Full Length Research Paper

Acute and subchronic toxicity evaluation of the hydroethanolic extract of germinated brown rice

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Accepted 24 July, 2012

The hydroethanolic extract obtained from a germinated brown rice (*Oryza sativa* L. indica) grown under conditions that favor high concentration of γ -aminobutyric acid (GABA) was evaluated for acute and subchronic toxicities in rodents. Intragastric administration of this extract to Swiss albino mice of both sexes at a single dose of 2 g/kg body weight produced no toxicity signs during 7 days of observation. For the subchronic toxicity study, the germinated brown rice extract at 75, 150, and 300 mg/kg body weight was administered daily to Wistar rats by oral gavage for 12 weeks. Ten male and ten female rats were used for each dose. In all instances, consumption of the extract showed no adverse effects on behavior, growth or health status of these animals throughout the study period. After 12 weeks of feeding, no significant dose-related differences in blood biochemical parameters were detected among the groups. Hematological profiles of the test groups did not alter from those of the controls. In addition, either gross necropsy or histopathological examination of their visceral organs did not reveal any abnormal appearances. These results suggest that either short or long-term exposure of the extract from GABA-rich germinated brown rice grains may have no negative influence on general well-being of our experimental animals.

Key words: Acute toxicity, brown rice extract, germinated brown rice, subchronic toxicity.

INTRODUCTION

Rice (*Oryza sativa* L.) is the main staple food of Asia, making it the second most consumed cereal grain worldwide (Patil and Khan, 2011). Rice is not only a good source of protein and carbohydrate, but it also contains plenty of bioactive substances beneficial for health, which exist mainly in the germ and bran layers of the grain (Juliano, 1992). Generally, rice is milled to improve its

taste and texture before consumption. Milling or polishing process, however, removes the outer bran layer of the rice grain causing a significant decrease in its nutritional and bio-functional components. Despite the fact that the unpolished rice or brown rice has high nutritional value, its intake is unpopular. Unlike the ordinary milled rice or white rice, brown rice requires long cooking time due to the hard texture. Cooked brown rice is also inferior to cooked white rice in terms of both appearance and flavor.

During germination, biochemical activities in rice kernel change drastically in order to produce essential elements and energy for the formation of seedling. As a result, many nutrients and bioactive components are highly

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increased in the germinated rice seeds (Khampang et al., 2009). Germinated brown rice (GBR) is achieved by soaking the whole kernel of brown rice in water until its embryo begins to bud. The process of germination makes GBR much more nutritious than ordinary brown rice as polysaccharides and proteins are hydrolyzed leading to an increase of simple sugars and amino acids in addition to a high production of bio-functional substances, such as γ -aminobutyric acid (GABA), inositols, α -tocopherol, tocotrienols, ferulic acid, and γ -oryzanol (Kayahara et al., 2001; Komatsuzaki et al., 2007; Moongngarm and Saetang, 2010). The reaction between phytic acid and minerals during the birth of sprout helps soften the texture of GBR and thus causes it to be cooked easily (Kayahara et al., 2001). In comparison to cooked non-germinated brown rice, cooked GBR is sweeter, softer, more swelled, and cohesive (Jiamyangyuen and Oorikul, 2008). Despite the fact that GBR is known for its high nutritional value and biological properties that offer health benefits, the main reason that GBR gains much attention lately is the high GABA content. GABA is a nitrogenous compound produced primarily from decarboxylation of glutamic acid by enzyme glutamate decarboxylase which is highly active in rice seedlings during germination (Liu et al., 2005). In human, its major role as an inhibitory neurotransmitter involving in the regulation of brain functions is well-established (Watanabe et al., 2002). This amino acid derivative also possesses other health effects such as anti-hypertension (Aoki et al., 2003), anti-cancer (Oh and Oh, 2004), anti-hyperlipidemia (Roohinejad et al., 2010), and lowering blood sugar level (Shallan et al., 2010). Although, biological properties of GBR and its contents, especially GABA, have been extensively studied (Patil and Khan, 2011) and the popularity of GABA and GBR products among health-conscious consumers becomes increased, a systemic toxicological evaluation of GABA derived from GBR for human consumption remains to be established. Earlier study has demonstrated that GBR does not affect the blood parameters in mice (Shirai et al., 2010). In this study, we carried out both acute and subchronic toxicity assays to evaluate the safety of GABA containing extract from germinated brown rice. The data obtained from this study would increase confidence on GBR as well as GABA consumptions.

MATERIALS AND METHODS

Plant and preparation of GBR extract

The brown rice grains of a traditional Southern Thailand variety called "Chor Lung" were obtained from Rice Research Center in Pattani province, Thailand. The voucher specimen (SKP 081151901) is kept at the Herbarium of the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand. They were germinated under conditions that give the highest GABA content by soaking in citrate buffer, pH 3 at 40°C (Banchuen et al., 2010). After soaking for 72 h, the germinated rice grains were washed with distilled water, and were dried at 50°C, and then

ground to powder. To prepare the extract, 2 kg of the sample were successively macerated with 1:1 (v/v) of water:ethanol (3 L $\times$ 3 times). The extract was filtered, concentrated to dryness under reduced pressure, and freeze-dried. Its yield was 0.7% (w/w). Dried extract was then stored at 4°C in sealed plastic bags and were kept away from light after preparation. The same batch of material was used throughout this study.

Experimental animals

Swiss albino mice weighing 28 to 35 g of both sexes and Wistar rats weighing, on average, 200 g for female and 260 g for male were obtained from the Laboratory Animal Facility Unit, Faculty of Science, Prince of Songkla University, Hat Yai, Thailand. They were housed in identical wire-mesh-bottomed stainless-steel cages containing 5 or 10 rats per cage and were maintained in an air-conditioned room at 25 $\pm$ 2°C, 50 to 60% relative humidity, and artificial illumination between 06:00 and 18:00 h. Commercial chow diets (C.P. Mice Feed[®], Charoen Phokphand Group, Bangkok, Thailand) and drinking water were provided *ad libitum*. All procedures concerning animal treatments and experimentations in this study were reviewed and approved by the Institutional Committee for Ethical Use of Experimental Animals at Prince of Songkla University (approval no. 2412010).

Acute toxicity study

The acute toxicity test in this study was performed in Swiss mice using an up and down procedure (Bruce, 1985). Ten mice per sex were randomly assigned to the control and treated groups. The GBR extract dissolved in distilled water was orally given to each mouse in the test groups at a single dose of 2 g extract/kg body weight. The control animals, however, received the water vehicle instead. Following administration, animals were observed closely during the first day, and occasionally, thereafter, for 7 days, for toxic signs and symptoms, and death. At the end of the period, all survivors were sacrificed to examine gross changes in their vital organs.

Subchronic toxicity study

The method was performed based on the Organization of Economic Co-operation and Development (OECD) guideline for the testing of chemicals: Test No. 408 (OECD, 1998). Male and female Wistar rats were randomly divided into four groups of ten. They were acclimatized for 7 days before beginning the experiments. During the entire 12 weeks, the extract solution prepared as mentioned earlier was administered orally to the animals in each treatment group daily at doses of 0, 75, 150, and 300 mg extract/kg body weight. The animals were observed daily for their clinical signs and any behavioral changes. They were weighed initially and then once a week until termination.

At the end of the study, the rats were anesthetized with ether following an overnight fasting. Their blood was collected by heart puncture for hematology and biochemical analyses. All hematological measurements were performed by an automatic analyzer of Nihon Kohden Model Celltac E (MEK-7222) (Nihon Kohden Corp., Tokyo, Japan). Plasma samples were assayed for their biochemical levels using a BioSystems Lab Automation Model A15 (BioSystems S.A., Barcelona, Spain).

Each rat was sacrificed by cervical dislocation and then an autopsy was performed. The heart, liver, spleen, and kidneys were macroscopically observed and weighed immediately after removal. Samples of these organs were fixed in 10% (v/v) neutral buffered formalin and were kept in the solution for further histopathological

examination.

Statistical analysis

All data are presented as means $\pm$ standard error of mean (SEM). They were subjected to one-way analysis of variance (ANOVA) and differences between means were evaluated by Duncan's multiple range test at 95% confidence level. Significance was judged at $P \leq 0.05$.

RESULTS

Acute toxicity study

All mice treated with the GBR extract at a single dose of 2 g/kg body weight by intragastric gavage survived until the end of experimental period. They did not exhibit any signs of toxicity during the 7 days of observation. There were no obvious differences between the treated and untreated animals. The gross examinations of their internal organs also found no pathological abnormalities.

Subchronic toxicity study

Ingestion of the extract at any tested dose did not cause mortality or toxic symptoms in the rats. The average daily food and water intake among the groups of each sex were similar throughout this study. There was no unusual behavior or physical appearance among these animals. In all cases, their feces were dry and dark indicating no abnormalities in their digestive tracts. Measurements of the body weight over the whole experimental period found no differences among the four male groups at any time point (Figure 1). The female rats, however, hardly gained weight as the experiment progressed. Between the control and treatment groups, their rates of weight increase were not significantly different (Figure 1).

As shown in Table 1, feeding of the extract up to the highest dose of 300 mg/kg/day for consecutive 12 weeks did not produce any significant dose-related effects on blood chemistry parameters in female rats. In male groups receiving the extract at low dose (75 mg/kg/day) and middle dose (150 mg/kg/day), however, their plasma triglycerides, cholesterol, and aspartate aminotransferase (AST) concentrations were markedly increased from the control values at the end of the treatment ($P \leq 0.05$), whereas protein, blood urea nitrogen (BUN), and uric acid levels were slightly but significantly higher ($P \leq 0.05$) (Table 1).

To determine if the extract could affect blood cells and the bone marrow activity of the rats, hematological examinations were performed. As presented in Table 2, plasma of both male and female groups receiving the extract at low and middle doses contained more platelets, but significant differences were not found among groups ($P > 0.05$). Also, eosinophil counts in all treated female

groups seem to become lower with increasing doses, but they were not significantly different from the control value ($P > 0.05$). Despite the mentioned changes, no hemolyzed plasma was obtained and normal blood smears were observed in all of the animals.

At autopsy, macroscopic examinations of vital organs including heart, liver, kidney, and spleen in our experimental rats did not show any abnormality in their gross appearances and weights as a result of the consumption of the extract (Table 3). In addition, we did not detect any damage in their gastrointestinal tracts, the potential and direct target for toxic effects of ingested foods. The results from gross examination were also confirmed by histopathological assessment. The extract did not produce any significant histological changes in the organ tissues of any of the animals (Figure 2).

DISCUSSION

In this study, the GABA containing extract was prepared from germinated grains of "Chor Lung", one of the popular traditional rice varieties planted in Southern Thailand (Rice Department, Ministry of Agriculture and Cooperatives, Thailand, available at <http://ricethailand.go.th>). The results of our acute toxicity study indicate that this GBR extract is non-toxic to mice after they have been exposed for short time to a high dosage. In the subchronic experiments, rats were given the extract up to 300 mg/kg/day which is four-fold higher than the level obtained from an estimated human consumption (50 g brown rice/kg/day). During the 12 week-period, we did not observe any treatment-associated effects on the body weights of both male and female rats suggesting that the extract did not alter their metabolic rates or improved digestion and absorption of foods in these animals. In a previous study, Oh et al. (2005) found that the intake of GBR water extract containing 841 nmoles of GABA/ml for 8 weeks effectively reduces abdominal fat without lowering the weight and rate of weight increase in mice fed a high fat diet. Although, the water extract of GBR as well as the whole GBR improve the serum lipid profiles in high fat diet-induced hyperlipidemic animals (Oh et al., 2005; Miura et al., 2006; Roohinejad et al., 2010), GBR intake does not reduce plasma lipids in normal mice (Shirai et al., 2010). In the present study, we did not observed any significantly different blood lipid results among our female rats. The measurably increased amount of plasma triglycerides in both male groups is unknown. It could have been the results of biological variation among rats rather than the treatment effect, because their dose levels did not vary with the extract doses and were still within the reference intervals for Wistar rats (Boehm et al., 2007). Our finding that GBR extract did not suppress plasma lipids when animals were not fed additional fat is in agreement with that of Shirai et al. (2010), and thus suggests that a reduction in blood lipids found in animals

Figure 1. Body weights of male (M) and female (F) rats fed daily for 12 weeks with the hydroethanolic extract of germinated brown rice at various doses. Values are mean $\pm$ SD; $n = 10$. The body weights of untreated female group at the 4 to 12th week and those of all male groups at the 3 to 12th week were significantly different ($P \leq 0.05$) from their corresponding initial values.

fed a high fat diet may be due to inhibitory effects of some ingredient(s) rather than GABA in GBR on dietary fat absorption. Hypoglycemic action of GBR on post-prandial blood in healthy subjects has been reported (Ito et al., 2005; Seki et al., 2005; Shallan et al., 2010) and insoluble fiber in GBR has been suggested to be mainly responsible for such effect (Seki et al., 2005). However, GBR intake does not decrease fasting blood glucose in normal mice (Shirai et al., 2010). The same evidence was also observed in this study. Therefore, it is conceivable that GBR intake would lower blood glucose concentration

by suppressing its absorption from the diet. In diabetes, the hypoglycemic effects of GBR have been reported (Hagiwara et al., 2004; Hsu et al., 2008) and acylated steryl glucoside has been proposed to be an active ingredient in this case (Usuki et al., 2008).

AST (serum glutamic oxaloacetic transaminase (SGOT)) is commonly measured clinically as a part of diagnostic liver function tests. This enzyme is found in the liver, heart, skeletal muscle, kidneys, brain, and red blood cells. As a result, its level in plasma may be elevated in diseases affecting these organs and is less specific

Table 1. Blood chemistry values in rats treated with the germinated brown extract at various doses for 12 weeks.

Parameter	Administered doses (mg/kg/day)			
	0 (Control)	75	150	300
Male rats				
Glucose (mg/dl)	88.50 ± 3.72 ^a	75.44 ± 5.50 ^a	66.71 ± 10.52 ^a	86.00 ± 5.08 ^a
BUN (mg/dl)	26.46 ± 1.45 ^c	21.24 ± 1.27 ^a	21.80 ± 0.94 ^{ab}	25.03 ± 0.77 ^{bc}
Creatinine (mg/dl)	0.64 ± 0.02 ^{bc}	0.68 ± 0.04 ^c	0.43 ± 0.46 ^a	0.56 ± 0.03 ^b
Cholesterol (mg/dl)	54.00 ± 4.26 ^a	77.00 ± 5.83 ^c	69.88 ± 5.97 ^{bc}	60.40 ± 3.62 ^a
Triglycerides (mg/dl)	47.70 ± 3.50 ^a	90.56 ± 10.73 ^b	82.00 ± 16.40 ^b	52.10 ± 3.66 ^a
HDL-C (mg/dl)	42.30 ± 4.07 ^a	40.22 ± 4.99 ^a	42.33 ± 7.33 ^a	32.56 ± 2.49 ^a
Total protein (g/dl)	7.10 ± 0.11 ^a	8.29 ± 0.12 ^c	7.75 ± 0.23 ^b	7.10 ± 0.10 ^a
Total bilirubin (mg/dl)	0.50 ± 0.07 ^a	0.53 ± 0.08 ^a	0.75 ± 0.22 ^a	0.42 ± 0.10 ^a
AST (U/L)	191.80 ± 9.78 ^a	257.00 ± 16.18 ^b	239.38 ± 15.14 ^b	178.00 ± 8.71 ^a
ALT (U/L)	45.90 ± 2.84 ^a	43.44 ± 1.44 ^a	47.75 ± 4.84 ^a	51.70 ± 4.44 ^a
ALP (U/L)	65.10 ± 2.48 ^a	61.44 ± 2.65 ^a	76.13 ± 14.17 ^a	67.70 ± 3.53 ^a
Albumin (g/dl)	3.49 ± 0.04 ^a	3.86 ± 0.04 ^b	3.52 ± 0.26 ^a	3.47 ± 0.06 ^a
Uric acid (mg/dl)	1.20 ± 0.15 ^a	1.92 ± 0.14 ^b	1.99 ± 0.20 ^b	1.56 ± 0.12 ^{ab}
Female rats				
Glucose (mg/dl)	68.30 ± 4.48 ^a	131.83 ± 24.42 ^b	55.50 ± 9.43 ^a	72.00 ± 6.99 ^a
BUN (mg/dl)	27.85 ± 1.71 ^a	22.33 ± 5.58 ^a	30.40 ± 5.79 ^a	24.72 ± 1.49 ^a
Creatinine (mg/dl)	0.74 ± 0.10 ^b	0.43 ± 0.10 ^a	0.73 ± 0.05 ^b	0.58 ± 0.02 ^{ab}
Cholesterol (mg/dl)	50.30 ± 4.41 ^a	85.43 ± 22.78 ^a	103.12 ± 43.76 ^a	75.50 ± 7.32 ^a
Triglycerides (mg/dl)	52.90 ± 4.12 ^a	83.50 ± 21.79 ^a	212.88 ± 37.31 ^a	60.60 ± 5.62 ^a
HDL-C (mg/dl)	47.10 ± 4.00 ^a	28.00 ± 0.00 ^a	28.25 ± 5.15 ^a	35.78 ± 4.64 ^a
Total Protein (g/dl)	7.19 ± 0.26 ^a	5.20 ± 1.09 ^a	11.03 ± 3.69 ^a	7.22 ± 0.92 ^a
Total Bilirubin (mg/dl)	0.82 ± 0.19 ^a	1.18 ± 0.43 ^a	0.68 ± 0.43 ^a	0.78 ± 0.33 ^a
AST (U/L)	157.40 ± 8.70 ^a	151.67 ± 55.27 ^a	216.20 ± 15.63 ^a	164.33 ± 13.84 ^a
ALT (U/L)	53.10 ± 9.65 ^a	134.50 ± 85.34 ^a	32.83 ± 5.48 ^a	34.67 ± 2.68 ^a
ALP (U/L)	35.40 ± 1.92 ^a	35.50 ± 5.32 ^a	34.17 ± 3.82 ^a	39.67 ± 4.18 ^a
Albumin (g/dl)	3.62 ± 0.07 ^b	2.70 ± 0.86 ^a	4.19 ± 0.28 ^b	3.71 ± 0.54 ^b
Uric Acid (mg/dl)	1.99 ± 0.34 ^a	2.03 ± 0.34 ^a	2.42 ± 0.54 ^a	1.58 ± 0.11 ^a

AST= aspartate aminotransferase, ALT = alanine aminotransferase, ALP = alkaline phosphatase, BUN = blood urea nitrogen, and HDL-C = high density lipoprotein-cholesterol. Values are mean ± SEM, n = 8 to 10. Values with different superscripts (a, b, c) in the same row are significantly different ($P \leq 0.05$).

indicator than alanine aminotransferase (ALT; serum glutamic pyruvic transaminase (SGPT)) for hepatocellular damage (Rej, 1989). In our study, we found increased AST in plasma of male rats receiving the extract at 75 and 150 mg/kg/day as compared to the control animals. The reason behind these findings remains unknown. Since the differences were not dose-related and did not appear in female groups, they are not likely to be treatment-associated. Also, the alterations of plasma proteins, BUN, and uric acid values among our male rats were not dose-dependent. The effects were also slight and thus seem to have no clinical implication. Despite the aforementioned changes, their heart, liver, and kidneys did not reveal any abnormalities after histopathological examinations.

In this study, the hematological values detected in all blood samples appeared to be within the normal range and lymphocytes which are the central cells of the immune system, were the major leukocyte count (white blood cell, WBC) populations as reported in the literature (Matsuda et al., 2000). Previously, immune cell-stimulating activities of GBR extracts have been demonstrated in mice (Oh and Oh, 2003). A preliminary study of our GBR extract on nitric oxide production in cultured macrophages however revealed that it had anti-inflammatory activity and hydroxyphenyllactic acid could be one of candidate components for such inhibitory action.

The increased circulating platelets detected in low-dose and middle-dose groups of both sexes are probably

Table 2. Hematological values in rats treated with the germinated brown rice extract at various doses for 12 weeks.

Parameter	Administered doses (mg/kg/day)			
	0 (Control)	75	150	300
Male rats				
WBC (μ)	5,680.0 $\pm$ 214.9 ^a	5,677.8 $\pm$ 462.4 ^a	7,575.0 $\pm$ 2,403.4 ^a	5,510.0 $\pm$ 502.9 ^a
HB (g/dl)	14.81 $\pm$ 0.19 ^{ab}	16.03 $\pm$ 0.22 ^c	15.46 $\pm$ 0.55 ^{bc}	14.48 $\pm$ 0.27 ^a
HCT (%)	43.50 $\pm$ 0.69 ^{ab}	47.56 $\pm$ 0.44 ^c	45.38 $\pm$ 1.59 ^{bc}	42.20 $\pm$ 0.83 ^a
MCV (fL)	52.50 $\pm$ 0.22 ^{ab}	54.00 $\pm$ 0.53 ^b	52.88 $\pm$ 0.61 ^{ab}	51.40 $\pm$ 0.60 ^a
MCH (pg)	18.00 $\pm$ 0.00 ^a	18.22 $\pm$ 0.22 ^a	18.00 $\pm$ 0.19 ^a	17.60 $\pm$ 0.27 ^a
MCHC (g/dl)	34.20 $\pm$ 0.13 ^a	33.78 $\pm$ 0.22 ^a	34.33 $\pm$ 0.24 ^a	34.10 $\pm$ 0.31 ^a
PMN (%)	70.00 $\pm$ 1.25 ^a	73.33 $\pm$ 1.17 ^a	70.75 $\pm$ 1.63 ^a	68.40 $\pm$ 2.09 ^a
Lymp (%)	27.30 $\pm$ 1.27 ^a	24.33 $\pm$ 1.12 ^a	25.63 $\pm$ 1.41 ^a	28.50 $\pm$ 1.70 ^a
Mono (%)	1.30 $\pm$ 0.15 ^a	1.22 $\pm$ 0.15 ^a	2.13 $\pm$ 0.40 ^a	2.10 $\pm$ 0.50 ^a
Eo (%)	1.40 $\pm$ 0.22 ^a	1.11 $\pm$ 0.20 ^a	1.50 $\pm$ 0.33 ^a	1.00 $\pm$ 0.15 ^a
Baso (%)	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a
Plt (μ /L)	561,300 $\pm$ 21,687 ^a	770,625 $\pm$ 27,306 ^a	728,750 $\pm$ 52,925 ^a	561,600 $\pm$ 45,553 ^a
RBC (μ /L)	8,252,000 $\pm$ 153,947 ^a	8,796,667 $\pm$ 121,747 ^a	8,591,250 $\pm$ 327,080 ^a	8,249,000 $\pm$ 146,799 ^a
Female rats				
WBC (μ /L)	3,070.0 $\pm$ 282.5 ^a	4,080.0 $\pm$ 377.4 ^a	3,188.9 $\pm$ 524.3 ^a	4,250.0 $\pm$ 571.2 ^a
HB (g/dl)	14.07 $\pm$ 0.47 ^a	15.00 $\pm$ 0.47 ^a	15.30 $\pm$ 0.36 ^a	14.23 $\pm$ 0.30 ^a
HCT (%)	39.90 $\pm$ 1.36 ^a	42.30 $\pm$ 1.25 ^a	43.00 $\pm$ 0.82 ^a	40.80 $\pm$ 1.07 ^a
MCV (fL)	53.90 $\pm$ 0.46 ^a	55.30 $\pm$ 0.47 ^a	52.44 $\pm$ 2.16 ^a	53.00 $\pm$ 0.33 ^a
MCH (pg)	19.00 $\pm$ 0.21 ^a	21.70 $\pm$ 2.04 ^a	19.00 $\pm$ 0.36 ^a	19.00 $\pm$ 0.33 ^a
MCHC (g/dl)	35.40 $\pm$ 0.22 ^a	35.70 $\pm$ 0.30 ^a	35.67 $\pm$ 0.44 ^a	35.70 $\pm$ 0.65 ^a
PMN (%)	68.60 $\pm$ 1.59 ^a	69.00 $\pm$ 2.56 ^a	68.89 $\pm$ 1.40 ^a	73.40 $\pm$ 0.79 ^a
Lymp (%)	26.10 $\pm$ 1.38 ^a	26.30 $\pm$ 2.54 ^a	27.44 $\pm$ 1.82 ^a	23.80 $\pm$ 1.08 ^a
Mono (%)	2.20 $\pm$ 0.49 ^a	2.50 $\pm$ 0.62 ^a	1.89 $\pm$ 0.31 ^a	1.80 $\pm$ 0.55 ^a
Eo (%)	3.00 $\pm$ 0.54 ^a	2.20 $\pm$ 0.59 ^a	1.78 $\pm$ 0.40 ^a	1.00 $\pm$ 0.47 ^a
Baso (%)	0.10 $\pm$ 0.10 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a
Plt (μ /L)	549,500 $\pm$ 70,107 ^a	742,000 $\pm$ 77,419 ^a	800,890 $\pm$ 10,578 ^a	616,400 $\pm$ 70,434 ^a
RBC (μ /L)	7,406,000 $\pm$ 260,888 ^a	7,661,000 $\pm$ 265,173 ^a	7,735,600 $\pm$ 245,821 ^a	7,697,000 $\pm$ 197,130 ^a

WBC = leukocyte count, HB = hemoglobin concentration, HCT = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, PMN = polymorphonuclear leukocyte, Lymp = lymphocyte, Mono = monocyte, Eo = eosinophil, Baso = basophil, Plt = platelet count, and RBC = erythrocyte count. Values are mean $\pm$ SEM, n = 8 to 10. Values with different superscripts (a, b, c) in the same row are significantly different ($P \leq 0.05$).

transient since it did not occur in the high-dose group and the differences were significant only among the male groups. In the female groups, however, decreased eosinophil counts are likely to be treatment-associated due to their dose-variable manner, but need to be further investigated.

In conclusion, we have investigated both acute and subchronic toxicities of the extract from GABA-rich GBR of a Southern Thailand variety. With respect to our results, the extract at the doses tested did not produce any significant undesirable effects in the experimental animals. Their livers and kidneys which are the most sensitive organs to toxic factors were apparently normal and showed no signs of dysfunction. The toxicological data obtained from this study are of significance in

relation to increasing the consumption of GBR and its products for health benefits and remedial purposes worldwide. An additional study to evaluate chronic toxicity is needed to determine the long-term safety of the same kind of rice extract.

ACKNOWLEDGEMENTS

The authors are grateful to Thailand Research Fund and Prince of Songkla University for their financial supports (Grant No. DBG5080023). The authors thank Miss Worrapanit Chansuwan for performing statistical analyses of all the data obtained and Dr. Brian Hodgson for reading our manuscript.

Table 3. Organ weights in rats treated with the germinated brown rice extract at various doses for 12 weeks.

Organ weight (g)	Administered doses (mg/kg/day)			
	0 (Control)	75	150	300
Male rats				
Liver	8.95 ± 0.46 ^a	7.99 ± 0.23 ^a	8.17 ± 0.32 ^a	8.90 ± 0.29 ^a
Kidney	2.02 ± 0.07 ^a	1.91 ± 0.08 ^a	1.84 ± 0.06 ^a	2.02 ± 0.06 ^a
Heart	1.29 ± 0.05 ^a	1.25 ± 0.06 ^a	1.28 ± 0.06 ^a	1.26 ± 0.05 ^a
Spleen	0.73 ± 0.02 ^a	0.67 ± 0.04 ^a	0.69 ± 0.05 ^a	0.67 ± 0.03 ^a
Female rats				
Liver	5.84 ± 0.18 ^a	5.20 ± 0.20 ^b	5.26 ± 0.22 ^b	5.28 ± 0.14 ^b
Kidney	1.40 ± 0.05 ^a	1.31 ± 0.05 ^{ab}	1.31 ± 0.04 ^{ab}	1.25 ± 0.03 ^b
Heart	0.83 ± 0.02 ^a	0.84 ± 0.03 ^a	0.84 ± 0.03 ^a	0.80 ± 0.04 ^a
Spleen	0.55 ± 0.02 ^a	0.50 ± 0.03 ^a	0.59 ± 0.08 ^a	0.52 ± 0.02 ^a

Values are mean ± SEM, n = 9 to 10. Values with different superscripts (a, b) in the same row are significantly different ($P \leq 0.05$).

Figure 2. The histological morphology of internal organs of a male rat treated with extract at 300 mg/kg/day dosage, as shown by hematoxylin and eosin staining. (A) Heart (magnification, ×40); (B) kidney (magnification, ×40); (C) spleen (magnification, ×10); (D) liver (magnification, ×10).

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