

# Antioxidant and Anti Inflammation Effect of Snack Bars from Black Glutinous Rice and Pumpkin Powder

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**Abstract.** The study was design to evaluate the antioxidant and anti-inflammatory effect of snack bars that made of black glutinous rice and pumpkin powder and study on the male Sprague Dawley rats. Twenty-five male rats in between the age of 2 and 3 months were selected and randomly divided into 5 groups, which were positive control, negative control, group with sylimarin treatment, snack bars A and snack bars B. Carbon tetrachloride was used to induce hepatic damage. The results showed that group with CCl<sub>4</sub> treatment has  $3.04 \pm 0.16$   $\mu\text{mol/L}$  MDA while group with Sylimarin, snack bars A, snack bars B were  $0.49 \pm 0.05$ ;  $0.54 \pm 0.05$ ; and  $1.15 \pm 0.16$   $\mu\text{mol/L}$  MDA, respectively. The snack bars had the capacity to decline the damage in liver as much as Sylimarin (standardized natural medicine for hepatotoxicity). The SGPT (Serum Glutamin Pyruvic Transaminase) of the two treatment was  $22.24 \pm 0.80$  U/I for Sylimarin and  $22.33 \pm 1.03$  U/I for snack bars while SGOT (Serum Glutamic Oxaloacetic Transaminase) was  $40.78 \pm 0.77$  U/I for Sylimarin and  $40.88 \pm 1.25$  U/I for snack bars A. Snack bars B has a significantly different value on final SGOT and SGPT.

Keywords: antioxidant, anti inflammation, black glutinous rice, pumpkin powder, snack bars

Received 13 November 2019 | Revised 20 November 2019 | Accepted 29 November 2019

## 1. Introduction

Black glutinous rice was a popular food and consume as functional food because of its health effect, including antioxidant [1] [2] [3]; anti hypercholesterolemia [1] and anticancer [2]. Anthocyanin also reported as a substance which have anti-inflammatory effect [6]. Meanwhile pumpkin was known as natural sources of antioxidant and pro-Vitamin A because of its  $\beta$  carotene content. Studies about the antioxidant and anti-inflammatory effect of product using the local product were very limited. In vivo antioxidant test sometimes has no correlation with in vitro test due to the issue of the bioavailability. Food preparation and food processing could change the bioactive compound and affect their functional properties [7]. Processing black glutinous rice and pumpkin into snack bars probably could change their biological activity.

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Itthivadhanapong and Sangnark [8] using black glutinous rice powder to produce cake. The results showed that their sensory characteristics were not significantly differed with cake, which using wheat flour while the specific volume and specific gravity tend to decreased when using black glutinous rice. Meanwhile, Thoif [9] using black glutinous rice for wheat flour substitution in cookies. The results showed that cookies with red rice had a higher fiber content and a lower antioxidant activity than black glutinous rice. Pumpkin flour were developed as a functional food according to the  $\beta$  carotene compound, such as cookies [10], cake [11], or snack foods [13].

Development of ready-to-eat food using black glutinous rice and pumpkin flour as a functional food were never done before. The information about the antioxidant and anti-inflammatory properties processed from black glutinous rice also lack. Snack bars are dietary supplements which was consumed as a replacement of a meal or between meals. Some snack bars were created to retard hungriness or to supply energy while exercising. This snack bars were called energy bars. Snack bars were made from 3 to five materials which could support the need for energy, protein, fats, mineral, vitamin, fiber, and other nutrients. Modern snack bars should be delicious and nutritious, not just sweet and tasty. Snack bars were made from cereal, grain, rice, soy, fruits, and so on [13] [14] [15].

Carbon tetrachloride ( $\text{CCl}_4$ ) was a substance that commonly used to induce the damage in the liver [16] [17] [18].  $\text{CCl}_4$  bind covalently to cell components and involved in the peroxidation damage that causing injury. Cytochrome P450 activate the reductive halogenation of  $\text{CCl}_4$  and produce  $\text{CCl}_3^*$  and  $\text{OO}^*$  free radical. This leads to the destruction of intracellular membranes and the plasma membrane via unsaturated phospholipids binding. Oxidation produces mostly reactive aldehydes and increase membrane permeability [19]. Sylimarin was a natural compound derived from *Sylibum marianum*, which is commonly known as Milk thistle. The use of medicinal plants such as *Sylibum marianum* to threatened illnesses has reported for ancient. Sylimarin could inhibit the formation of free radicals caused by ethanol, acetaminophen, paracetamol, or carbon tetrachloride. Sylimarin enhance the production of glutathione which is contribute to the protective role liver damage [20]. The aims of the research were (1) to determine the physicochemical and organoleptic properties of snack bars from black glutinous rice and pumpkin flour and (2) to evaluate the antioxidant and anti-inflammatory properties of the snack bars in SD rats using carbon tetrachloride as a modulator.

## 2. Materials and Methods

Black glutinous rice and pumpkin were collected from local market. Black glutinous rice was ground and sifted with mesh 40 while pumpkins were process into pumpkin flour. Fresh pumpkins were peeled and shredded and dried at 50 oC for 5h using cabinet drier, grind, and sifted in mesh 80. The final moisture content of the powder was below 8%. Constituents using

in the snack bars processing were collected from local market, especially honey, palm sugar, fructose, can sugar, margarine, egg, and ingredients. Carbon tetrachloride purchased from Sigma Aldrich. Syllimarin from Kalbe (Hepamax) where used as standard medicinal plants. All chemical was analytical grade.

Snack bar from black glutinous rice and yellow pumpkin powder was made using method that has describe from the previous research by Putri [29]. Snack bars were analyzed for moisture content, ash, protein (Kjeldahl method), fats (Soxhlet), total sugar (Nelson Somogyi), fiber content [21], and antioxidant activity using DPPH as radical [22]. Analysis were done in three replicates.

Antioxidant and anti-inflammatory potential were analyzed based on true randomized experimental design with post test only controlled goup. Rats were randomly chosen and divided into five groups. Twenty five healthy Sprague Dawley male rats aged 2-3 months were used. The five groups were healthy groups (negative group), positive group (induced with CCl<sub>4</sub>), groups treat with Syllimarin, while the forth and the last groups were fed with snack bars. Blood were collected via retro orbitalis before treatment and after induction of CCl<sub>4</sub>. Malondialdehyde (MDA) test were done using TBARS C18 methods [23] while SGPT and SGOT using spectrophotometry UV DiaSys [24].

This experimental used factorial completely randomized design with the first factor was the ratio between black glutinous rice and pumpkin flour (25:75; 50:50; and 75:25) and the second factor was sweeteners, including honey, palm sugar, and fructose. Data were show as mean  $\pm$  SD (One-way ANOVA, Windows SPSS version 25) using 5% significance continue with Tukey tests ( $P < 0.05$ )

### **3. Results and Discussion**

#### **3.1. Chemical and Sensory Evaluation**

Moisture content of snack bar from black glutinous rice and pumpkin flour were in the range of 11.67 to 15.65 g/100 g. Ho et al. 16 formulating “energy” snack bars using “dodol” and the moisture content was 13.23 g/100 g (db). From [25], snack bar should not exceed 11.26% of moisture content, 10.91% of fats, and 9.3% of protein and the calorie 120.93. The lower the moisture content, the long the shelf life will be. Snack bar from glutinous rice and yellow pumpkin has relatively high moisture content. The batter have high moisture value because of this product contain high starch from black glutinous rice and also high sugar and presumably consider as “intermediate moisture food”. Sweeteners variant did not affect the moisture content of “sticky bars”.

Ash content of sticky bars range from 1.50 to 2.53 g/ 100 g (db). Ash have a correlation with the mineral content of the product or contamination indication from kitchen tools. Mineral in pumpkin reach 5.5% (db) [26], mainly by potassium, sodium, magnesium, phosphor, zinc, calcium, iron, and cobalt. The ash content of low amylose black rice (var Niaw Dam Pleuak Khao, Thailand) was 1.42 g/100 g (db) [27]. The ash content of snack bars was relatively high. Ash content of “energy” snack bars from banana and coconut milk was 1.13 g/100 g (wb) [15]. “Sticky” snack bar resulting high ash content, presumably have a potent as mineral source.

**Table 1.** Proximate Composition (g/100 g of dry matter)

Parameters	Black Glutinous Rice and pumpkin flour Ratio / Sweeteners								
	25 : 75			50 : 50			75 : 75		
	Honey	Palm Sugar	Fructose	Honey	Palm Sugar	Fructose	Honey	Palm Sugar	Fructose
Moisture (%)	14.56 ± 0,00 <sup>bcd</sup>	14.30 ± 0,00 <sup>bc</sup>	15.65 ± 0,00 <sup>d</sup>	15.58 ± 0,00 <sup>cd</sup>	11.67 ± 0,28 <sup>a</sup>	14.41 ± 0,00 <sup>bcd</sup>	13.68 ± 0,97 <sup>b</sup>	12.03 ± 0,03 <sup>a</sup>	12.25 ± 0,00 <sup>a</sup>
Ash (%)	2.53 ± 0,05 <sup>d</sup>	2.22 ± 0,07 <sup>bcd</sup>	2.41 ± 0,01 <sup>cd</sup>	1.88 ± 0,05 <sup>abc</sup>	2.35 ± 0,06 <sup>cd</sup>	1.92 ± 0,09 <sup>abc</sup>	1.66 ± 0,19 <sup>ab</sup>	1.65 ± 0,37 <sup>ab</sup>	1.50 ± 0,04 <sup>a</sup>
Protein (%)	6.09 ± 0,01 <sup>bc</sup>	6.19 ± 0,05 <sup>cd</sup>	6.33 ± 0,12 <sup>d</sup>	5.71 ± 0,05 <sup>a</sup>	5.85 ± 0,04 <sup>ab</sup>	6.79 ± 0,03 <sup>c</sup>	6.23 ± 0,04 <sup>cd</sup>	6.77 ± 0,10 <sup>e</sup>	6.26 ± 0,00 <sup>cd</sup>
Fat (%)	8.57 ± 0,06 <sup>a</sup>	8.36 ± 0,18 <sup>a</sup>	7.98 ± 0,20 <sup>a</sup>	8.73 ± 0,30 <sup>a</sup>	8.48 ± 0,05 <sup>a</sup>	9.74 ± 0,28 <sup>b</sup>	10.96 ± 0,27 <sup>c</sup>	11.32 ± 0,29 <sup>c</sup>	10.54 ± 0,05 <sup>bc</sup>
Sugar (%)	39.4 ± 0,02 <sup>a</sup>	52.7 ± 0,07 <sup>b</sup>	55.0 ± 0,05 <sup>b</sup>	54.9 ± 0,08 <sup>b</sup>	56.5 ± 0,08 <sup>bc</sup>	53.0 ± 0,18 <sup>b</sup>	53.8 ± 0,18 <sup>b</sup>	59.0 ± 0,00 <sup>c</sup>	55.5 ± 0,04 <sup>bc</sup>
Calori (kcal)	117,19 ± 0,52 <sup>a</sup>	121,11 ± 1,50 <sup>a</sup>	119,14 ± 2,50 <sup>a</sup>	123,34 ± 2,13 <sup>a</sup>	122,31 ± 0,24 <sup>a</sup>	136,01 ± 3,11 <sup>b</sup>	145,12 ± 3,29 <sup>cd</sup>	152,60 ± 2,97 <sup>d</sup>	142,06 ± 0,29 <sup>bc</sup>
Fiber (%)	1.69 ± 0,05 <sup>ab</sup>	1.73 ± 0,30 <sup>ab</sup>	2.50 ± 0,00 <sup>b</sup>	2.43 ± 0,51 <sup>b</sup>	1.03 ± 0,30 <sup>a</sup>	1.59 ± 0,43 <sup>ab</sup>	1.72 ± 0,33 <sup>ab</sup>	1.12 ± 0,07 <sup>a</sup>	0.87 ± 0,23 <sup>a</sup>

\*Means in the same column were significantly different according to Tukey test at P < 5%

Previous research by Putri et al. [28] reported that the ash content of the snack bars was between 0.94 to 1.71 g/100 g (wb). This research did not use the whole grain of black glutinous rice but only use grain from the by product in the cake producers or starchy part of the grain. While the endosperm layer and the bran used for cake production. Part of the grain which rich in mineral was in the bran [27].

Protein content of “sticky” snack bars was between 5.71 to 6.79 g/100 g (wb). Sweeteners significantly did not affect the protein content of the snack bars while ratio of the black glutinous rice and pumpkin powder did. While the fats content was different as affected by the sweetener. Snack bars using palm sugar has higher fat than that using honey or fructose. Snack bars using by product from cake production has higher fat (9.18-12.44 g/ 100 g wb) [28], while [29] reported that the fats content of the snack bars using banana puree reach 11.47-12.65 g/ 100 g (wb) or 22.39 g/100 g as reported by Ho et al. [15].

Total sugar in the “sticky” snack bars reach to value of 39.41-59.04 g/100 g. Snack bars produced by [15] reach 56.89 g/100 g while [28] have a lower total sugar (from 20.22 – 30.88 g/100 g. Even the total sugars of snack bars from whole grain of black glutinous rice was lower, it does not have a strong correlation with the total calories. Snack bars from whole grain have more advantage to health because of the anthocyanin content. Akkarachiyasit et al. [30] reported that pigment in black glutinous rice (cyanidin-3-glucoside, cyanidin-3-galactose, or cyaniding-3,5-diglucoside) have an important factor for modulating in carbohydrate metabolism. Thus,

this pigment could provide a prevention for diabetes patient. By using the by-product of cake production, the prevention mechanism of the snack bars also lacks.

Snack bars known as food that could eat between meals. It could retard hungry. For some athletes, snack bars could supply a lot of energy without giving heavy duty for stomach. So they can run or bicycling for a long period without feel exhausted or having a vomiting while exercise. In this case, snack bars have to supply mineral also for homeostatic. “Sticky” snack bars have a low calories comparing to “energy” snack bars from [15] which have 454.51 kcal. Even though have lower calories, “sticky” bars have higher mineral value. Higher content of black glutinous rice resulting in higher calories while the sweetener did not affect the calories of the “sticky” snack bars. The ratio of black glutinous rice and pumpkin flour did not significantly affect the fiber content of the “sticky” snack bars as well as the sweeteners variety. Fiber was an important nutrient which have beneficial effect to intestinal microflora.

### 3.2. Organoleptic Assays

Organoleptic assays were need to evaluate the preference of the product in the market. The organoleptic assays including color, texture, black glutinous rice flavor and hedonic test (Table 2). The results showed that ratio of black glutinous rice and pumpkin flour and the sweeteners have a significant difference in the color of “sticky” snack bars while the texture, black glutinous rice and pumpkin flavor and preference were not affected by the treatments.

**Table 2.** Organoleptic Assays of Sticky Snack Bars

Black Glutinous Rice and pumpkin flour Ratio	Sweetener	Color	Texture	Black glutinous rice flavor	Pumpkin Flavor	Preference
25 : 75	Honey	1.44±0.63 <sup>a</sup>	2.56±0.81 <sup>ab</sup>	1.81±0.66 <sup>a</sup>	3.25±0.86 <sup>c</sup>	2.31±1.01 <sup>a</sup>
	Palm Sugar	1.38±0.62 <sup>a</sup>	2.50±0.63 <sup>ab</sup>	2.13±0.81 <sup>a</sup>	3.00±0.82 <sup>bc</sup>	2.88±0.34 <sup>a</sup>
	Fructose	1.25±0.58 <sup>a</sup>	2.44±0.81 <sup>ab</sup>	2.00±0.89 <sup>a</sup>	3.19±1.17 <sup>c</sup>	2.31±0.79 <sup>a</sup>
50 : 50	Honey	2.00±1.03 <sup>ab</sup>	2.31±1.01 <sup>ab</sup>	2.13±0.62 <sup>a</sup>	2.00±0.73 <sup>a</sup>	2.81±0.91 <sup>a</sup>
	Palm Sugar	2.69±0.70 <sup>b</sup>	2.13±1.15 <sup>a</sup>	2.31±0.70 <sup>ab</sup>	2.75±0.93 <sup>abc</sup>	2.94±0.57 <sup>a</sup>
	Fructose	2.56±0.89 <sup>b</sup>	2.44±1.21 <sup>ab</sup>	2.13±0.62 <sup>a</sup>	2.63±0.72 <sup>abc</sup>	3.06±0.68 <sup>a</sup>
75 : 25	Honey	3.56±0.81 <sup>c</sup>	3.75±0.45 <sup>c</sup>	3.19±0.91 <sup>b</sup>	1.94±0.94 <sup>a</sup>	2.56±0.81 <sup>a</sup>
	Palm Sugar	3.56±0.81 <sup>c</sup>	3.13±0.89 <sup>bc</sup>	3.06±0.93 <sup>b</sup>	2.06±0.85 <sup>ab</sup>	2.75±0.93 <sup>a</sup>
	Fructose	3.69±0.48 <sup>c</sup>	2.94±0.77 <sup>ab</sup>	3.06±1.06 <sup>b</sup>	2.31±1.14 <sup>abc</sup>	2.56±0.63 <sup>a</sup>

Note:

\*Means in the same column were significantly different according to Tukey test at P < 5%

Color: value represent brown color

Texture: value represent smoothness, the higher the value, product not smooth

Black glutinous rice flavor: higher value represents more flavor of black glutinous rice

Pumpkin flavor: higher value represents high pumpkin flavor

Preference: high value showed high preference by the panelist

The organoleptic evaluation showed that the flavor of black glutinous rice was still tasted in the ratio of 80% black glutinous rice and 20% of pumpkin using fructose as a sweetener. The flavor of black glutinous rice was not exist in the ratio of 50% of black glutinous rice using palm sugar as sweetener. The more the black glutinous rice in the batter, the more the flavor of black glutinous rice in the snack bar. The ratio of black glutinous rice and pumpkin flour significantly affect the color of the snack bars while the sweetener did not. Meanwhile the texture, the flavor of black glutinous rice and pumpkin flour were not affected by the ratio of the batter. The preference of the panelist was not significantly detected in all treatments ( $P < 5\%$ ).

### 3.3. Antioxidant and Antiinflammation Assays

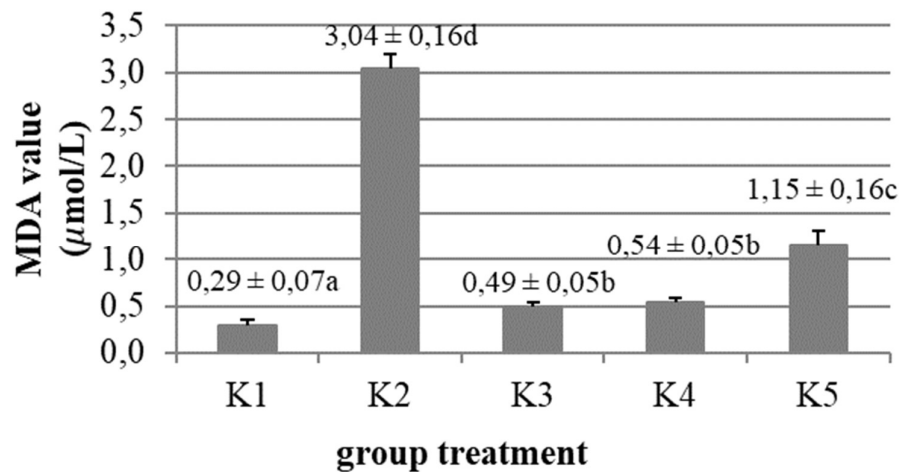
Liver is the largest organ in the body that plays an important role in metabolic processes and detoxification. According to Stine & Brown [31], liver contained an enzyme system that is chemically able to convert toxic compounds into less toxic forms. However, in certain cases such as hepatotoxicity by CCl<sub>4</sub>, this substance will become more toxic than the original compound, this reaction is often called CCl<sub>4</sub> biotransformation. An increase in liver enzymes such as SGOT or SGPT is an indicator of liver disorders. Carbon tetrachloride in the body of an organism undergoes reactive biotransformation catalyzed by the cytochrome P450 enzyme. The reactive metabolite is a trichloromethyl free radical (CCl<sub>3</sub>). Trichloromethyl with oxygen will form a trichloromethyl peroxy (CCl<sub>3</sub>O<sub>2</sub>) radical which can attack endoplasmic membrane lipids at a speed that exceeds trichloromethyl free radicals. Furthermore, trichlorometilperoxy causes lipid peroxidation and thus disrupts Ca<sup>2+</sup> homeostasis, and ultimately causes cell death [19].

### 3.4. Measurement of MDA Levels

Malondialdehyde is formed due to degradation of OH free radicals to unsaturated fatty acids, which are converted into highly reactive free radicals. In addition, MDA is also a metabolite of cell components produced by free radicals. High MDA levels will reflect the oxidation process in cell membranes, and conversely low MDA levels prove high antioxidant enzyme activity or antioxidant status. MDA measurements have long been used as indicators of the presence of free radicals [32].

Figure 1 shows that MDA levels for group I or negative controls (-) of  $0.29 \pm 0.07 \mu\text{mol/l}$ , this group I is an indicator of normal MDA levels, this is according to normal standards of MDA levels according to Yang et al. [32], that the normal level of MDA is  $1.04 \pm 0.43 \mu\text{mol/l}$ . MDA level in group II or positive control was  $3.04 \pm 0.16 \mu\text{mol/l}$ , which was the group that had the greatest increase in MDA levels, because this group was induced by CCl<sub>4</sub> without any additional treatment, so it did not get antioxidant and anti-inflammatory compounds. There were three groups induced by CCl<sub>4</sub> with the addition of different treatments showing the highest MDA levels in group V of  $1.15 \pm 0.16 \mu\text{mol/l}$ , which was the CCl<sub>4</sub> induced group and treated with black sticky rice B snack bars, p. this is due to the presence of anthocyanin in 50 g of black

glutinous rice, so that there is an intake of antioxidant compounds that can potentially reduce MDA levels but are not optimal and still high compared to groups III and IV. The lowest MDA level in group III was  $0.49 \pm 0.05 \mu\text{mol/l}$ , which was a  $\text{CCl}_4$  induced group and was given a treatment in the form of a comparative drug containing silymarin, this could occur because silymarin has a very effective ability and has therapeutic effects for liver damage [19]. Whereas MDA group IV levels were not significantly different from third group III of  $0.54 \pm 0.05 \mu\text{mol/ml}$ , were  $\text{CCl}_4$  induced groups and were treated in the form of black glutinous rice snack bars A, this proves that black glutinous rice A snack bars have the ability is equal to silymarin in reducing MDA levels, because of its antioxidant content.



**Figure 1.** MDA Value of Rats Blood in All Treatment

Note:

- Group I (negative control) : Healthy rats
- Group II (positive control) : Rats induced by  $\text{CCl}_4$  in the day of 14<sup>th</sup>
- Group III : Rats induced by  $\text{CCl}_4$  in the day of 14<sup>th</sup> and treated with 100 mg/kg BB of silymarin for 14 days
- Group IV : Rats induced by  $\text{CCl}_4$  in the day of 14<sup>th</sup> and treated with feed using snack bars based cake formula A for 14 days
- Group V : Rats induced by  $\text{CCl}_4$  in the day of 14<sup>th</sup> and treated with feed using snack bars based cake formula B for 14 days

Giving additional treatment with black glutinous rice A and B snack bars together can reduce MDA levels. This proves that black glutinous rice snack bars as a whole have the potential to reduce MDA levels. This is because the presence of anthocyanin which is an antioxidant compound has a protective effect that has the potential as a free radical therapy agent in the liver, which can reduce MDA levels. Boll et al.20 stated that antioxidants would neutralize free radical compounds from  $\text{CCl}_4$  and prevent the occurrence of chain reactions by donating electrons to trichloromethyl and trichloromethylperoxyl free radicals to become more stable compounds. The decrease in MDA levels associated with the provision of black glutinous rice snack bars related to anthocyanin activity which is an antioxidant compound has a protective effect that has the potential as a free radical therapy agent in the liver, which can reduce MDA

levels. This is reinforced by the research that the results of the in-vivo test [33], his research showed that the consumption of anthocyanin extract doses of 50 mg/kg BB and 100 mg/kg BB was able to reduce MDA levels to 3.52 mmol/l and 2.78 mmol/l. Fitriyati [35] showed that administration of black rice anthocyanin extract and purple sweet potato anthocyanin extract at a dose of 13.5 mg/kg BW at the initial 3 weeks and 67.5 mg/kg body weight at the last 3 weeks could reduce blood MDA levels by 52.43 - 55.38%.

### 3.5. Measurement of SGPT and SGOT Levels

Increased levels of liver enzymes in the serum, including increased SGPT levels and SGOT are one indicator of liver damage. Increased aminotransferase enzymes (also known as transaminases), SGPT and SGOT, usually lead to hepatocellular or inflammatory treatment, their increase is in accordance with hepatocyte inflammation [16].

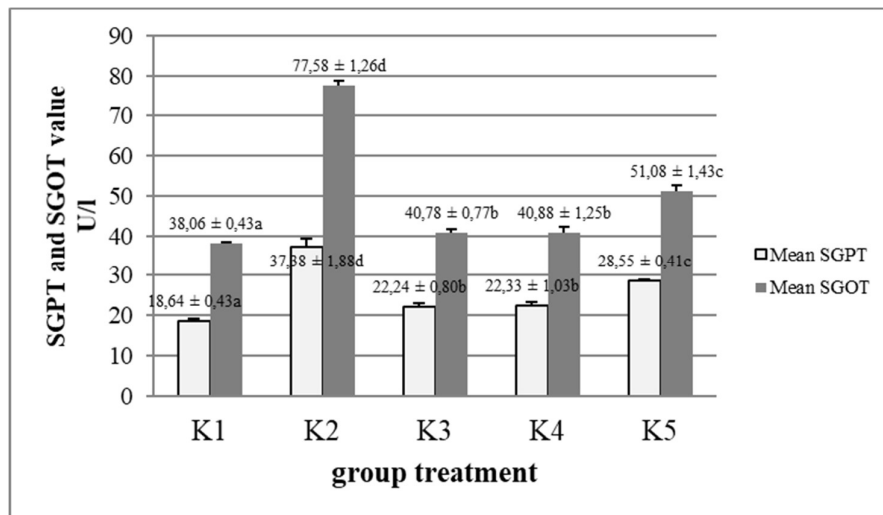


Figure 2. SGPT and SGOT Level of Rats in All Treatments

Note:

- Group I (negative control) : Healthy rats
- Group II (positive control) : Rats induced by CCl<sub>4</sub> in the day of 14<sup>th</sup>
- Group III : Rats induced by CCl<sub>4</sub> in the day of 14<sup>th</sup> and treated with 100 mg/kg BB of silymarin for 14 days
- Group IV : Rats induced by CCl<sub>4</sub> in the day of 14<sup>th</sup> and treated with feed using snack bars based cake formula A for 14 days
- Group V : Rats induced by CCl<sub>4</sub> in the day of 14<sup>th</sup> and treated with feed using snack bars based cake formula B for 14 days

Figure 2 shows that the levels of SGPT and SGOT for group I or negative controls (-) of 18.64 ± 0.43 and 38.06 ± 0.43 U/l, this group I is a normal indicator of SGPT and SGOT levels. Normal levels of SGPT are 17.5 - 30.2 U/l and SGOT which are 30.2 - 45.7 U/l. SGPT and SGOT levels in group II or positive controls were 37.38 ± 1.88 and 77.58 ± 1.26 U/l, which were the groups that had the greatest increase in SGPT and SGOT levels, because this group was induced by CCl<sub>4</sub> without additional treatment. There were three groups induced by CCl<sub>4</sub> with the addition of different treatments showing the highest SGPT and SGOT levels in group V of 28.55 ± 0.41



and  $51.08 \pm 1.43$  U/l, which were CCl<sub>4</sub> induced and given treatment in the form of black glutinous rice snack bar B, this was due to the presence of anthocyanin in 50 g of black glutinous rice, so that there was an intake of anti-inflammatory compounds that could potentially reduce SGPT and SGOT levels but were less optimal and highest than groups III and IV. The lowest SGPT and SGOT levels in group III were  $22.24 \pm 0.80$  and  $40.78 \pm 0.77$  U/l, were CCl<sub>4</sub> induced groups and were given comparative drugs containing silymarin, this could occur because of silymarin was a therapeutic effect for liver damage [20]. Whereas SGPT and SGOT IV group levels were not significantly different with group III of  $22.33 \pm 1.03$  and  $40.88 \pm 1.25$  U/l, were CCl<sub>4</sub> induced groups and were treated as black glutinous rice snack bars A. This proves that black sticky rice A snack bars have the same ability as silymarin in reducing SGPT and SGOT levels as liver anti-inflammatory agent.

Giving additional treatment with black glutinous rice A and B snack bars together can reduce SGPT and SGOT levels. This proves that black glutinous rice snack bars as a whole have the potential to reduce SGPT and SGOT levels. This is because the presence of anthocyanin which can act as an anti-inflammatory compound has a protective effect that has the potential as a liver damage therapy agent, so that it can reduce SGPT and SGOT levels compared to group II which has the highest SGPT and SGOT levels.

The decrease in SGPT and SGOT levels associated with the administration of black glutinous rice snack bars is related to anthocyanin activity as a potential anti-inflammatory agent. Burkie [36] showed that the administration of black rice bran extract at dose of 540 mg/ 200 g BB has an effective hepato-protective effect. It can reduce SGPT levels till  $56.08 \pm 7.50$  U/l compared to the negative treatment group with an average yield of SGPT  $\pm$  SD,  $176.18 \pm 118.80$  U/l.

#### 4. Conclusion

The most popular snack bars are panelists with a comparison of black glutinous rice flour and 50:50 pumpkin flour with the use of honey sugar. In terms of nutritional value the best is the snack bars with a comparison of black glutinous rice flour and pumpkin flour 75:25 with the use of honey sugar, where the water content is 13.667%; ash content of 1.657%; protein content 6.234%; fat content 10.962%; total sugar content of 53.80%; total calories 338,794 kcal; 1.72% fiber content and snack bars with a comparison of black glutinous rice flour and pumpkin flour 75:25 with the use of the type of palm sugar potentially as a free radical therapy agent in the liver which is characterized by a decrease in malondialdehyde (MDA) and potentially an inflammatory therapy agent in the liver with decreased serum levels of glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) in CCl<sub>4</sub> induced mice.

## Acknowledgments

Many thanks to General Ministry of Higher Education via Applied Grants to explore Black Glutinous Rice as functional food. Also for research team who always support this topics.

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