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พิษกึ่งเฉียบพลันของผงคีเฟอร์จากข้าวกล้อง

Sub-acute Toxicity of Brown Rice Kefir Powder

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บทคัดย่อ

การวิจัยในครั้งนี้เพื่อศึกษาพิษกึ่งเฉียบพลันของผงคีเฟอร์ข้าวกล้องจากข้าวขาวดอกมะลิ 105 (KDMLKP) ข้าวหอมแดง (RHKP) และข้าวหอมนิล (HNKP) โดยการป้อนผงคีเฟอร์ขนาด 500, 1,000 และ 2,000 มิลลิกรัมต่อกิโลกรัม ทุกๆ 2 วัน เป็นระยะเวลา 14 วัน พบว่า ผงคีเฟอร์ทุกขนาดไม่ก่อให้เกิดการตายและอาการความเป็นพิษ และไม่เปลี่ยนแปลงน้ำหนักสัมผัสของอวัยวะของหนู KDMLKP ปริมาณสูงทำให้น้ำหนักตัวหนูเพิ่มขึ้น แต่ RHKP และ HNKP ไม่มีผลต่อน้ำหนักของหนู ผงคีเฟอร์จากข้าวกล้องทั้ง 3 ชนิด ทำให้อัตราการแลกเนื้อของหนูทดลองดีกว่า PBS ยกเว้น KDMLKP ขนาด 500 และ 1,000 มิลลิกรัมต่อกิโลกรัม ผงคีเฟอร์ขนาด 1,000 และ 2,000 มิลลิกรัมต่อกิโลกรัม มีผลต่อการลดระดับน้ำตาลในเลือด และการทำงานของไตและตับของหนูทดลอง โดย KDMLKP ทำให้ระดับกลอบูลินในเลือดเพิ่มขึ้น RHKP ทำให้ระดับครีเอตินิน และกรดยูริกในเลือดลดลง แต่ระดับกลอบูลิน, aspartate aminotransferase (AST) และ alanine aminotransferase (ALT) ในเลือดเพิ่มขึ้น และ HNKP ทำให้ระดับไนโตรเจน กรดยูริก และอัลบูมินในเลือดลดลง แต่ระดับกลอบูลิน, AST และ ALT ในเลือดเพิ่มขึ้น นอกจากนี้ ผงคีเฟอร์ขนาดดังกล่าวยังทำให้ปริมาณเม็ดเลือดขาวนิวโตรฟิลล์ลดลงแต่ลิมโฟไซต์เพิ่มขึ้น

ผลการศึกษาชี้ให้เห็นว่า ผงคีเฟอร์จากข้าวกล้องขนาด 500 mg/kg ที่ให้หนูทุกๆ 2 วัน เป็นระยะเวลา 14 วัน ไม่ก่อให้เกิดพิษกึ่งเฉียบพลัน แต่ขนาด 1,000 และ 2,000 mg/kg มีผลต่อการลดระดับน้ำตาลในเลือด และการทำงานของไตและตับของหนูทดลอง

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คำสำคัญ: ข้าวกล้อง ข้าวขาวดอกมะลิ 105 ข้าวหอมแดง ข้าวหอมนิล ผงคีเฟอร์จากข้าวกล้อง พิษกึ่งเฉียบพลัน

Abstract

The present study was designed to determine sub-acute toxicity of kefir powders from Khao Dawk Mali 105 (KDMLKP), Red Hawm (RHKP) and Hawm Nil (HNKP) brown rice. Kefir powders at the doses of 500, 1,000 and 2,000 mg/kg were given orally to the rats every 2 days for 14 days. The results showed that all the doses of the kefir powders did not produce mortality and any symptoms of toxicity. And also, kefir powders did not alter relative organ weight in the treated rats. Increasing KDMLKP significantly increased body weight gain in contrast to RHKP and HNKP. Kefir powders produced feed conversion ratio better than that PBS, but this did not KDMLKP at the doses of 500 and 1,000 mg/kg. Kefir powders at the doses of 1,000 and 2,000 mg/kg decreased blood sugar and affected renal and/or hepatic functions as KDMLKP significantly increased globulin, RHKP significantly decreased creatinine and uric acid but increased globulin, serum aspartate aminotransferase (AST), and serum alanine aminotransferase (ALT) and HNKP significantly decreased blood urea nitrogen, uric acid, and albumin but increased globulin, AST and ALT. Kefir powder also significantly decreased neutrophils, but increased lymphocytes.

These findings indicate that kefir powders at the dose of 500 mg/kg exert non sub-acute toxicity when the doses of 1,000 and 2,000 mg/kg administering every 2 days for 14 days decreased blood sugar and may cause renal and hepatic dysfunctions.

Keywords: Brown rice, Khao Dawk Mali 105, Red Hawm, Hawm Nil, kefir powder, sub-acute toxicity

1. Introduction

Kefir is a fermented milk product [1]. It has a long tradition of being regarded as good benefit for health in many countries. It has high nutritional value as a source of proteins and calcium [2], and high antioxidant activity [3]. Recently, kefir from rice milk has been reportedly possessed higher antioxidant activity than that from cow milk [4]. In addition, kefir products also exhibited antitumor [5], anti-allergic [6], antidiabetic [7-8], anti-inflammatory [6, 9-10], and immunomodulatory activities [11].

Rice (*Oryza sativa*, L.) is one of the most important economic plants in Thailand and all most countries in Asia. It is an important nourishes nutrition resource. Phytochemicals such as protein, total free amino acids, α-tocopherol, γ-oryzanol, thiamine, niacin, and pyridoxine in brown rice are higher than those in ordinary milled rice [12]. Several compounds such as γ-aminobutyric acid (GABA), α-tocopherol, γ-tocopherol and total phenolic compounds (TPC) in rice and rice products exerting pharmacological and antioxidant activities have been reported [12-14].

Although, rice kefir products exerted high nutrition resource, scientific report on toxicity of the rice kefir is limited. The present study was therefore, designed to determine sub-acute toxicity of kefir powders from Khao Dawk Mali 105, Red Hawm and Hawm Nil brown rice in the rat model.

2. Materials and Experiment

2.1 Brown rice, fermentation and kefir powder preparation

Brown rice of Khao Dawk Mali 105, Red Hawm and Hawm Nil, harvested during November 2013- January 2014 from Selaphum District, Roi Et Province, Thailand was used in this study. The brown rice fermentation and kefir powder preparation was conducted following the method described by Chunchom *et al.* [15]. The rice was dried, weighed, soaked in distilled water (1:5, w:v) at 25°C for 2 h and grinded thoroughly by using blender and filtered to obtain rice milk. The rice milk was pasteurized at 70°C for 15 min and then cooled directly at 4°C.

Brown rice fermentation: A 0.2 g freeze-dried kefir grain from Department of Biotechnology, Faculty of Technology, Mahasarakham University, Thailand was inoculated into 250 mL flask with 200 mL of Lactobacilli de Man, Rogosa, and Sharpe (MRS) broth (Acc. ISO 15214, Merck KGaA, Darmstadt, Germany) and incubated under anaerobic conditions. The flask was put into a 5L anaerobic jar. After that, the sample jars were kept at 30°C for 24 h, and then centrifuged (1000×g, 15 min at 4°C) to provide the cells. The cells were washed and re-suspended in sterile saline solution (0.85% NaCl) and diluted with sterile 0.85% NaCl (1:10; v:v). Kefir was sub-cultured by inoculating kefir starter into fresh milk (20:200; v:v) and incubated under anaerobic conditions at 30°C for 48 h to obtain activated kefir grain. The activated kefir grain were cultured and fermented by inoculating into brown rice milk adding with 2.5% sucrose (100:1,000, v:v) and incubated under anaerobic conditions at 30°C for 24 h to obtain the final rice milk kefir, pH 4.8-4.9.

Brown rice kefir powder: KDMLKP, RHKP and HNKP were prepared by freeze-drying and powdering the Khao Dawk Mali 105, Red Hawm and Hawm Nil rice milk kefir. The kefir powder was kept at -20°C until be used.

2.2 Animals

Eighty male Wistar rats weighing 280-300 g purchased from National Laboratory Animal Center, Mahidol University, Thailand and were used in the study. The rats were kept in animal laboratory and acclimatized for 7 days in environmental conditions of 22-25°C, 50%-55% humidity and under a 12-hour light/dark cycle. The rats were fed with rodent diet from Perfect Companion Group Co., Ltd., and water *ad libitum*. All experimental protocols were maintained in accordance with the Guidelines of Committee Care and Use of Laboratory Animal Research, National Research Council of Thailand and advice of the Institutional Animal Care and Use Committee, Mahasarakham University, Thailand (ID: 0008/2557).

2.3 Sub-acute toxicity study

Sub-acute toxicity study was conducted according to the organization for OECD guideline 407 [16]. The rats were randomly divided into 10 groups with 8 rats in each; group 1; rats received PBS (control group), group 2, 3 and 4; rats received 500, 1000 and 2000 mg/kg KDMLKP respectively, group 5, 6 and 7; rats received 500, 1000 and 2000 mg/kg RHKP respectively, and group 8, 9 and 10; rats received 500, 1000 and 2000 mg/kg HNKP, respectively. The kefir powder was given orally to the rats every 2 days for 14 days. Symptoms of toxicity and mortal rats were monitored during a period of observation within 14 days. Body weight gain, food intake, relative organ weight, and feed conversion ratio were investigated.

Body weight and food intake were recorded daily for a calculation of feed conversion ratio (FCR). At the end of experiments, the rats were fasted, weighed and then euthanasia by using cervical dislocation technique. Blood samples were collected from the rat hearts for the determination of blood biochemistry and hematological values by using the commercial kits (Stanbio Liqui Color®). Visceral organs including liver, lungs, heart, kidneys and spleen were removed and weighed for a calculation of relative organ weight (ROW).

ROW of each organ was calculated using the following equation;

$$\text{Row} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} x100$$

Feed conversion ratio (FCR) of each animal was calculated using the following equation;

$$\text{FCR} = \frac{\text{Food intake (g)}}{\text{Body weight gain (g)}}$$

2.4 Determination of blood biochemistry and hematological values

Blood samples were put into heparinized and non-heparinized tubes. Non-heparinzed blood was centrifuged at 1500 g for 10 min to separate serum. The serum was assayed for biochemistry including total protein (TP), blood sugar (BS), blood urea nitrogen (BUN), creatinine (Crea), uric acid (UA), albumin (Alb), globulin (Glob), total bilirubin (TB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP).

Heparinized blood was used for a hematological analysis including red blood cell (RBC), white blood cell (WBC), hematocrit (Hct), hemoglobin (Hb), platelets (Plt), neutrophils (Neu), and lymphocytes (Lym).

2.5 Statistical analysis

The data were presented as mean±SEM and analyzed using one-way ANOVA. The differences among means were detected by using the Duncan's Multiple Range Test. The values of $p \leq 0.05$ were considered statistically significant.

3. Results and Discussion

3.1 Symptoms and mortality

The rice kefir powder treated rats did not show any symptom of toxicity and mortality during the period of observation lasting 14 days.

3.2 Body weight gain, food intake, FCR and ROW

Table 1, the highest dose of KDMLKP and all the doses of RHKP and HNKP significantly ($p<0.05$) increased the body weight gain in the rats. Food intake of the kefir powder treated rats did not differ from that in the controls, but this did not show in 500 mg/kg of RHKP group. In contrast, all the doses of RHKP and HNKP significantly ($p<0.05$) decreased FCR, but only a dose at 2,000 mg/kg KDMLKP significantly ($p<0.05$) decreased FCR when compared to that in controls. However, ROW in the kefir powder treated rats did not differ from that in the controls (data not shown).

These findings indicate that brown rice kefir may be a good nourish nutrition resource. Brown rice exerts more potent antioxidant activity and provides higher level of phytochemicals than polished rice [12]. Thus, brown rice kefir is possibly suitable for further development as supplement diet for growth promotion.

Table 1 Body weight gain, food intake and FCR in the rats treated with PBS and kefir powder.

Groups	Body weight gain (g)	Food intake (g)	FCR
PBS	85.00±3.65^{a}	248.67±1.31^{ab}	2.95±0.13^{c}
KDMLKP			
500 mg/kg	90.83±2.01^{ab}	249.17±1.^{51ab}	2.75±0.07^{bc}
1,000 mg/kg	90.00±3.87^{ab}	251.67±1.93^{ab}	2.82±0.14^{c}
2,000 mg/kg	94.17±3.96^{b}	250.00±1.12^{ab}	2.68±0.11^{b}
RHKP			
500 mg/kg	95.00±2.24^{bc}	257.50±1.12^{c}	2.72±0.06^{b}
1,000 mg/kg	94.17±2.01^{b}	255.00±1.53^{bc}	2.71±0.04^{b}
2,000 mg/kg	93.33±3.07^{b}	253.00±1.79^{b}	2.72±0.09^{b}
HNKP			
500 mg/kg	101.67±3.80^{c}	248.67±1.11^{ab}	2.46±0.08^{a}
1,000 mg/kg	96.67±4.77^{bc}	240.83±0.98^{a}	2.52±0.12^{a}
2,000 mg/kg	93.33±2.79^{b}	237.50±0.85^{a}	2.56±0.09^{ab}

Mean values within each column with specify the difference among these superscripts ($^{a, b, c}$) are significantly different, Duncan's test at $p< 0.05$.

3.3 Blood biochemistry

Blood biochemistry including BS, BUN, CREA, UA, TP, Alb, and Glob are involved in renal function and AST, ALT, and ALP enzymes are involved in hepatic function [17]. A dose 2,000 mg/kg of KDMLKP significantly decreased ($p<0.05$) BS while increased Glob. And also, at a dose of 500 mg/kg RHKP and HNKP did not change these parameters in treated rats when compared to those in the controls (Table 2).

KDMLKP did not alter AST, ALT and ALP while a dose 2,000 mg/kg of RHKP significantly increased ($p<0.05$) AST and ALT enzymes in the treated rats compared to those in the controls In addition, the rats treated with HNKP at dose of 1,000 mg/kg also increased ($p<0.05$) AST enzyme (Table 3).

These findings indicate that the low doses of KDMLKP (500 and 1,000 mg/kg), RHKP (500 mg/kg) and HNKP (500 mg/kg) did not affect renal function. Moreover, KDMLKP (all the doses), RHKP (500 and 1,000 mg/kg) and HNKP (500 mg/kg) did not affect hepatic function.

3.4 Hematological values

The dose at 2,000 mg/kg of KDMLKP significantly ($p<0.05$) increased WBC. In contrast, the dose at 2,000 mg/kg of RHKP significantly ($p<0.05$) decreased WBC when compared with controls. Moreover, the increasing in amount of brown rice kefir powder significantly ($p<0.05$) decreased Neu, but increased Lym (Table 4). These results suggest that the kefir powder possibly acts in opposite way in the differentiation of hematopoietic cells by suppressing

neutrophils and stimulating lymphocytes. According to previous reports, the kefir induced the helper T-lymphocytes type 2 proliferations by increasing the number of immunoglobulin A (IgA) [11], in agreement with the increasing of lymphocyte and globulin in this study. On the other hand, the significant increasing in globulin may be the results of B-lymphocytes were induced to IgA secreting cells. However, the rats treated with 2,000 mg/kg RHKP and HNKP significantly ($p<0.05$) decreased Hb and Hct without the alteration of RBCs. These suggest the RBCs decreased ability on carry Hb so read to Hct changing.

Table 2 Blood biochemistry; BS, BUN, Crea, UA, TP, Alb, and Glob in the rats treated with PBS and kefir powder.

Groups	BS (mg/dl)	BUN (mg/dl)	CREA (mg/dl)	UA (mg/dl)	TP (g/dl)	Alb (g/dl)	Glob (g/dl)
PBS	185.83±7.25^{c}	20.45±1.12^{b}	0.82±0.03^{bc}	2.65±0.34^{b}	5.72±0.13^{ab}	3.28±0.06^{b}	2.48±0.09^{a}
KDMLKP							
500 mg/kg	176.83±7.78^{c}	21.10±1.03^{b}	0.87±0.02^{c}	2.72±0.36^{b}	5.92±0.10^{b}	3.25±0.08^{b}	2.60±0.06^{ab}
1,000 mg/kg	160.67±7.53^{bc}	20.12±0.91^{b}	0.80±0.02^{b}	2.28±0.14^{ab}	5.77±0.12^{ab}	3.22±0.08^{b}	2.72±0.17^{ab}
2,000 mg/kg	139.83±8.95^{b}	20.07±0.59^{b}	0.80±0.04^{b}	2.20±0.36^{ab}	5.70±0.14^{ab}	3.12±0.05^{b}	2.85±0.04^{bc}
RHKP							
500 mg/kg	190.50±9.35^{c}	19.22±0.70^{b}	0.85±0.06^{b}	2.37±0.38^{ab}	5.60±0.14^{ab}	2.77±0.06^{ab}	2.72±0.07^{ab}
1,000 mg/kg	144.17±14.15^{b}	19.22±0.66^{b}	0.77±0.02^{ab}	2.02±0.28^{ab}	5.58±0.10^{ab}	2.75±0.07^{ab}	2.80±0.06^{bc}
2,000 mg/kg	99.00±9.02^{a}	19.15±0.53^{b}	0.73±0.03^{a}	1.53±0.17^{a}	5.52±0.12^{ab}	2.73±0.04^{ab}	2.88±0.09^{bc}
HNKP							
500 mg/kg	156.50±19.70^{bc}	19.27±0.71^{b}	0.90±0.07^{c}	2.62±0.28^{b}	5.63±0.15^{ab}	2.72±0.05^{ab}	2.73±0.05^{ab}
1,000 mg/kg	128.67±9.30^{ab}	17.53±0.55^{ab}	0.78±0.03^{ab}	1.65±0.25^{a}	5.43±0.08^{a}	2.67±0.03^{a}	2.73±0.08^{ab}
2,000 mg/kg	85.50±7.64^{a}	16.47±0.27^{a}	0.83±0.03^{bc}	1.48±0.19^{a}	5.73±0.18^{ab}	2.67±0.05^{a}	3.02±0.06^{c}

Mean values within each column with specify the difference among these superscripts ($^{a, b, c}$) are significantly different, Duncan's test at $p< 0.05$. BS= blood sugar; BUN = blood urea nitrogen; CREA = creatinine; UA= uric acid TP = total serum protein; Alb = albumin; Glob = globulin.

Table 3 Blood biochemistry; AST, ALT and ALP in the rats treated with PBS and kefir powder.

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
PBS	137.00±1.53^{ab}	49.17±1.66^{a}	132.83±4.66^{ab}
KDMLKP			
500 mg/kg	114.67±5.13^{a}	51.33±2.58^{ab}	137.50±3.82^{b}
1,000 mg/kg	117.33±3.56^{a}	51.00±3.13^{ab}	133.33±3.05^{ab}
2,000 mg/kg	144.67±4.52^{bc}	50.50±1.38^{a}	131.00±3.47^{ab}
RHKP			
500 mg/kg	141.33±1.93^{b}	49.33±1.11^{a}	128.83±9.19^{ab}
1,000 mg/kg	152.83±6.36^{bc}	49.50±1.69^{a}	125.83±8.09^{ab}
2,000 mg/kg	164.83±3.50^{cd}	57.67±2.59^{b}	114.17±3.59^{a}
HNKP			
500 mg/kg	140.00±3.18^{b}	47.33±1.41^{a}	119.83±6.82^{ab}
1,000 mg/kg	151.67±5.16^{bc}	50.33±1.33^{a}	116.67±3.37^{a}
2,000 mg/kg	157.33±4.58^{cd}	53.83±3.70^{ab}	112.67±3.25^{a}

Mean values within each column with specify the difference among these superscripts ($^{a, b, c}$) are significantly different, Duncan's test at $p \leq 0.05$. AST = serum aspartate aminotransferase; ALT = serum alanine aminotransferase; ALP = alkaline phosphatase.

Table 4 Hematological values in the rats treated with PBS and kefir powder.

Groups	WBC (10^3 cell/mm^3)	RBC (10^6cell/mm^3)	Hb (g/dl)	Hct (%)	Plt (10^3cell/mm^3)	Neu (%)	Lym (%)
PBS	6.42±0.28^{b}	8.76±0.13	17.52±0.23^{b}	53.33±0.76^{b}	923.17±24.11	8.83±1.01^{d}	80.83±1.25^{a}
KDMLKP							
500 mg/kg	6.93±0.44^{bc}	8.46±0.20	16.78±0.32^{ab}	51.83±0.87^{ab}	882.83±4.82	8.00±0.58^{c}	89.83±1.22^{ab}
1,000 mg/kg	6.95±1.10^{bc}	8.40±0.13	16.95±0.32^{ab}	51.50±0.76^{ab}	888.33±8.35	6.50±0.56^{bc}	93.17±0.79^{b}
2,000 mg/kg	8.63±0.74^{c}	8.37±0.13	16.90±0.27^{ab}	51.83±0.87^{ab}	922.83±14.71	3.67±0.56^{a}	95.50±0.81^{b}
RHKP							
500 mg/kg	7.00±0.45^{bc}	8.28±0.12	16.83±0.28^{ab}	50.67±1.11^{ab}	837.00±68.15	8.00±1.37^{c}	90.67±1.47^{b}
1,000 mg/kg	5.53±0.28^{ab}	8.24±0.09	16.63±0.24^{ab}	49.83±0.48^{ab}	890.33±29.69	7.00±1.79^{bc}	91.00±3.88^{b}
2,000 mg/kg	5.13±0.28^{a}	8.19±0.19	16.30±0.28^{a}	48.50±0.92^{a}	947.50±31.95	5.00±1.12^{ab}	92.50±1.98^{b}
HNKP							
500 mg/kg	5.77±0.43^{ab}	8.38±0.14	16.70±0.35^{ab}	51.00±0.68^{ab}	887.33±24.78	7.33±1.54^{bc}	91.83±1.87^{b}
1,000 mg/kg	6.50±0.26^{b}	8.63±0.41	16.88±0.44^{ab}	50.67±1.38^{ab}	893.67±42.25	5.83±0.94^{b}	95.17±0.87^{b}
2,000 mg/kg	5.60±0.40^{ab}	8.13±0.18	16.32±0.47^{a}	48.67±1.36^{a}	905.67±46.40	4.83±1.05^{ab}	96.33±0.71^{b}

Mean values within each column with specify the difference among these superscripts ($^{a, b, c}$) are significantly different, Duncan's test at $p< 0.05$. WBC = white blood cells; RBC = red blood cells; Hb = hemoglobin; Hct = hematocrit; Plt = platelets; Neu = neutrophils; Lym = lymphocytes.

4. Conclusions

KDMLKP, RHKP and HNKP possess no sub-acute toxicity when the dose of 500 mg/kg (LD50≤500 mg/kg) is administered every 2 days for 14 days. Long term administration of the kefir powder at the doses higher than 500 mg/kg may cause abnormal function of liver and kidney as they produce the alteration of the enzymes and many parameters in blood biochemistry monitoring. Furthermore, its activity on decreasing neutrophils and increasing lymphocytes which results in increasing globulin leads to activated immunomodulatory activity. Moreover, the significant increasing in globulin may be the results of B-lymphocytes were induced to IgA secreting cells.

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