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Research Article

Hepatoprotective Effect of Germinated Sang-Yod Rice in Obese Mice

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Abstract

Background: Obesity can be predisposed to Non-Alcoholic Fatty Liver Disease (NAFLD). The pathogenesis of NAFLD shows multiple-hit effects, but mainly causing inflammation and apoptosis to the liver. γ-Aminobutyric acid (GABA) in foods has been advantageous for decreasing liver inflammation.

Aim: Germinated Sang-Yod rice was evaluated for abilities of improving serum lipid profiles and fat accumulation in the livers of obese mice.

Methods: Four groups of mice (n=8) were independently fed with a distinct diet for 12 weeks, including normal diet (CONTROL group), High Fat Diet (HFD group), high fat diet plus 20 mg/kg/day simvastatin (POSITIVE group), and high fat diet plus 0.5% germinated Sang-Yod rice per kg per day (HFD-GR group). Mice body weights were recorded weekly. Measurements of % visceral fat, blood glucose, serum lipid profiles, and histological analysis were also conducted.

Results: The body weights of mice in HFD-GR and HFD groups were comparable. Less body weight was apparent for mice in POSITIVE group. The visceral fat of mice in HFD-GR group was significantly higher than those of other HFD-feeding groups, including CONTROL group. Concentrations of blood glucose and serum lipids, such as triglycerides, LDL, total cholesterols, and HDL, of all HFD-feeding mice were found to be in similar ranges. But LDL and total cholesterol levels were significantly lowered in CONTROL mice. By feeding mice with 0.5% germinated Sang-Yod rice per kg per day, the degrees of fat degeneration and lipid accumulation in liver tissues were improved to be comparable to those of CONTROL mice.

Conclusion: Germinated Sang-Yod rice possibly possessed some roles in lipid metabolisms and underlining mechanisms are going to find out.

Keywords: Sang-Yod Rice; Germination; Aminobutyric Acid; Hepatoprotection; Obese Mice

Abbreviations

HFD: High Fat Diet; GR: Germinated Sang-Yod Rice; NAFLD: Non-Alcoholic Fatty Liver Disease; GABA: γ-Aminobutyric Acid; TG: Triglycerides; LDL: Low-Density Lipoprotein; TC: Total Cholesterol; HDL: High-Density Lipoprotein; H&E: Hematoxylin and Eosin

Background

Changes to modern life styles of people, in particular, to the consumption of high fat diets mostly contribute to obesity. Consistently with animal studies, high fat diets containing ≥ 30% of total energy from fats causes obesity in rats, mice, dogs and primates as a result of increased energy intake and efficient energy storage. Obesity is a predisposing factor to a variety of metabolic diseases, including type 2 diabetes, cardiovascular diseases, and Non-Alcoholic Fatty Liver Disease (NAFLD). The incidence of NAFLD ranges between 6 and 35 % worldwide. Recently, this trend is considerably increasing due to western dietary preference [1]. The pathogenesis of NAFLD shows multiple-hit effects, causing moderate to severe inflammation and apoptosis of various tissues and organs, including the liver [2]. The safety and efficacy of western medication in treating NAFLD are currently limited. Rather, diet control is proof better for prevention and curing of liver diseases by modulating lipid metabolisms and associated pathways. Nevertheless, a combination of medications and lifestyle changes is a common treatment plan to correct blood cholesterol levels [3].

Simvastatin is an HMG-CoA reductase inhibitor and administered to hyperlipidemic patients for decreasing LDL and triglyceride levels and increasing HDL level. The drug is on the World Health Organization's list of essential medicines because it elicits a lower risk to cardiovascular system [4]. Accordingly, simvastatin was used in this study as a positive control by supplemented with high fat diet at a dose of 20 mg/kg body weight/day entire the period of 12 weeks [5].

The US Food and Drug Administration has approved soy protein and isoflavones as healthy foods for controlling triglyceride and total cholesterol levels [6]. In Chinese medicine, soup containing mung bean (Vigna radiata) has been prepared to detoxify the body [7]. Because of containing large amounts of protein, essential amino acids, and -Aminobutyric Acid (GABA), mung bean is consequently potential as antioxidant and hepatoprotective agent [8]. Germinated brown rice is a rich source of GABA [9] and has been consumed for controlling blood lipid profiles [10]. To date, *in vivo* hypolipidemic

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and hepatoprotective effects of germinated Sang-Yod rice have not been investigated. In this project, we evaluated such health benefits of germinated Sang-Yod rice through determinations of blood glucose, serum lipid profiles, and liver histopathology in obese mice induced by high fat diet.

Methods

Preparation of germinated Sang-Yod rice

Sang-Yod is one of red rice varieties of southern Thailand. The dehulled seeds with perfect germs were soaked in tap water for 12 h in the dark at room temperature and replaced by new water every 4 h. After that the rice was wrapped in cheesecloth and maintained in moist atmosphere with good ventilation for 18 h to achieve germination. The germinated rice was dried in a hot air oven at 45°C for 8 h, ground to fine powder by using multi-function disintegrator (WF-20B), and kept at 4°C until use.

Animals

Thirty-two male C57BL/6J mice, aged 6 weeks old and weighed 22±3 g, were purchased from Monash University, Kuala Lumpur, Malaysia. They were housed 4 per cage in a room at 22± 2°C with 12 h lighting and accessible to food and water ad libitum. Normal and High Fat Diets (HFD) were obtained from Altromin Spezialfutter GmbH & Co., KG, Germany. The diets' compositions were detailed in Table 1. HFD-GR was prepared by thoroughly mixing 0.5% of germinated Sang-Yod rice per kilogram body weight per day with HFD described above and pelleted in a mold to imitate its previous form. After 2 weeks of adaptation, mice were divided into 4 groups by keeping an equal starting body weight per group. These included CONTROL (a group of mice that fed with normal diet); HFD (a group of mice that fed with HFD); POSITIVE (a group of mice that fed with HFD plus 20 mg/kg/day simvastatin; and HFD-GR (a group of mice that fed with HFD-GR diet). The feeding programs were continued for 12 weeks. Changes of mice body weight and the amount of intake were recorded weekly. Experiments involving animals were performed in accordance with the ethical guidelines for laboratory animal at Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia.

Manipulations of visceral fat, blood sample, and liver tissue

Mice were anaesthetized intra-peritoneal by using an overdose of pentobarbital (80mg/kg) with a volume of 0.1 ml per 10 g of body weight in 1 ml syringe 23-gauge, 5/8-inch needle, and sacrificed by cardiac puncture. Visceral fat was removed and weighed immediately by using a weighting machine. Livers were kept in suitable containers at -80°C until use. Blood samples were collected and serums were separated by centrifugation at 2,000 rcf for 15 min.

Determinations of blood glucose and serum lipids

Glucose levels in blood samples were measured by using Accu-Chek kit, according to procedures as recommended by the manufacturer (Roche Diagnostic Co., Ltd). Serum samples were used for analysis of Triglycerides (TG), Low-Density Lipoprotein (LDL), Total Cholesterol (TC), and High-Density Lipoprotein (HDL). They were sent to and assayed by Pathlab, Petaling Jaya, Selangor, Kuala lumper, Malaysia.

Histological analysis

Liver tissues were cut into 5-mm thick slices, fixed in 10% neutral buffered formalin overnight, and paraffin-embedded. The embedded sample was sectioned at a 3-μm thickness by using Leica RM2235 manual rotary microtome. The section was expanded on a slide and kept at -20°C for 1 h. Next, it was stained with Hematoxylin and Eosin (H&E) (Sigma-Aldrich, St. Louis, MO, USA) according to established standard procedures, and visualized under a light microscope (Olympus-bx53-ccd). For Oil red O staining, the embedded section at a thickness range of 5-10 μm was prepared by using cryo-section method. It was air-dried for 30 min at room temperature, fixed in 10% iced-cold formalin and air-dried again. After washing with dH_2O and completely dried, the section was stained with Oil red O by using standard techniques. Slice's samples were visualized by using a light microscope.

Statistical analysis

Data were expressed as mean ± SEM (n=8). Individual group difference between the means was evaluated by one-way analysis of variance (ANOVA), followed by Tukey's multiple range tests using SPSS 19.0 statistical software packages. Differences were statistically significant at $p<0.05$.

Results

Body weight

The weekly body weights of mice entire 12 weeks of the feeding programs were summarized in Table 2. The mean body weight of mice corresponding to CONTROL, HFD, and HFD-GR groups were not significantly different. But there was statistical difference of the mean body weight of mice in POSITIVE and HFD groups after two weeks of the trial. Increased body weight of mice in each group was significantly apparent after 12 weeks.

It was noted that a time period required to adapt to each diet for each group of mice was different, during which mice did not gain weight. These were respectively corresponding to 3 weeks for CONTROL group, 4 weeks for HFD group, 7 weeks for HFD-GR group, and 8 weeks for POSITIVE group.

Visceral fat content

Figure 1 shows % visceral fat of mice fed with different diets for 12 weeks. Increased visceral fat content was found in an order as CONTROL < HFD < POSITIVE < HFD-GR, respectively. The fat

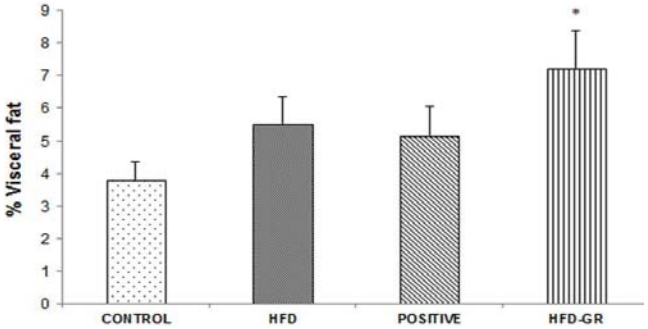

Figure 1: The percentage of visceral fat at 12 weeks of the feeding program; mice were fed with normal diet (CONTROL), HFD, HFD plus 20 mg/kg/day simvastatin (POSITIVE), and HFD plus 0.5% germinated Sang-Yod rice per kg per day (HFD-GR); Data were mean ± SEM; *, $p < 0.05$ compared to CONTROL group.

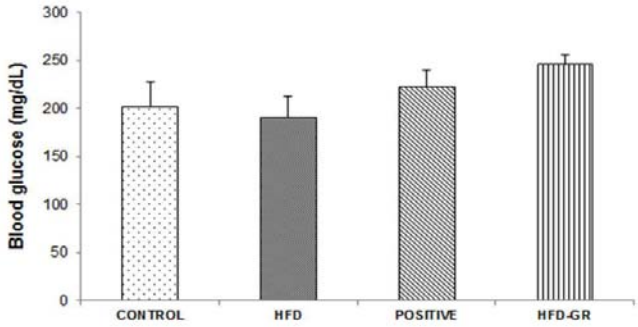

Figure 2: Blood glucose levels of mice fed with normal diet (CONTROL), HFD, HFD plus 20 mg/kg/day simvastatin (POSITIVE), and HFD plus 0.5% germinated Sang-Yod rice per g per day (HFD-GR) for 12 weeks.

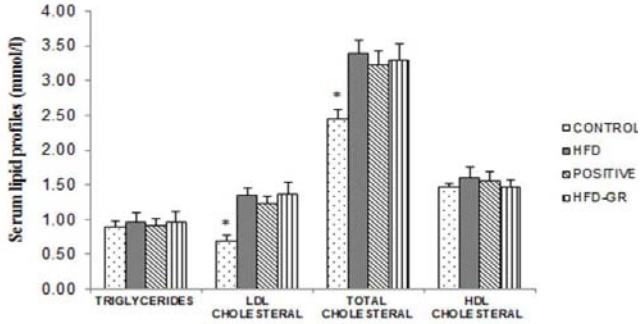

Figure 3: Levels of TG, LDL, TC, and HDL in serums of mice fed with normal diet (CONTROL), HFD, HFD plus 20 mg/ kg/day simvastatin (POSITIVE), and HFD plus 0.5% germinated Sang-Yod rice per kg per day (HFD-GR) for 12 weeks; Data were mean ± SEM; *, $p < 0.05$ compared to CONTROL group.

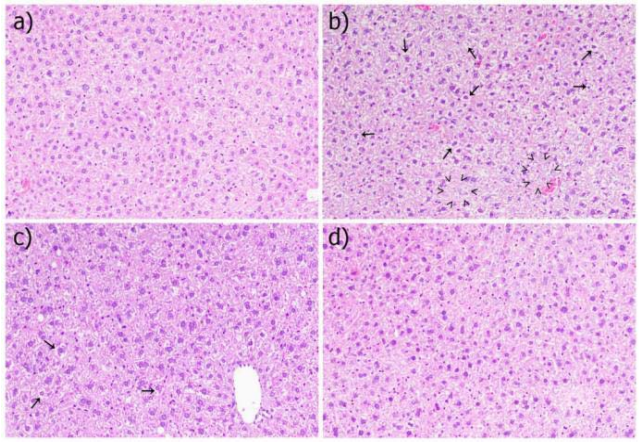

Figure 4: Representative photomicrographs of H&E staining of the livers in each group; mice were fed with (a) normal diet, (b) HFD, (c) HFD plus 20 mg/kg/day simvastatin, and (d) HFD plus 0.5% germinated Sang-Yod rice per kilogram per day for 12 weeks; Magnification, 100x;, fat degeneration;, cytoplasmic vacuole (For color interpretation, readers may refer to the web version of this article).

deposition was significantly enhanced in mice of HFD-GR group in compared to those of CONTROL and HFD groups. The increase of % visceral fat was not proportionally related to the final body weight of mice, however.

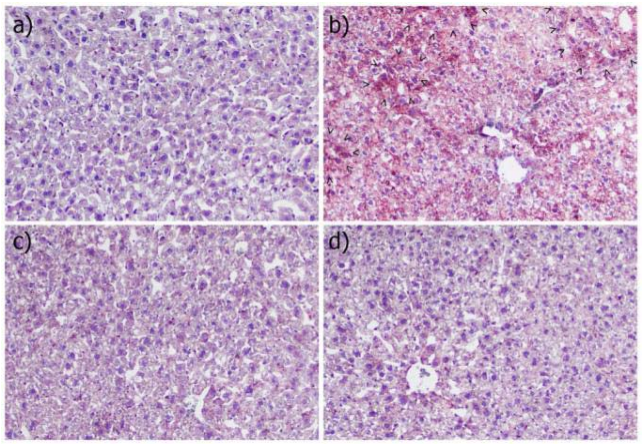

Figure 5: Representative photomicrographs of Oil red O staining of the livers in each group; mice were fed with (a) normal diet, (b) HFD, (c) HFD plus 20 mg/kg/day simvastatin, and (d) HFD plus 0.5% germinated Sang-Yod rice per kilogram per day for 12 weeks; Magnification, 100x;, lipid droplet (For color interpretation, readers may refer to the web version of this article).

Table 1: Compositions of normal diet and HFD used for feeding mice.

On a caloric basis	Normal diet (C1090–10)	HFD (C1090–60)
(per 100 g)	(weight per 10% energy from fat)	(weight per 60% energy from fat)
Moisture	7.90%	2.90%
Crude Ash	4.30%	3.20%
Crude Fiber	3.10%	4.70%
Crude Fat	4.00%	35.00%
Crude Protein	20.70%	21.00%
Nitrogen free extractives	60.00%	33.20%
Total calories	**351 kcal**	**523 kcal**

Blood glucose

Blood glucose levels of mice in CONTROL, HFD, POSITIVE, and HFD-GR groups were determined and displayed in Figure 2. Differences in blood glucose levels among these groups of mice were not revealed by 12 weeks of the feeding program (see Methods).

Serum lipid profiles

Serum lipids, including TG, LDL, TC, and HDL, of mice fed with distinct diets for 12 weeks were shown in Figure 3. There was resemblance in TG and HDL levels for all groups of mice. LDL levels of mice in HFD, POSITIVE and HFD-GR groups were fairly higher than those of CONTROL group. In addition, mice of HFD, POSITIVE and HFD-GR groups distinctly presented higher levels of TC in compared to CONTROL mice.

Histological analysis of the livers

There were no histological abnormalities in liver tissues of CONTROL mice (Figure 4a). In contrast, fat degeneration and cytoplasmic vacuoles in hepatocytes were clearly observed in HFD-treated mice (Figure 4b). Simvastatin treatment was unable to alleviate fat degeneration of the livers (Figure 4c). However, as supplemented with 0.5% germinated Sang-Yod rice per kilogram body weight per day, decreased hepatic steatosis was exhibited (Figure 4d). It was slightly different in histopathological signs for simvastatin treatment

Table 2: Body weight[a] of mice measured weekly entire 12 weeks of feeding.

Week No.	CONTROL	HFD	POSITIVE	HFD-GR
0	21.33±0.5	23.76±0.6[b]	19.78±0.6[b]	22.65±0.7
1	22.30±0.6	24.46±0.6	21.04±1.0[b]	23.51±0.6
2	23.12±0.6	25.52±0.9	22.28±1.1[b]	24.54±0.9
3	24.38±0.4*	26.19±0.8	22.84±1.0[b]	25.67±0.9
4	25.01±0.4*	27.15±0.8*	22.83±1.2[b]	26.29±1.2
5	24.94±0.4*	27.74±0.9*	23.21±1.1[b]	27.33±1.3
6	25.46±0.6*	28.88±1.0*	24.32±1.3[b]	28.34±1.1
7	26.26±0.6*	29.47±1.1*	25.51±1.3[b]	29.07±1.2*
8	26.60±0.7*	29.43±1.1*	26.15±1.6	29.27±1.4*
9	26.79±0.7*	29.25±1.1*	26.07±1.6	29.92±1.6*
10	26.74±0.7*	29.63±1.0*	25.69±2.0	29.96±1.7*
11	27.39±0.8*	30.58±0.7*	26.91±1.5*	31.31±1.7*
12	27.39±0.7*	30.63±0.7*	26.92±1.5*	31.91±1.8*

[a] Data were % weight gain ± SEM; *, $p < 0.05$ compared to the body weight at the 1st week of feeding of each corresponding group; [b], $p < 0.05$ compared to HFD group.

in compared to the untreated control (Figure 4b and c).

In addition, cellular components in response to inflammation were not detected. Increased intracellular lipid deposition was considerably detected in HFD-feeding mice, in compared to CONTROL mice (Figure 5a and b). The lipid droplets were markedly decreased by treating with 20 mg/kg/day simvastatin (Figure 5c). Interestingly, such decreasing was much higher in HFD mice supplemented with 0.5% germinated Sang-Yod rice per kilogram body weight per day (Figure 5d).

Discussion

Feeding mice with high fat diet resulted in weight gain. The extent of weight change was corresponded to how much energy from fat in the intake (data not shown). However, it appeared that enhancing visceral fat was indirectly proportional to gaining of weight (Figure 1). In agreement with previous reports, changes of body weight are significantly dependent on what diets individuals consume. Nevertheless, augmentation of visceral fat and body weight is independently associating [11]. Whether changes in visceral fat are associated with future development of hyperlipidemia will be described subsequently.

NAFLD is conceded in accompany with changes of endogenous lipid metabolism [2]. In this study, serum TG, LDL, and TC levels increased significantly in HFD-feeding mice after 12 weeks, suggesting that hyperlipidemia was likely developed in these mice. It was noted that serum TG, LDL, and TC were not reduced to normal levels following treated with simvastatin and germinated Sang-Yod rice by 12 weeks (Figure 3). However, fewer lipid droplets were accumulated in the livers of HFD-GR mice in compared to those of HFD mice, as evidenced by diminution of Oil red O staining (Figure 5). Thus, germinated Sang-Yod rice significantly reversed liver damage and dysfunction caused by hyperlipidemia. In estimation, bioactive compounds present in this germinated rice might render antioxidant and anti-inflammatory activities able to recover damaged hepatic cells to more healthy counterparts, consisting with previous studies [12].

Elevation of blood glucose is considered as the "first hit" in multiple-hit hypothesis of NAFLD [13]. As indicated in Figure 2, mice of POSITIVE and HFD-GR groups exhibited higher blood glucose levels, although insignificantly different, in compared to those determined for CONTROL and HFD mice. It was noted that the liver cells of HFD mice were considerably malfunctioned even challenged by a relatively lower concentration of blood glucose, resulting in lipid metabolic abnormality with increased lipid droplets accumulation (Figure 5). Interestingly, decreased fat degeneration, cytoplasmic vacuoles, and lipid accumulation were indicated by feeding mice with HFD-GR (Figure 4 and 5). Such accomplishments as demonstrated by germinated Sang-Yod rice would result from unsaturated fatty acids, mostly oleic and linoleic acids therein (personal communication, data not shown), consisting with previous studies [14]. Accordingly, germinated Sang-Yod rice supplement at a dose of 0.5% per kilogram body weight per day was beneficial for NAFLD by returning abnormal liver cells to normal cell status, contributing to its hepatoprotective property.

Conclusion

In conclusion, NAFLD in our mice model demonstrated a significant increase of lipid accumulation in both serum and the liver. Germinated Sang-Yod rice showed a marked modulating effect on lipid metabolism, possibly concerning a number of pathways in the body. Molecular mechanisms of any presumed pathways that influenced by germinated Sang-Yod rice are going to be researched.

Ethics Approval

Animal use of this study was approved by the Ethics Committee for Laboratory Animal of Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia.

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Authors' Contributions

NW and SP were involved in study design, data collection, analysis, and interpretation, and writing a draft manuscript. JK, KH, AU, UK, and JK were involved in study design, protocol development, revision of data and the findings, and finalizing the manuscript. All authors read the final version of the manuscript before approval.

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