disease. There has been an increasing trend since 2004 and it is expected to continue until 2030 [1]. Exposure to psychological stressors can cause either eustress or distress. Individuals who are in a state of eustress are more likely to achieve their life goals, whereas those who are in a state of distress are more likely to suffer physical and mental harm. Distress might cause sequential hormonal and neurotransmission functional changes, including an increase in noradrenergic activity and cortisol levels. Chronic stress can alter brain structure by inducing pyramidal cells atrophy and reducing hippocampal volume. It can also increase the

hypothalamic-pituitary-adrenal axis (HPA axis) activity, which has a negative correlation with serotonergic neurotransmission in the brain. Research has shown that high cortisol levels are linked to the binding of serotonin 1A receptors in all parts of the brain [2]. Low levels of serotonergic activity were associated with depression or depressive-like behavior in people with chronic stress.

Chronic distress might lead to depression through disruption in serotonin pathways. The most common is serotonergic dysfunction, through several mechanisms involving the presence of hypercortisolemia conditions that induce tryptophan 2.3 dioxygenases, which will metabolize L-tryptophan thereby reducing the availability of L-tryptophan for serotonin synthesis and causing an increase in pro-inflammatory cytokines. Other studies [3], [4], [5] have shown that chronic stress can result in increased cortisol, resulting in a decrease in serotonin release, which is linked to activation of the glucocorticoid (GR) tryptophan dioxygenase in the liver. Chronic stress causes the body to produce large

amounts of glucocorticosteroids and results in high cortisol levels [6].

The role of nutrients in various mental health disorders, including depression, has been the subject of recent research. Some nutrients have significant relevance to depression, such as carbohydrates, protein (amino acid), and bioactive compounds [7], [8]. Carbohydrates have been shown to have an impact on mood and behavior [9], [10]. When people eat a carbohydrate-rich meal, the body releases insulin, which makes it easier for blood sugar to enter cells and be used as energy, as well as helping tryptophan to enter the brain when compared to a high Glycemic Index (GI) food, which provides immediate but temporary relief, fruits, and vegetables have a moderate and long-lasting effect on mood [11]. A fruit that is rich in complex carbohydrates and has been known to have anti-anxiety potential is bananas. Not only has the pulp proven beneficial, the peel has the potential to be further processed (the portion of the banana peel is about 40% of the total weight of the banana) because it contains phyto serotonin as an antidepressant of about 170,000 ng/g and the pulp contains about 35,000 ng/g [12] and is also high in tryptophan, an amino acid that aids in the production of serotonin in the brain. Tryptophan is a serotonin precursor that plays a role in regulating sleep and eating patterns, regulates locomotor and affects emotional states, and affects the occurrence of depression [13]. As the link between tryptophan and serotonin suggests, changes in the gut microbiota may play a role in the pathophysiology of human central nervous system disorders [14].

Banana peels are rapidly degenerate soon after harvest therefore requires further processing into more durable food products with low water content, such as banana peel floss. However, there are few studies concerning the link between diet quality and mental health variables such as depression levels, which are linked to cortisol and serotonin levels. Based on this background information, this study aimed to formulate the yellow *Kepok* banana peel floss appropriately and examine its antidepressant property, so it can become a reference for healthy diet choices that can provide a protective effect on the brain and mental health by increasing the availability of tryptophan in the body for use in serotonin synthesis.

Methods

Preparation of banana peel floss

Kepok bananas (*Musa balbisiana* Colla) were purchased from a traditional market in Sleman Regency, Yogyakarta, Indonesia. After sorting, the banana peels were washed with running water, soaked in a 0.5% citric acid solution for 10 min, then boiled with spices for 10 min

before being pounded and then stir-fry with added spices (50 ml coconut milk, 5 g salt, 6 g garlic, 5 g onion, 5 g lemongrass, and 20 g brown sugar for 100 g banana peel) until dry for 20 min, then bake for 100 min at 180°C.

Determination of proximate composition, antioxidant, tannin, and dietary fiber

The banana peel floss's proximate compositions were determined using the official method proposed by the Association of Official Agricultural Chemists (AOAC, 1995). Moisture content was analyzed with the oven-drying method (AOAC Official Method 977.11); for analyzing crude proteins, the Kjeldahl method (AOAC Official Method 955.04) was used. The crude fat content was determined using the Soxhlet method (AOAC Official Method 960.39), while the ash content was determined using the dry ashing method (AOAC Official Method 923.03). The crude fiber content was determined using the gravimetric method (AOAC Official Method 991.43). Dietary fiber analysis was carried out using the AOAC method 2011.25 [15]. Tannin content was determined according to the AOAC method [16]. Finally, banana peel floss was evaluated on antioxidant activity with DPPH radical scavenging activity [9]. The total carbohydrate content of the banana peel floss was calculated as follows:

$$(\text{total carbohydrate } [\% \text{ wet basis}] = 100\% - \% [\text{moisture} + \text{ash} + \text{crude protein} + \text{crude fat}]).$$

High-performance liquid chromatography for tryptophan analysis

The AOAC method was used to measure tryptophan [17] with 4.2M NaOH at 110°C under an atmosphere of N₂ for 20 h.

Animals and diets

The present experiment was approved by the Ethical Committee of the Faculty of Medicine, Universitas Islam Indonesia (approval number 19/Ka.Kom.Et/70/KE/II/2021). Adult male Wistar rats (mean weight 26826.22, provided by the Laboratory of Physiology, Universitas Islam Indonesia) were used, and they were acclimated for 7 days to get used to single housing, handling, and normal environmental conditions with a 12-h light-dark cycle before the experiments, 27.00 ± 0.57°C temperature, and 73% ± 0.03% humidity. The experiments were performed on male rats at the age of 3 months (90 days). The duration of the experiment was 73 days (42 days of stress exposure; day 1–42), 28 days of banana peel floss administration (day 43–71), 1 day of tail suspension test (TST) and Morris water maze (MWM) (day 72), and 1 day (day 73) of decapitation procedures, blood, and organ collection. Except during the deprivation feed and drink periods of the chronic mild stress (CMS) protocol, control diets and water were provided *ad libitum*.

This study aimed to see how effective banana peel floss was in treating behaviors parameters of depression in the CMS model. Explanation regarding the study protocol is shown in Figure 1. As a result, after the adaptation period, the animals were randomly assigned to control and intervention groups. The control groups were the negative control group (NC; without CMS protocol and food supplementation) and the positive control group (PC; received CMS protocol without food supplementation). There were three intervention groups, each received CMS protocol and different concentrations of food supplementation (CB 15%; CB 30%, and CB 60%; supplemented with 15%, 30%, and 60% banana peel floss).

Table 1: Experimental diet composition (%)

Components	Groups			
	NC and PC	CB 15	CB 30	CB 60
Moistures	6.80 ± 0.005 ^a	10.79 ± 0.60 ^b	10.09 ± 0.35 ^b	12.13 ± 0.35 ^c
Protein	4.7 ± 0.05 ^a	13.03 ± 0.44 ^b	11.56 ± 0.52 ^b	12.97 ± 0.38 ^b
Fat	18.14 ± 0.035 ^a	6.97 ± 1.71 ^b	8.94 ± 1.41 ^b	14.38 ± 1.42 ^b
Crude Fiber	5.16 ± 0.12 ^a	5.45 ± 0.44 ^a	5.40 ± 0.39 ^a	6.95 ± 0.42 ^b
Ash	10.92 ± 0.035 ^a	7.16 ± 0.42 ^b	7.82 ± 0.34 ^b	11.10 ± 0.47 ^a
Carbohydrate	54.30 ± 0.09 ^a	56.59 ± 0.01 ^b	56.19 ± 0.04 ^c	42.48 ± 0.06 ^d
Tannin	12.91 ± 0.08 ^a	1.94 ± 0.02 ^b	3.87 ± 0.03 ^c	7.74 ± 0.05 ^d
Phenolic	20.10 ± 0.22 ^a	3.01 ± 0.03 ^b	6.03 ± 0.06 ^c	12.06 ± 0.13 ^d
Dietary fiber	27.66 ± 0.17 ^a	13.50 ± 0.83 ^b	15.99 ± 0.65 ^c	24.29 ± 0.60 ^d
Tryptophan (ppm)	306.93 ± 0.93 ^a	46.04 ± 0.14 ^b	92.08 ± 0.28 ^c	184.16 ± 0.56 ^d

The data were presented as mean±SEM. Means with a different superscript in the same rows significantly differ (p ≤ 0.05) according to Duncan Multiple Range Test. NC: Control diet, without being exposed to CMS; PC: Control diet, CMS; CB 15: Control diet supplemented with 15% banana peel floss, CMS; CB 30: Control diet supplemented with 30% banana peel floss, CMS; and CB 60: Control diet supplemented with 60% banana peel floss, CMS).

Table 1 reports proximate composition and content of bioactive compounds of banana peel floss and control diet supplemented with banana peel floss examined in this study. Banana peel floss has significantly higher carbohydrate, tannins, phenolic, dietary fiber, and tryptophan as compared to the control diet supplemented with banana peel floss with varied concentrations. However, the control diet supplemented

with banana peel floss 60% demonstrated significantly higher tannin, phenolic, dietary fiber, and tryptophan as compared to the control diet supplemented with banana peel floss 15% and 30%.

CMS protocol

Except for the normal control (NC) group, all rats were exposed to chronic stress daily between 09:00 and 14:00 for 6 weeks. These stressors based on a previous study with slight modification [18] comprises damp bedding with 250 mL water in each cage (24 h), cold water spray (5 mL of 4°C, the animals' heads were suddenly drenched in water), tilted cage (45°, for 24 h), the hairdryer's hot-air steam (10 min), and sawdust removal (for 24 h). These stressors were applied daily, as shown in Figure 1.

Measurement of body weight and feed intake

intake

To determine what has changed in body weight and feed intake, in this study we measured body weight and feed intake before the CMS period (basal), at the days 7, 14, 21, 28, 35, and 42 during CMS and at the days 7, 14, 21, and 28 during banana peel intervention. The basal measurement was converted to 100 %, and changes were expressed as a percentage on the selected days. The amount of feed consumed was calculated using the formula [19]: weight of control diets given to rats per day - the weight of the leftover control diets the next day.

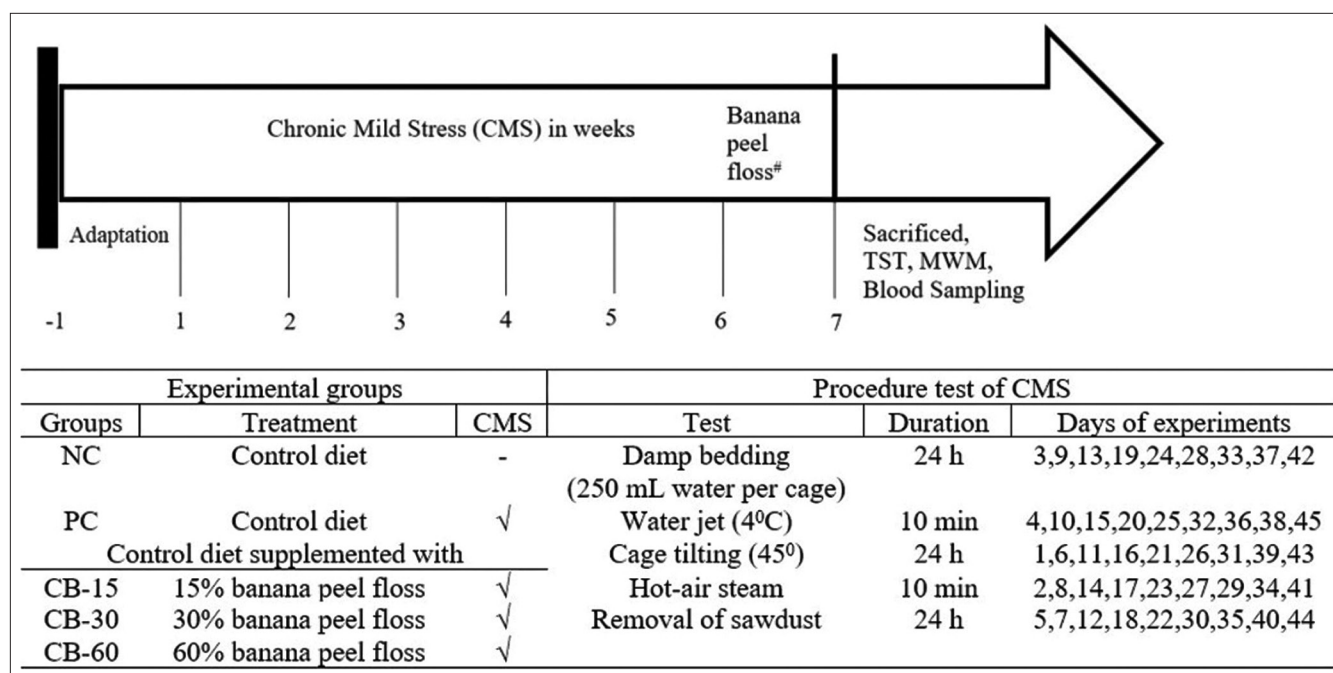


Figure 1: Experimental design and CMS procedure for 6 weeks. Control (NC) and CMS group received control diet supplemented with banana peel floss 15%, 30%, and 60% for 4 weeks

Behavioral tests

The MWM and TST were used to evaluate depressive-like behaviors for 1 day after CMS, with a 60-min interval between tests.

MWM

The MWM test was conducted according to the protocols described elsewhere [20], [21], [22]. The test apparatus was a large, white-painted circular pool with a diameter of 150 cm and a height of 40 cm. The pool was filled to a depth of 18 cm with water. 2 cm below the water's surface, a white circular platform was placed. Fresh milk was added to the water to make it opaque, which helped to hide the platform. The water was around 25°C. A video camera was placed above the pool's center and transmitted the image of the animals' movements to a laptop computer nearby. The pool was divided into four equal-sized imaginary quadrants. Around the pool's circumference wall, four equal-distance starting points were marked. The test began last day of banana peel floss intervention, the platform in the circular pool was positioned at the edge of the quadrant. It was expected that the rats might accidentally find the hidden platform and climb onto it. At each trial, the rat was given a maximum of 2 min to swim to the hidden platform. The rat's escape latency to find the platform was recorded, and if he was unable to do so within 2 min, he was given a latency score of 2 min.

TST

The TST is generally utilized in antidepressant preclinical testing [23], [24], [25]. Individual animals were suspended 50 cm above the ground using an adhesive tape-attached small metal hook attached to the tip of the tail. The total duration of immobility during the last 4 min of a 6-min testing period was used to determine the immobility time duration.

Plasma preparation

As much as 2 ml of blood samples were taken from a syringe retro-orbital with a sterile hematocrit capillary tube to help avoid periorbital infection [26]. A blood sample was taken in two stages: the pre-stage (after 6 weeks of CMS exposure) and the post-stage (after consuming banana peel floss for 4 weeks). The supernatant plasma was separated by centrifugation at 3000 rpm for 15 min at 4°C. The obtained plasma was used for an Enzyme-linked immunosorbent assay (ELISA).

ELISA

Cortisol Elisa Kit 96 wells (Calbiotech, California, USA) and General 5-Hydroxytryptamine ELISA Kit 96 wells (ABclonal, Wuhan, Tiongkok) were

used for cortisol and serotonin analysis to quantify the concentration of rats after exposure to CMS (after CMS exposure for 6 weeks or before banana peel floss intervention) and after banana peel floss intervention. After performing the experimental procedure according to the instruction of each kit, absorbance was measured at the wavelength recommended by each kit using a Bio-Rad iMark Elisa reader with a wavelength at 450 nm.

Statistical analysis

One-way analysis of variance (ANOVA) was performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Variance analysis of the experimental design for body weights as well as the change (delta) was tested using an independent sample t-test and $p < 0.05$ was considered statistically significant. Duncan's Multiple Range Test was used to measure the data of cortisol and serotonin levels; escape latency in MWM; immobility time duration in the TST.

Results

In the present study, 10 weeks of the results of the experiments were split into two stages: 6 weeks of chronic stress induction and 4 weeks of banana peel floss supplementation. The animals administered a control diet supplemented with banana peel floss 30% exhibited a significant increase in body weight in week 1 when compared to the NC group (without CMS and banana peel floss administered) and showed a significant decrease in body weight in weeks 2 and 3 when compared to PC groups, and a significant increase in body weight was observed in Group CB 30 when compared to group NC. However, there were no significant differences in the body weight changes of rats in the NC and PC groups when compared to Groups CB 15 and CB 60 as shown in Figure 2. This is closely related to the amount of feed intake, the results of average daily feed intake in Table 2 indicate that there was a significant lower in feed intake in the treatment group with control diet supplemented with 15%, 30, and 60% of banana peel floss when compared to Groups NC and PC. The groups that consumed banana peel floss with tryptophan intake content of 11.51; 23.02; and 46.04 mg/kg had a

Table 2: Average daily feed consumption of rats during banana peel floss intervention

Groups	Average feed intake, g
NC	20.00 ± 0.00
PC	20.00 ± 0.00
CB 15	17.57 ± 2.04 [#]
CB 30	17.63 ± 1.02 [#]
CB 60	18.18 ± 1.50 [#]

An independent sample t-test was used to perform statistical analysis of the data, * $p < 0.05$ was compared to the NC group. [#] $p < 0.05$ was compared to PC. NC (control diet, without exposed to CMS); PC (control diet, CMS); CB 15 (control diet supplemented with 15% banana peel floss, CMS); CB 30 (control diet supplemented with 30% banana peel floss, CMS); and CB 60 (control diet supplemented with 60% banana peel floss, CMS).

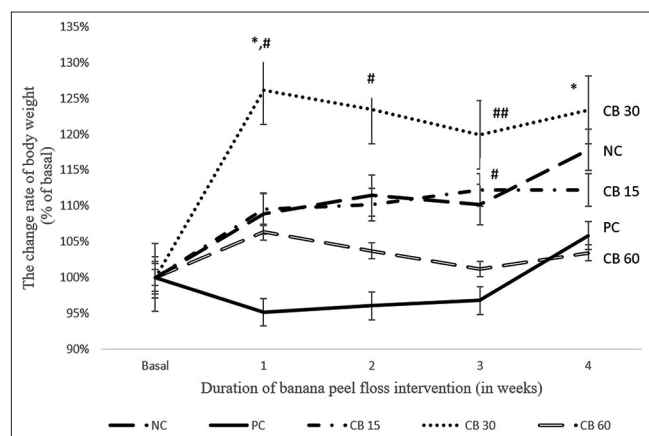


Figure 2: The effect of banana peel floss on body weight in all rat groups as a percentage of baseline body weight ($n = 6-8$). An independent sample *t*-test was used to perform statistical analysis of the data, $*p < 0.05$ compared to the NC group. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ were compared to PC group (each experiment, $n = 8$ except in group PC, $n = 6$ due to rats died during stress exposure). Data represented Mean \pm SEM. NC (control diet, without being exposed to CMS); PC (control diet, CMS); CB 15 (control diet supplemented with 15% banana peel floss, CMS); CB 30 (control diet supplemented with 30% banana peel floss, CMS); and CB 60 (control diet)

significantly lower feed intake compared to the group without banana peel floss consumption (Table 2).

We measured changes in cortisol and serotonin concentrations in the plasma of rats to see if treatment with banana peel floss affects the concentrations of stress-related hormones and depression-related neurotransmitters, respectively. The data from the pre-test revealed that CMS has effects on plasma cortisol and serotonin level (Table 3). Compared to the group of rats who had not been exposed to CMS for 6 weeks (NC group), the rats who had been exposed to CMS had significantly higher levels of cortisol and serotonin

Table 3: Effect of banana peel floss on plasma cortisol and serotonin levels

A. Cortisol levels (ng/mL)			
Groups	Pre	Post	Fold change [#] (Cortisol _{post/pre})
NC	8.81 \pm 1.03 ^a	12.03 \pm 1.15 ^a	1.37 \pm 0.10 ^a
PC	9.42 \pm 0.95 ^a	16.85 \pm 2.59 ^{ab}	1.80 \pm 0.26 ^c
CB 15	10.65 \pm 1.16 ^b	13.02 \pm 1.76 ^{ab}	1.23 \pm 0.20 ^a
CB 30	9.24 \pm 0.74 ^a	14.78 \pm 1.06 ^c	1.60 \pm 0.13 ^b
CB 60	10.76 \pm 1.15 ^b	14.60 \pm 0.75 ^{bc}	1.37 \pm 0.25 ^a
B. Serotonin levels (ng/mL)			
Groups	Pre	Post	Fold change [#] (Serotonin _{post/pre})
NC	40.70 \pm 7.81 ^{ab}	18.40 \pm 0.91 ^a	0.61 \pm 0.13 ^a
PC	53.52 \pm 5.78 ^b	21.29 \pm 6.45 ^b	0.41 \pm 0.11 ^{ab}
CB 15	22.99 \pm 2.83 ^a	23.24 \pm 1.38 ^a	1.09 \pm 0.11 ^{bc}
CB 30	33.16 \pm 8.07 ^a	25.64 \pm 5.14 ^a	0.90 \pm 0.10 ^{cd}
CB 60	22.85 \pm 6.22 ^a	28.08 \pm 3.80 ^a	1.48 \pm 0.16 ^d

[#]Change concerning the baseline levels. Values are presented as mean \pm S. Duncan's Multiple Range Test shows that mean values in a column separated by different superscript letters are significantly different ($p < 0.05$). NC (control diet, without being exposed to CMS); PC (control diet, CMS); CB 15 (control diet supplemented with 15% banana peel floss, CMS); CB 30 (control diet supplemented with 30% banana peel floss, CMS); and CB 60 (control diet supplemented with 60% banana peel floss, CMS).

Figure 3 shows MWM activity in control and banana peel floss supplemented animals, which shows that the MWM activity is expressed as a time to find the hidden platform. This test showed optimum doses of banana peel floss improved the cognitive dysfunction induced by CMS protocol. The rats with a control diet supplemented with 30% banana peel floss subjected to CMS showed a significantly shorter escape latency, and all doses of

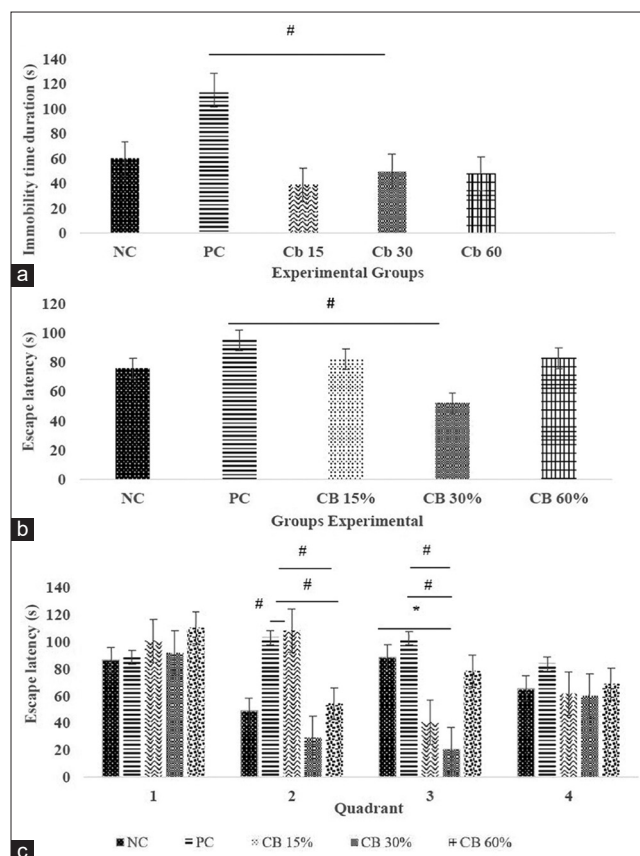


Figure 3: Effect of banana peel floss on the immobility time duration in the tail suspension test (a), one impaired learning (time latency to reach the platform in Morris water maze (b), on the time (s) spent each quadrant during the trial in Morris water maze test memory (c). Immobility duration was measured for the last 4 min of TST. Data represent the mean \pm SEM. Significance was determined by one-way ANOVA followed Duncan's Multiple Range Test. $*p < 0.05$ compared to NC group. $^{\#}p < 0.05$, as compared to PC group. NC (control diet, without being exposed to CMS); PC (control diet, CMS); CB 15 (control diet supplemented with 15% banana peel floss, CMS); CB 30 (control diet supplemented with 30% banana peel floss, CMS); and CB 60 (control diet supplemented with 60% banana peel floss, CMS).

banana peel floss showed not significantly compared with the negative control. They also spent shorter times in the target quadrant compared to all other groups.

Chronic stress significantly increased cortisol levels, the increased cortisol level was significantly higher in the PC group (1.80 ± 0.26) compared to other groups suggesting the effect on CMS on cortisol level. The CB 15 and CB 60 groups had a mean of 1.37-fold increase, similar to NC groups. The CB 30 group showed an increase in cortisol level up to 1.60 times the change (post/pre) in the banana peel intervention with various doses, which was significantly different from the CB 15 and CB 60 groups.

Discussion

The mechanism of tryptophan catabolism can cause a chemical link between mood, food, and obesity.

Mood changes can affect a person's food choices [27]. Tryptophan has been shown to have an anorexic effect on animals by some researchers [28], [29], [30]. Long-term administration of tryptophan to obese human subjects was found to reduce food intake. Tryptophan administration to non-fasted control rats (25–100 mg/kg i.p.) was found to cause a significant reduction in 24-h food intake [31]. High tryptophan diet, such as the Atkins diet and decreased body weight [29]. These findings are supported by the results of our study, which found that.

In addition to the tryptophan content in banana peel floss, the presence of dietary fiber also contributes to weight loss possibly through the following mechanisms: Soluble fiber produces glucagon-like peptide and peptide YY (PYY) when fermented in the large intestine, both hormones. The gut plays a role in inducing satiety [32] and dietary fiber has been shown to reduce energy intake significantly [33]. Even though dietary fiber contributes to a diet's total caloric content, it is much more resistant to digestion in the small and large intestines [34].

TST is a behavioral test to examine depressive-like behavior in the animal. The depressive-like behavior was measured using the immobility duration, which expresses the degree of depression or tension. In our study, experimental groups without banana peel floss supplementation exhibited depressive-like behavior, evidenced by higher immobility time when compared to experimental groups with banana peel floss supplementation. A control diet with 15% banana peel floss showed an optimal effect on the reduction of immobility time in TST. Lower immobility time showed that the rats actively pursue escape-directed behaviors, suggesting lower depressive-like behavior [35].

Our finding showed that the antidepressant effect is not dose-dependent. This finding is in concordance with fact that tryptophan metabolism involved rate-limiting enzyme, which means higher tryptophan supplementation will not further increase the antidepressant effect.

The HPA axis mediates the stress response, which is a common feature in people who are more likely to develop mental disorders [36]. The HPA axis controls the release of GRs (cortisol in humans and corticosterone in rodents) into the mind to bind with mineralocorticoid and GR receptors. These receptors are responsible for the negative feedback system on cortisol release in stress conditions. Studies showed that a lop-sidedness in this system can cause short-and long haul adverse consequences in the cerebrum, including neuronal demise, eased back neurogenesis, debilitated synaptic associations, expanded aggravation, and impeded learning and memory measures [37].

The presence of tryptophan, a precursor of serotonin, in banana peel floss may result in a decrease in cortisol levels and increased serotonin levels [38].

Other contents of the banana peel floss that might also affect the levels of cortisol and serotonin are carbohydrates (dietary fiber) and antioxidants (phenol and tannin). Consumption of carbohydrate compounds can alter growth hormone and cortisol levels, with high carbohydrate consumption lowering cortisol levels in the blood [39]. We observed a significant association between serotonin and cortisol levels and also between the duration of immobility time in TST and serotonin levels. These findings are consistent with recent research that found cortisol increases the expression of the gene encoding the serotonin transporter, associated with increased serotonin uptake and chronic activation of the serotonin transporter system. Exposure to intense or prolonged stress is characterized by persistent HPA activation and increased circulating cortisol. This will, in turn, reduce serotonin levels [40]. Other studies also reported an association between cortisol and serotonin. Serotonin interacts with dopamine and cortisol in complex ways, so that serotonin can increase dopamine production while inhibiting cortisol production in general [41]. These processes are most likely involved in the antidepressant effect of tryptophan, the precursor for serotonin.

Recent research has attempted to determine whether carbohydrate nutrition in the diet has an impact on mental disorders. A higher dietary GI and glycemic load are often associated with a higher risk of prevalence and incidence of depression and no significant association was found between dietary starch and the incidence of depression [42]. Increased fruit and vegetable consumption were also linked to a lower risk of depressive symptoms, while dietary fiber intake was linked to a lower risk of depressive symptoms, owing to dietary fiber's protective effect against the negative effects of postprandial hyperglycemia [42]. Dietary fiber also acts as an effective prebiotic, stimulating the growth of intestinal bacteria, particularly lactic acid bacteria, which can aid in the treatment of intestinal flora disorders caused by stressful conditions [43]. The brain gut axis concept depicts a two-way relationship between the gastrointestinal tract and the brain, which is followed by specific bacteria producing various metabolites including short chain fatty acids in improving mental health problems caused by chronic stress conditions [44].

Carbohydrate consumption has also been associated with an increased serotonin synthesis in the brain [45]. As previously described, tryptophan is a serotonin precursor that competes for the same transport system across the blood-brain barrier as larger amino acids. An increase in the ratio of tryptophan to other amino acids in plasma allows tryptophan to cross the blood-brain barrier and contribute to the formation of serotonin through the transport system. When carbohydrates are consumed, insulin is released, which can stimulate greater absorption of competing amino acids into muscle tissue, and an increase in the ratio

of tryptophan to other amino acids in plasma allows tryptophan to cross the blood-brain barrier through the transport system and aid in the production of serotonin [46].

Major depression is associated with decreased brain serotonin function and increased cortisol secretion, which may help to bridge the gap between these two characteristics. Consumption of banana peel floss for 4 weeks after a chronic stress intervention might restore cognitive function, therefore, enhanced the memory and decreasing the time to reach the hidden platform, similar to that of the negative control. Tryptophan metabolism is regulated by cortisol and is important in serotonin synthesis [47]. The availability of tryptophan, an amino acid precursor, in the plasma is required for serotonin synthesis in the brain. Tryptophan content in banana peels is expected [48] to increase the availability of tryptophan in the body and activate enzymatic machinery in the liver and blood, including TDO (tryptophan 2,3-dioxygenase enzyme), which is activated when blood tryptophan levels increase.

Conclusion

It may be concluded that banana peel floss has an antidepressant effect in the CMS rat model, which might occur through the mechanism of controlling cortisol and serotonin plasma through the agency of the phytochemical compounds. The compounds include antioxidants and tryptophan as precursors for the formation of serotonin and dietary fiber in normalizing the function of the gut-brain axis. The mechanisms that influence their effects are still unknown. Further research is needed to confirm these findings.

References

- Bishwajit G, Leary DP, Ghosh S, Sanni Y, Shangfeng T, Zhanchun F. Association between depression and fruit and vegetable consumption among adults in South Asia. *BMC Psychiatry*. 2017;17(1):15. <https://doi.org/10.1186/s12888-017-1198-1>
PMid:28088202
- Steinberg LJ, Rubin-Falcone H, Galfalvy HC, Kaufman J, Miller JM, Sublette ME, *et al*. Cortisol stress response and *in vivo* pet imaging of human brain serotonin 1A receptor binding. *Int J Neuropsychopharmacol*. 2019;22(5):329-38. <https://doi.org/10.1093/ijnp/pyz009>
PMid:30927011
- Santymire RM, Manjerovic MB, Sacerdote-Velat A. A novel method for the measurement of glucocorticoids in dermal secretions of amphibians. *Conserv Physiol*. 2018;6(1):coy008. <https://doi.org/10.1093/conphys/coy008>
PMid:29479435
- Ehiaghe FA, Digban KA, Ehiaghe IJ. Effect of stress of examination on serum cortisol level and cd4 cell count in male undergraduates at Igbinedion University, Nigeria. *African J Cell Pathol*. 2014;2(1):19-23.
- Badawy AA. Modulation of tryptophan and serotonin metabolism as a biochemical basis of the behavioral effects of use and withdrawal of androgenic-anabolic steroids and other image-and performance-enhancing agents. *Int J Tryptophan Res*. 2018;11:1178646917753422. <https://doi.org/10.1177/1178646917753422>
PMid:29487480
- Prete A, Taylor AE, Bancos I, Smith DJ, Foster MA, Kohler S, *et al*. Prevention of adrenal crisis: Cortisol responses to major stress compared to stress dose hydrocortisone delivery. *J Clin Endocrinol Metab*. 2020;105(7):2262-74. <https://doi.org/10.1210/clinem/dgaa133>
PMid:32170323
- Bell IR, Edman JS, Morrow FD, Marby DW, Mirages S, Perrone G, *et al*. B Complex vitamin patterns in geriatric and young adult inpatients with major depression. *J Am Geriatr Soc*. 1991;39(3):252-7. <https://doi.org/10.1111/j.1532-5415.1991.tb01646.x>
PMid:2005338
- Young SN. Folate and depression: A neglected problem. *J Psychiatry Neurosci*. 2007;32(2):80-2.
PMid:17353937
- Kim DO, Lee KW, Lee HJ, Lee CY. Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals. *J Agric Food Chem*. 2002;50(13):3713-7. <https://doi.org/10.1021/jf020071c>
PMid:12059148
- Swann OG, Kilpatrick M, Breslin M, Oddy WH. Dietary fiber and its associations with depression and inflammation. *Nutr Rev*. 2020;78(5):394-411. <https://doi.org/10.1093/nutrit/nuz072>
PMid:31750916
- Rao TS, Asha MR, Ramesh BN, Rao KS. Understanding nutrition, depression and mental illnesses. *Indian J Psychiatry*. 2008;50(2):77-82. <https://doi.org/10.4103/0019-5545.42391>
PMid:19742217
- Rayne S. Concentrations and profiles of melatonin and serotonin in fruits and vegetables during ripening: A mini-review. *Nat Preced*. 2010;4722. <https://doi.org/10.1038/npre.2010.4722.1>
- Ittiyavirah S, Anurenj DA. Adaptogenic studies of acetone extract of *Musa paradisiaca* L. Fruit peels in albino Wistar rats. *Int J Nutr Pharmacol Neurol Dis*. 2014;4(2):88-94. <https://doi.org/10.4103/2231-0738.129592>
- Jenkins TA, Nguyen JC, Polglaze KE, Bertrand PP. Influence of tryptophan and serotonin on mood and cognition with a possible role of the gut-brain axis. *Nutrients*. 2016;8(1):56. <https://doi.org/10.3390/nu8010056>
PMid:26805875
- Tobaruela EC, O Santos AO, de Almeida-Muradian LB, Araujo ED, Lajolo FM, Menezes EW. Application of dietary fiber method AOAC 2011.25 in fruit and comparison with AOAC 991.43 method. *Food Chem*. 2018;238:87-93. <https://doi.org/10.1016/j.foodchem.2016.12.068>
PMid:28867106
- Atanassova M, Christova-Bagdassaria V. Determination of tannins content by titrimetric method for comparison of different plant species. *J Univ Chem Technol Metall*. 2009;44(4):413-5.
- Allred MC, MacDonald JL. Determination of sulfur amino acids and tryptophan in foods and food and feed ingredients: Collaborative study. *J Assoc Off Anal Chem*. 1988;71(3):603-6.
PMid:3391969
- Gáll Z, Farkas S, Albert Á, Ferencz E, Vancea S, Urkon M, *et al*.

- Effects of chronic cannabidiol treatment in the rat chronic unpredictable mild stress model of depression. *Biomolecules*. 2020;10(5):801. <https://doi.org/10.3390/biom10050801>
PMid:32455953
19. Omotola MB, Adeosun IO, Obuotor EM, Akomolafe RO, Ayannuga OA. Assessment of the effects of graded doses of polyphenolic-rich fraction of *Garcinia kola* seeds on pituitary-Testicular axis of male wistar rats. *Dose Response*. 2017;15(4):1559325817729260. <https://doi.org/10.1177/1559325817729260>
PMid:29051716
 20. Yan T, He B, Wan S, Xu M, Yang H, Xiao F, et al. Antidepressant-like effects and cognitive enhancement of *Schisandra chinensis* in chronic unpredictable mild stress mice and its related mechanism. *Sci Rep*. 2017;7(1):6903. <https://doi.org/10.1038/s41598-017-07407-1>
PMid:28761074
 21. Lam V, Takechi R, Albrecht MA, D'Alonzo ZJ, Graneri L, Hackett MJ, et al. Longitudinal performance of senescence accelerated mouse prone-strain 8 (SAMP8) mice in an olfactory-visual water maze challenge. *Front Behav Neurosci*. 2018;12:174. <https://doi.org/10.3389/fnbeh.2018.00174>
PMid:30210312
 22. Hermawati ER, Arfian NU, Mustofa M, Partadiredja G. Spatial memory disturbance following transient brain ischemia is associated with vascular remodeling in hippocampus. *Kobe J Med Sci*. 2018;64(3):E93-106.
PMid:30666039
 23. Zhua WL, Shia HS, Weic YM, Wang SJ, Suna CY, Dinga ZB, et al. Green tea polyphenols produce antidepressant-like effects in adult mice. *Pharmacol Res*. 2012;65(1):74-80. <https://doi.org/10.1016/j.phrs.2011.09.007>
PMid:21964320
 24. Akkol EK, Dereli FT, Ilhan M. Assessment of antidepressant effect of the aerial parts of *Micromeria myrtifolia* Boiss. and Hohen on mice. *Molecules*. 2019;24(10):1869. <https://doi.org/10.3390/molecules24101869>
PMid:31096603
 25. Aslam M. Tail suspension test to evaluate the antidepressant activity of experimental drugs. *Bangladesh J Pharmacol*. 2016;11(2):292-4. <https://doi.org/10.3329/bjp.v11i2.26517>
 26. Donovan J, Brown P. Blood collection. *Curr Protoc Neurosci*. 2005;33(1):171-9. <https://doi.org/10.1002/0471142735.im0107s73>
PMid:18432965
 27. Strasser B, Gostner JM, Fuchs D. Mood, food, and cognition: Role of tryptophan and serotonin. *Curr Opin Clin Nutr Metab Care*. 2016;19(1):55-61. <https://doi.org/10.1097/MCO.0000000000000237>
PMid:26560523
 28. Nieuwenhuizen AG, Hochstenbach-Waelen A, Veldhorst MA, Westerterp KR, Engelen MP, Brummer RJ, et al. Acute effects of breakfasts containing α -lactalbumin, or gelatin with or without added tryptophan, on hunger, "satiety" hormones and amino acid profiles. *Br J Nutr*. 2021;101(12):1859-66. <https://doi.org/10.1017/S0007114508131774>
PMid:19017422
 29. Coşkun Ş, Özer Ç, Gönül B, Take G, Erdoğan D. The effect of repeated tryptophan administration on body weight, food intake, brain lipid peroxidation and serotonin immunoreactivity in mice. *Mol Cell Biochem*. 2006;286(1-2):133-8. <https://doi.org/10.1007/s11010-005-9103-5>
PMid:16432760
 30. Niesink RJ, Van Ree JM. Short-term isolation increases social interactions of male rats: A parametric analysis. *Physiol Behav*. 1982;29(5):819-25. [https://doi.org/10.1016/0031-9384\(82\)90331-6](https://doi.org/10.1016/0031-9384(82)90331-6)
PMid:7156221
 31. Ju CY, Tsai CT. Serotonergic mechanisms involved in the suppression of feeding by 5-HTP in rats. *Chin J Physiol*. 1995;38(4):235-40.
PMid:8925676
 32. Keenan H, Doria A, King G, Aiello L. Positivity of C-peptide, GADA and IA2 antibodies in Type 1 diabetic patients with extreme duration. *Diabetes*. 2006;55:A65.
 33. Tucker LA, Thomas KS. Increasing total fiber intake reduces risk of weight and fat gains in women. *Nutrition*. 2009;139(3):567-81. <https://doi.org/10.3945/jn.108.096685>
PMid:19158230
 34. Lattimer JM, Haub MD. Effects of dietary fiber and its components on metabolic health. *Nutrients*. 2010;2(12):1266-89. <https://doi.org/10.3390/nu2121266>
PMid:22254008
 35. Cryan JF, Mombereau C, Vassout A. The tail suspension test as a model for assessing antidepressant activity: Review of pharmacological and genetic studies in mice. *Neurosci Biobehav Rev*. 2005;29(4-5):571-625. <https://doi.org/10.1016/j.neubiorev.2005.03.009>
PMid:15890404
 36. Hori H, Ozeki Y, Teraishi T, Matsuo J, Kawamoto Y, Kinoshita Y, et al. Relationships between psychological distress, coping styles, and HPA axis reactivity in healthy adults. *J Psychiatr Res*. 2010;44(14):865-73. <https://doi.org/10.1016/j.jpsychires.2010.02.00>
PMid:20334880
 37. Lippi SL. Brain sciences: chronic mild unpredictable stress and high-fat diet given during adolescence impact both cognitive and noncognitive behaviors in young adult mice. *Brain Sci*. 2021;11(2):260. <https://doi.org/10.3390/brainsci11020260>
PMid:33669543
 38. Capello AE, Markus CR. Effect of sub chronic tryptophan supplementation on stress-induced cortisol and appetite in subjects differing in 5-HTTLPR genotype and trait neuroticism. *Psychoneuroendocrinology*. 2014;45:96-107. <https://doi.org/10.1016/j.psyneuen.2014.03.005>
PMid:24845181
 39. Stachowicz M, Lebidzińska A. The effect of diet components on the level of cortisol. *Eur Food Res Technol*. 2016;242(12):2001-9. <https://doi.org/10.1007/s00217-016-2772-3>
 40. Tafet GE, Idayaga-Vargas VP, Abulafia DP, Calandria JM, Roffman SS, Chiovetta A, et al. Correlation between cortisol level and serotonin uptake in patients with chronic stress and depression. *Cogn Affect Behav Neurosci*. 2001;1(4):388-93. <https://doi.org/10.3758/cabn.1.4.388>
PMid:12467090
 41. Kim YR, Park BK, Kim YH, Shim I, Kang IC, Lee MY. Antidepressant effect of *Fraxinus rhynchophylla* hance extract in a mouse model of chronic stress-induced depression. *Biomed Res Int*. 2018;2018:8249563. <https://doi.org/10.1155/2018/8249563>
PMid:30065945
 42. Gangwisch JE, Hale L, Garcia L, Malaspina D, Opler MG, Payne ME, et al. High glycemic index diet as a risk factor for depression: Analyses from the women's health initiative. *Am J Clin Nutr*. 2015;102(2):454-63. <https://doi.org/10.3945/ajcn.114.103846>
PMid:26109579
 43. Madison A, Kiecolt-Glaser JK. Stress, depression, diet, and the gut microbiota: Human-bacteria interactions at the core of psychoneuroimmunology and nutrition. *Curr Opin Behav Sci*.

- 2019;28:105-10. <https://doi.org/10.1016/j.cobeha.2019.01.011>
PMid:32395568
44. Van de Wouw M, Boehme M, Lyte JM, Wiley N, Strain C, O'Sullivan O, *et al.* Short-chain fatty acids: Microbial metabolites that alleviate stress-induced brain-gut axis alterations. *J Physiol.* 2018;596(20):4923-44. <https://doi.org/10.1113/JP276431>
PMid:30066368
45. Wurtman RJ, Wurtman JJ. Brain serotonin, carbohydrate-craving, obesity and depression. *Obes Res.* 1995;3 Suppl 4:477S-80. <https://doi.org/10.1002/j.1550-8528.1995.tb00215.x>
PMid:8697046
46. Wurtman RJ, Hefti F, Melamed E. Precursor control of neurotransmitter synthesis. *Pharmacol Rev.* 1980;32(4):315-35.
PMid:6115400
47. Cowen PJ. Cortisol, serotonin and depression: All stressed out? *Br J Psychiatry.* 2002;180:99-100. <https://doi.org/10.1192/bjp.180.2.99>
PMid:11823315
48. Meliala A, Andwi Y, Sumiwi AR, Narwidina P, Sulisty SR, Setyaningsih W. Banana peel flakes alleviate blood glucose and stress in a dose-dependent manner. *Int J Pharm Pharm Sci.* 2020;12(8):75-81. <https://doi.org/10.22159/ijpps.2020v12i8.37659>