Total compounds of Secondary Metabolites Soy-Yamghurt Formula and Nephropathy Effect in Male White Rats

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Abstract

Complications of diabetes mellitus cause diabetic nephropathy. Soy-yamghurt is made from a combination of Banggai sweet potato juice and fermented soybean juice to be a functional food for diabetic nephropathy sufferers. This study aimed to analyze quantitatively the secondary metabolites contained in soy-yamghurt and to determine the effectiveness of soy-yamghurt in reducing urea and creatinine levels. This study used an experimental method with a total of 25 rats divided into 5 groups, namely normal control, negative control, soy-yamghurt treatment group with a comparison of yam and soybean extract, namely F1(1:1), F2(1:2), and F3(2:1). The parameters observed were urea and creatinine levels on days 0, 7, 14, 21, and 28. The results obtained were the total levels of secondary metabolites of Alkaloid compounds F1 0.10% v/v, F2 0.01% v/v, and F3 0.01% (v/v), Flavonoid compounds F1 0.14% (v/v), F2 0.12% (v/v), F3 0.13% (v/v), Tanin compounds F1 0.27% (v/v), F2 0.26% (v/v), F3 0.14% (v/v) and saponins F1 1.15% (v/v), F2 1.22% (v/v), F3 1.25% (v/v). Administration of soy-yamghurt F2 was effective in lowering urea and creatinine levels. With an average value of 14.66 mg/dL urea and 0.40 mg/dL creatinine.

Keywords: Creatinine, secondary metabolites, soy-yamghurt, urea.

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1. INTRODUCTION

Kidneys are organs that have an excretory function, including excreting metabolic waste products. Some end products of metabolism that the kidneys can excrete are urea and creatinine. When the levels of urea and creatinine in the body increase, this indicates kidney damage, causing kidney failure (Hermawan, 2016). Diabetic nephropathy is a disease of kidney failure that occurs due to microvascular complications in people with diabetes mellitus (Preguica et al., 2020).

Banggai sweet potato (Dioscorea alata L.) contains bioactive compounds such as dioscorin, dioscin, diosgenin, inulin, and PLA, which function as antioxidants and sources of prebiotics (Yuniastuti et al., 2017; Estiasih et al., 2017). The soybean plant (Glycine max (L.) Merr.) contains isoflavone flavonoid compounds with antioxidant activity. Isoflavones in soybeans are generally glycosides but will be converted into aglycone compounds through a fermentation process by certain bacteria. One of the processed, fermented soybeans is soyghurt. Along with the development of methods in the food sector, soyghurt has begun to be widely formulated into functional food that is beneficial for health with the addition of prebiotics (Rahmawati et al., 2017).

Soy-yamghurt is a synbiotic drink containing probiotics and prebiotic components with antioxidant activity. The dosage administration of soy-yamghurt with antidiabetic properties was 3 mL/160 g BW and 3.5 mL/160 g BW. It is because the oligosaccharides contained in soy-yamghurt can form a gel and can be degraded into short-chain fatty acids by microbes with the help of enzymes. Short-chain fatty acids will inhibit the
absorption of blood glucose (Kwanariesta et al., 2018).

Therefore, it is necessary to research the effect of treated soy-yamghurt fermented from Banggai sweet potato extract and soybean juice on rat urea and creatinine levels.

2. MATERIALS AND METHODS

Test Animal Experiment

The experimental animal protocol was approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Tadulako University, with the number 983/UN 28.1.30/KL/2021. Male Wistar rats weighing 200-250g were obtained from Vaila Wistar and acclimatized in local animal cages for 2 weeks.

Testing of Urea and Kreatinin Activity in Test Animals

A total of 25 rats were divided into 5 groups, each group divided into 5 test animals with each group, namely Normal control (NaCMC), negative control (NaCMC+streptozotocin), formula 1 (1:1) given a volume 3.4 mL, formula 2 (1:2) was given a volume 3.4 mL and formula 3 (2:1) administration of volume 3.4 mL. Before the test animals were used, the test animals fasted for 16 hours, and then blood urea and kreatinin levels were analyzed with UV-Vis spectrophotometer evolution 201. After the urea and kreatinin level was analyzed (on day 0), streptozotocin was induced at 40 mg/kg BW intraperitoneally except for the normal control group. On the seventh day, the rats fasted for 16 hours, and then the blood urea and kreatinin levels were analyzed with UV-Vis spectrophotometer evolution 201 after streptozotocin induction. After the fasting, blood urea and kreatinin levels of the mice reached a state of affect urea and kreatinin level, after that the mice of each group were given a Normal control (NaCMC), negative control (NaCMC+streptozotocin), formula 1 (1:1) given a volume 3.4 mL, formula 2 (1:2) was given a volume 3.4 mL and formula 3 (2:1) administration of volume 3.4 mL for 28 days. After treatments, all blood samples were recorded and analyzed with UV-Vis spectrophotometer evolution 201.

Yogurt Starter Preparation

Sixteen grams of skim milk was dissolved in 100 mL of hot water while stirring well, and then the temperature was lowered to 45 °C. Commercial yogurt starter culture (biokul plain) was added as much as 5% of the volume of the mixture and stirred, then covered with polyethylene plastic. The resulting mixture was incubated at 37 °C for 12 hours. The yogurt starter that had been produced was stored in the refrigerator at 4 °C.

Banggai Yam Extraction

The obtained Banggai yam was cleaned by peeling the Banggai yam tuber. Furthermore, washing was carried out to remove the remaining dirt on the tuber flesh. After washing, the tubers were refined using a blender by adding boiled water in a ratio of 1:3 w/v. Then filtered using a filter cloth that had been blanching, and then the extract was taken (Kwanariesta et al., 2018).

Soybean Extraction

The soybean was boiled for 30 minutes and then soaked in 0.2% NaHCO₃ solution for 30 minutes. After that, it was boiled again for 30 minutes. Then the soybean skin was separated by kneading and washing it many times until clean. Hot water (100 °C) was added to skinless soybeans in a ratio of 1:6, then blended. The resulting soybean porridge was filtered using a filter cloth that had been blanching to obtain soybean juice and then left on low heat at 80 °C for 20 minutes (Kwanariesta et al., 2018).

Soy-Yamghurt Fabrication

Soy-yamghurt was made from a mixture of Banggai yam extract and 79.4% soybean extract, with a ratio of 1:1, 1:2, and 2:1 (v/v), 15% skim milk, and 2% honey. Then, 3% starter was added to the mixture and mixed with 0.6% Na CMC. Then it was incubated at 43 °C for 6 hours. Then, the formed Soy-yamghurt was stored in the refrigerator at 4 °C under anaerobic conditions (Kwanariesta et al., 2018). The Soy-Yamghurt formula can be seen in Table 1.
Table 1. Soy-yamghurt formulation in 100 mL

<table>
<thead>
<tr>
<th>Composition</th>
<th>Function</th>
<th>Amount (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweet Potato Juice</td>
<td>Source of prebiotics</td>
<td>F1 (1:1): 39.7</td>
</tr>
<tr>
<td>Soy Sauce</td>
<td>Basic material</td>
<td>F2 (1:2): 52.92</td>
</tr>
<tr>
<td>Skim Milk</td>
<td>Source of lactose</td>
<td>F3 (2:1): 26.46</td>
</tr>
<tr>
<td>Honey</td>
<td>Starter</td>
<td></td>
</tr>
<tr>
<td>Biokul Plain Yogurt Starter (S. Thermophilus, L. Bulgaricus, L. Acidophilus, Bifidobacterium)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na CMC</td>
<td>Stabilizer</td>
<td></td>
</tr>
</tbody>
</table>

**Determination of Total Flavonoid**

0.5 mL diluted sample (1:10 g/mL ethanol 96%), added 1.5 mL of ethanol (96%), 0.1 mL of 10% AlCl₃, 0.1 mL of sodium acetate 1 M, and 2.8 mL of distilled water. It was left for 30 minutes, then the sample was read at 417 nm.

**Determination of Total Alkaloid**

0.5 mL diluted sample (1:10 g/mL ethanol 96%). After that filled into a volume 100 mL volumetric flask, diluted with 96% ethanol on the mark, and homogenized. Then the sample was read at 275 nm.

**Determination of Total Saponin**

0.5 mL diluted sample (1:10 g/mL ethanol 96%) and 2 mL of 25% H₂SO₄ were transferred to the autoclave. After that, the autoclaved was heated for 120 minutes at 110 °C. Then, the mixture was extracted using ether and dried the filtrate. Add distilled water as much as 1 mL, vortex extraction for 5 minutes, 50 l of anisaldehyde, shaking, and let stand for 10 minutes. Add 2 mL of 50% sulfuric acid, then heat on a water bath at 60 °C for 10 minutes. Then add up to 10 mL of distilled water, diluted 10 times. Then the absorbance of the sample was read at 435 nm.

**Determination of Total Tannin**

0.5 mL diluted sample (1:10 g/mL ethanol 96%) was extracted with diethyl ether for 20 hours and then filtered. The remaining diethyl ether was evaporated, followed by adding distilled water to a volume of 10 mL. Then 1 mL of sample solution was taken. 0.1 mL of Folin Ciocalteu reagent was added to the sample solution and vortexed for 5 minutes. After that, distilled water was filled into it to a volume of 10 mL and diluted 5 times. Then, the absorbance of the sample was read at 760 nm after being incubated for 30 minutes at room temperature.

**Blood Sampling**

Blood sampling was conducted in the laboratory STIFA Pelita Mas Palu. Blood collection was carried out on days 0, 7, 14, 21, and 28 through rats using a tube that had been given 2 mL of EDTA to be centrifuged into a serum.

**Urea Analysis**

The test tubes were prepared for sample, standard, and blank. Then 10 µl serum and 2000 µl of urea reagent were added to the sample and then allowed to stand at 25 °C. Then the absorbance of the sample, standard, and blank solutions were measured using UV-Vis spectrophotometer evolution 201 at 340 nm.

**Creatinine Analysis**

Then the test tubes were prepared for sample, standard, and blank. Then 50 µl serum and 2000 µl of creatinine reagent were added to the sample tube and allowed to stand at 25 °C. The absorbance of the sample, standard, and blank solutions were measured using UV-Vis spectrophotometer evolution 201 at 492 nm.

**Data Analysis**

The measurement data of urea and creatinine levels using a UV-Vis spectrophotometer were calculated and statistically analyzed using one-way ANOVA and Kruskal-Wallis tests. If the data was normal and homogeneous, it was analyzed using the one-way ANOVA test. Then, if there were differences, it continued with Duncan’s further test. If the data was not normal or homogeneous, then the data was analyzed using the Kruskal-Wallis test.
3. RESULTS AND DISCUSSION

The total levels of secondary metabolites contained in the soy-yamghurt formula were carried out by quantitative analysis using UV-Vis spectrophotometry, including total flavonoids, alkaloids, saponins, and tannins (Table 2).

Table 2. Quantitative results of Soy-Yamghurt formula

<table>
<thead>
<tr>
<th>No.</th>
<th>Test Parameters</th>
<th>Formula 1:1</th>
<th>Formula 1:2</th>
<th>Formula 2:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total alkaloids equivalent caffeine</td>
<td>Formula 1:1</td>
<td>0.10</td>
<td>0.01</td>
</tr>
<tr>
<td>2</td>
<td>Total flavonoid equivalent quercetin</td>
<td>Formula 1:1</td>
<td>0.14</td>
<td>0.12</td>
</tr>
<tr>
<td>3</td>
<td>Total tannic equivalent quercetin</td>
<td>Formula 1:1</td>
<td>0.27</td>
<td>0.26</td>
</tr>
<tr>
<td>4</td>
<td>Saponin from Quailaja bark</td>
<td>Formula 1:1</td>
<td>1.15</td>
<td>1.22</td>
</tr>
</tbody>
</table>

Determination of total alkaloid content obtained by regression equation Y = 0.2793-0.0132 and correlation coefficient (R²)= 0.9892 (Figure 1). Based on the regression equation, the total alkaloid content was calculated, and the results for formula 1 was 0.10%, formula 2 was 0.01%, and formula 3 was 0.01% (w/w) (Table 2).

In determining the total flavonoid content, the regression equation is Y=0.0125+0.047, and the correlation coefficient (R²)= 0.9996 (Figure 2). After calculating, the total flavonoid content of all formulas was 0.14%, 0.12%, and 0.13% (w/w) for formula 1, 3 and 3, respectively (Table 2).

Determination of total tannin content obtained by regression equation Y = 0.0037x - 2E-05, R² = 0.9972 (Figure 3). After calculating, the total tannin content of all formulas was 0.14%, 0.12%, and 0.13% (w/w) for formula 1, 3 and 3, respectively (Table 2).
0.000285 and correlation coefficient ($R^2$) = 0.9972 (Figure 3). The regression equation calculated the total tannin content for formula 1 at 0.27%, formula 2 at 0.26%, and formula 3 at 0.14% (w/w) (Table 2). Determination of total saponin levels obtained by regression equation $Y=0.0037-1.90476E^{-5}$ and correlation coefficient ($R^2$)=0.9972 (Figure 4). The regression equation obtained that the total saponin content in all formulas were 1.15%, 1.22%, and 1.25% (w/w) for formula 1, 2, and 3, respectively (Table 2).

Table 3. Average levels of urea (mg/dL) in male white rats (Rattus norvegicus) (days: 0, 7, 14, 21, and 28).

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 0 (y)</th>
<th>Day 7 (y)</th>
<th>Day 14 (x)</th>
<th>Day 21 (y)</th>
<th>Day 28 (y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>18.21±2.30 a</td>
<td>16.43±0.49 a</td>
<td>15.99±1.68 a</td>
<td>19.77±4.74 a</td>
<td>15.55±0.00 a</td>
</tr>
<tr>
<td>Negative Control</td>
<td>18.66±1.45 a</td>
<td>45.10±5.86 b</td>
<td>50.66±7.05 c</td>
<td>37.55±6.72 b</td>
<td>28.66±1.99 b</td>
</tr>
<tr>
<td>Soy-yamgurt F1</td>
<td>18.21±1.68 a</td>
<td>40.21±6.05 b</td>
<td>30.84±2.04 b</td>
<td>32.88±3.97 b</td>
<td>21.55±1.27 c</td>
</tr>
<tr>
<td>Soy-yamgurt F2</td>
<td>19.33±1.68 a</td>
<td>41.55±5.42 b</td>
<td>30.43±3.10 b</td>
<td>30.21±1.99 bc</td>
<td>14.66±1.99 a</td>
</tr>
<tr>
<td>Soy-yamgurt F3</td>
<td>18.43±0.99 a</td>
<td>43.77±6.78 b</td>
<td>29.33±4.05 b</td>
<td>24.66±3.63 ac</td>
<td>22.89±2.56 c</td>
</tr>
</tbody>
</table>

Note: (x) = One way ANOVA test. (y) = Kruskal-Wallis test. Different lowercase letters in each group indicate significant differences between treatments.

Figure 5. Urea level profile of male white rats in each group on day 0, day 7 (after induction), day 14, 21, and 28 (after soy-yamgurt treatment).

Table 4. Average creatinine levels (mg/dL) of male white rats (Rattus norvegicus) Day 0, 7, 14, 21, and 28.

<table>
<thead>
<tr>
<th>Group</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>0.36±0.16 a</td>
<td>0.46±0.24 a</td>
<td>0.36±0.07 a</td>
<td>0.44±0.10 a</td>
<td>0.58±0.17 ab</td>
</tr>
<tr>
<td>Negative Control</td>
<td>0.40±0.05 a</td>
<td>1.44±0.09 b</td>
<td>1.42±0.08 b</td>
<td>1.47±0.04 b</td>
<td>2.18±0.37 c</td>
</tr>
<tr>
<td>Soy-yamgurt F1</td>
<td>0.56±0.36 a</td>
<td>1.60±0.15 b</td>
<td>1.24±0.04 c</td>
<td>1.04±0.08 c</td>
<td>0.62±0.13 b</td>
</tr>
<tr>
<td>Soy-yamgurt F2</td>
<td>0.44±0.07 a</td>
<td>1.39±0.19 b</td>
<td>1.21±0.13 c</td>
<td>1.04±0.08 c</td>
<td>0.40±0.13 a</td>
</tr>
<tr>
<td>Soy-yamgurt F3</td>
<td>0.49±0.14 a</td>
<td>1.53±0.03 b</td>
<td>1.42±0.13 c</td>
<td>1.04±0.05 c</td>
<td>0.64±0.10 b</td>
</tr>
</tbody>
</table>

Note: Different lowercase letters in each group indicate significant differences between treatments.
Figure 6. Creatinine level profile of male white rats in each group on day 0, day 7 (after induction), day 14, 21, and 28 (after soy-yamghurt treatment)

The urea and creatinine levels decreasing in the soy-yamghurt treatment group was thought to be due to the content of lactic acid bacteria (LAB) and bioactive compounds like dietary fiber, saponins, alkaloids, and flavonoids in soy-yamghurt which act as antioxidants. Kwanariesta et al. (2018) stated that soy-yamghurt has a performance of polysaccharides and antioxidants that can trap free radicals and reduce oxidative stress. Figure 5 and 6 shows all groups that were treated with a volume of 3.4 mL/200g BW of soy-yamghurt F1, F2 and F3 for 21 days reduced urea and creatinine levels to reach normal levels, with the average value of urea levels being 14.66 mg/dL and creatinine 0.40 mg/dL. (Table 3 and 4), where the normal value of rat urea levels is 13.9–28.3 mg/dL and normal creatinine levels are 0.30–1.00 mg/dL (Anshar et al., 2018). Pei et al., (2018) reported that treated probiotics, prebiotics, and symbiotics could reduce inflammation in kidney disorders and improve kidney function by reducing urea levels in rat test animals. Ban et al. (2019) also explained that symbiotic yogurt could reduce glucose levels in type 2 diabetic rats, regenerate the islets of Langerhans, and repair liver and kidney damage in rats. Guntiyastutik and Nuhrawangsa (2020) stated that dietary fiber significantly reduces urea and creatinine levels.

The content of dietary fiber inulin and Polylactic acid (PLA) as a source of prebiotics will be fermented by LAB. According to Oh et al. (2020), adding prebiotics can increase the viability of LAB so that the total LAB increases and the antioxidant activity increases. It is supported by research conducted by Diastini et al. (2020), which states that total LAB affects the increase in antioxidant activity, phenolics, anthocyanins, and flavonoids in a black soyghurt food. LAB will produce protease enzymes that break down milk proteins and then produce bioactive peptides as antioxidants to repair damage to kidney tissue cells (Chalid et al., 2021).

Alkaloids work by stimulating the hypothalamus to increase the secretion of Growth Hormone Releasing Hormone (GHRH) so that high secretion of Growth Hormone (GH) will stimulate the secretion of Insulin-Like Growth Factor-1 (IGF-1). IGF-1 can induce hypoglycemia and reduce gluconeogenesis, so glucose levels and insulin requirements decrease (Tandi et al., 2019).

Flavonoids are one of the phenol groups that work to inhibit hydrolytic and oxidative enzymes and complement the lack of electrons in free radicals so that they can prevent damage to glomerular cells (Tungmunnithum et al., 2018). In the mechanism of healing diabetes mellitus, flavonoids have a significant role in increasing antioxidant enzyme activity and being able to regenerate damaged pancreatic beta cells so that insulin deficiency can be overcome. A repair of kidney tissue cells causes a decrease in urea and creatinine levels (Tandi et al., 2020).

Tannin compounds have antioxidant activity and inhibit tumor growth. Tannins also have hypoglycemic activity by increasing
glycogenesis. In addition, tannins also function as astringents or chelators that can shrink the epithelial membrane of the small intestine, thereby reducing the absorption of food juices and inhibiting sugar intake. As a result, the rate of increase in blood sugar is low (Tandi et al., 2020). On the other hand, saponins inhibit the increase in vascular permeability to prevent inflammation in kidney cells and inhibit super peroxides through hydroperoxide intermediates formation, thereby preventing biomolecules damaging by free radicals (Tandi et al., 2020).

Soy-yamghurt F2 is a formula with variations of sweet potato extract and soybean juice (2:1). This indicates that the amount of soybean juice is higher than that of Banggai sweet potato extract. Soybeans contain isoflavone flavonoid compounds which are the primary antioxidants in soybeans. Isoflavones will weaken the reactivity of free radicals and increase the activity and expression of antioxidant enzymes (Yoon & Park, 2014). Isoflavones in soybeans are glycosides. They will be converted into aglycone compounds through a fermentation process by lactic acid bacteria (Izaguirre et al., 2021). Aglycone compounds have a higher bioavailability than glycosides. So the fermentation process will increase the bioavailability of isoflavones in soy-yamghurt.

Genistein is an aglycon isoflavone that is more easily absorbed by the small intestine with higher antioxidant activity. Genistein helps repair cells, metabolizes glucose in fat, and protects pancreatic cells (Yulifianti et al., 2019). Dafriani (2016) states that aglycone isoflavones, especially genistein, can inhibit NFB, a transcription factor for inflammation cytokines. It can reduce the inflammatory process and prevent kidney fibrosis so that diabetic nephropathy can be controlled. In addition, repairing damaged kidney tissue cells causes a decrease in urea and creatinine levels.

4. CONCLUSIONS

The provision of soy-yamghurt fermented from Banggai sweet potato extract and soybean extract contains secondary metabolites, reducing the urea and creatinine levels of white male rats in the formula F2 with a ratio of Banggai sweet potato juice and soybean extract, namely 1:2 (26.46: 52.92%).

REFERENCES


