Enzymatic virgin coconut oil effect on urea and creatinine levels of hypercholesterolemia-diabetics induced Wistar male rats

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ABSTRACT

Coconut (Cocos nucifera L.) is an Indonesian commodity that has high economic value tall. Virgin Coconut Oil (VCO) is one of the processed coconut products whose selling value is very high, because the composition of VCO consists of medium-chain fatty acids that can maintain a healthy body and prevent various diseases. The process of making VCO used in this research is an enzymatic method using pineapple weevil as a bromelain enzyme. This study aims to evaluate the impact of different doses of enzymatic VCO in reducing urea and creatinine levels in hypercholesterolemic diabetic male white rats (Rattus norvegicus). This study was an experimental laboratory with a modified pretest and posttest randomized controlled group design using 30 test animals which were divided into 6 groups. Each group consisted of 5 test animals, namely normal control, negative control, positive control receiving branded VCO, and test group given 0.2, 0.4, and 0.8 mL/kg BW of enzymatic VCO. The animals were administered enzymatic VCO orally every day for 14 days. Urea and creatinine levels were measured in blood samples taken from the tail at each observation time point, which included day 0, 21, 28, and 35. The results showed that enzymatic VCO at a dose of 0.8 mL/kg BW significantly reduced urea and creatinine blood levels with an average decrease of 17.40 mg/dL and 0.36 mg/dL. Based on the findings, VCO produced through enzymatic methods shows potential for controlling blood urea and creatinine levels in hypercholesterolemic-diabetic conditions. Further research regarding its formulation into a convenient and practical product would be highly advantageous for its use as a supplement.

Keywords: VCO enzymatically, Streptozotocin, Urea, Creatinine

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INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by high blood sugar (glucose) levels (hyperglycemia) exceeding normal levels, diabetes mellitus is caused by a lack of insulin in the body (Elmuizghi, 2023; Pai et al., 2023; Pathak et al., 2023). Lack of insulin makes the body unable to use glucose as energy. Hyperglycemia is an early sign of diabetes mellitus caused by impaired insulin secretion, hyperglycemia is known to increase the formation of free radicals and Reactive Oxygen Species (ROS) which cause lipids peroxidation and cell membrane damage then can cause long-term complications such as kidney damage (Asmat et al., 2016).

Kidneys are the main organs for the excretion of metabolic waste products that are no longer used by the body and control the volume of fluid composition in the body. The smallest functional unit of the kidney is the nephron. The nephron consists of the glomerulus, proximal convoluted tubule, loop of Henle, distal convoluted tubule, and collagenous duct. Overall kidney function is based on nephron function and impaired kidney function due to decreased nephron work. Laboratory examination method which is used to evaluate the function of the kidneys is to measure the body's metabolic waste substances excreted through the kidneys in the form of urea and creatinine (Pandya, 2016).

Creatinine is the final endogenous product of creatine phosphate metabolism where the levels are relatively more constant and creatinine is also a product of muscle metabolism which is secreted through serum by the body every day, while urea is the main product of protein metabolism in the body. Serum urea levels depend on the catabolism (breakdown) of proteins and amino acids in the liver which are secreted into the kidneys and then excreted through the urine. The body will form antioxidant compounds to control urea and creatinine levels through the release of electrons so that metabolism can take place properly (Sulistyani & Nurkhasanah, 2017).

Diabetes mellitus has a significant scientific correlation with altered urea and creatinine levels, primarily attributed to its proclivity for causing diabetic nephropathy, a progressive kidney disease. This condition, often associated with poorly managed diabetes, leads to a diminished glomerular filtration rate, subsequently impeding the efficient clearance of urea and creatinine from the bloodstream. Consequently, elevated levels of urea and creatinine, termed azotemia and hypercreatininemia, serve as critical clinical markers for renal impairment in diabetics, necessitating vigilant monitoring and diligent glycemic control to mitigate the risk of diabetic kidney disease (Vallon & Komers, 2011). In the other hand, hypercholesterolemia increases the risk of renal failure by promoting conditions such as atherosclerosis, which reduces blood flow to the kidneys (Kon et al., 2011). This compromised blood supply, along with associated factors like inflammation and oxidative stress, impairs renal function and contributes to the progression of chronic kidney disease (CKD).

Virgin Coconut Oil (VCO) is one of the food sources of fat that is in great demand by the wider community because of its potential role in addressing diabetes and hypercholesterolemia, showcasing promising therapeutic effects. The medium-chain fatty acids present in VCO are believed to contribute to improved insulin sensitivity, aiding in the management of diabetes (Narayanankutty et al., 2016). Additionally, VCO has demonstrated the ability to enhance lipid metabolism, leading to a reduction in hypercholesterolemia (Chinwong et al., 2017). Previous studies suggest that the unique composition of VCO, including lauric acid, may positively influence lipid profiles by increasing high-density lipoprotein (HDL) cholesterol while reducing low-density lipoprotein (LDL) cholesterol levels (Marcus, 2013; Savva & Kafatos, 2016). Furthermore, the antioxidant properties of VCO are thought to mitigate oxidative stress, a factor implicated in the progression of diabetes and associated complications (Garkuwa et al., 2023; Iranloye et al., 2013; Narayanankutty et al., 2016).

The role of bromelain in the production of Virgin Coconut Oil (VCO) is pivotal and closely associated with the enzymatic method of VCO production (Rahmalia & Kusumayanti, 2021). Bromelain is a protease enzyme naturally occurring in pineapple plants, and it plays a key role in catalyzing the hydrolysis of peptide bonds within proteins (Agrawal et al., 2022). The enzyme is primarily found in the extract of the pineapple's pulp or stem. In the enzymatic VCO production process, specifically the fermentation method, pineapple stem extract containing bromelain is

**Enzymatic virgin coconut oil... (Dewi et al.,)***
introduced into coconut milk. During this enzymatic process, bromelain’s proteolytic activity becomes instrumental. It acts upon the protein layer within the coconut milk emulsion, breaking down these proteins. As a result, the oil and water components are effectively separated, allowing for the extraction of pure VCO. The use of bromelain in this process enhances the efficiency of VCO production by aiding in the complete separation of oil from water, ensuring a higher-quality end product (Harimurti et al., 2022).

Previous research on VCO that compared with olive oil and red fruit oil at a dose of 0.2 mL/KgBW, VCO is more effective in reducing blood glucose levels in mice. The antidiabetic effect of virgin coconut at a dose of 0.8 mL/KgBW can reduce blood glucose levels (Sulistyani & Nurkhasanah, 2017). Nonetheless, research investigating the effects of enzymatic Virgin Coconut Oil (VCO) on the regulation of urea and creatinine levels in the context of hypercholesterolemia-diabetics complications has not been previously conducted. Hence, this study was conducted to assess the impact of enzymatic VCO at various dosages on blood urea and creatinine levels in hypercholesterolemia-diabetic-induced Wistar male rats.

MATERIALS AND METHOD

Materials

Preparation of test materials

Coconut (Cocos nucifera L.) and young pineapple (Ananas comosus) used in this study were obtained from the Dolo area in the Central Sulawesi region. 15 coconuts (Cocos nucifera L.) were taken and 5 pineapples (Ananas comosus) were then cleaned of skin and other impurities and the required parts were taken. The material is washed with running water and then drained so that it is free from the rest of the washing water and then weighed.

Methods

Cream preparation

Mature coconuts, once peeled, their flesh were extracted, grated using a grating machine, and then combined with water in a 1:1 ratio. This mixture was then kneaded and squeezed until all the coconut milk was extracted. The resulting coconut milk was placed in a jar and sealed tightly for 2 hours, allowing two distinct layers to form. The upper layer is referred to as cream, while the lower layer is known as skim or coconut milk. For the production of VCO, 2000 mL of coconut cream was extracted.

Preparation pineapple juice

The pineapple fruit was cut into small pieces and mashed using a blender then filtered with filter paper to obtain the juice. The pineapple weevil juice was then taken as much as 500 mL.

Preparation of VCO by enzymatic method

The 2000 mL of coconut cream was put into a jar and add 500 mL of pineapple weevil juice. The mixture was stirred well and covered with aluminum foil and then labeled. The mixture was standed for 22 hours until three layers are formed, namely oil, blonde and water. The oil was separated by centrifugation at 3000 rpm for 10 minutes. The yield of VCO obtained is calculated (Palilingan, & Pungus, 2018).

\[
\text{% Yield} = \frac{\text{Oil volume}}{\text{Cream volume}} \times 100 \%
\]

Bromelain enzyme identification test

The Bromelain Enzyme Identification Test was conducted by first preparing 2 mL of pineapple weevil juice. Subsequently, 5 mL of 10% NaOH was added, and the mixture was heated for 5 minutes. Following this, 2 drops of 5% Pb-acetate solution were introduced. The heating process was sustained until a noticeable change in color took place within the solution. The outcome of this test yielded a
positive result, characterized by the development of a brownish solution and the formation of a black precipitate within the solution.

**Fat feed manufacturing**

The high-fat diet utilized consisted of a mixture of pig oil (50%) and quail egg yolk (50%). The diet was prepared as follows: The pig oil was heated until it transformed into a liquid state. Meanwhile, the quail eggs were separated into yolks and whites, and the yolks were then combined with the liquified pig oil. The mixture was thoroughly stirred until it achieved a uniform consistency. Each rat had a maximum daily food intake of 20 grams, and a fresh batch of the diet was prepared and administered orally using a probe every day for a period of 14 days (Anggraeni et al., 2021).

**Preparation of streptozotocin (STZ) solution**

Streptozotocin powder at a dose of 40 mg/kg BW was weighed as much as 0.32 grams and then dissolved using citrate-buffer saline with a pH of 4.5 and then injected into rats intraperitoneally (ip).

**In vivo test**

All experiments were conducted in strict accordance with the animal welfare guidelines established by the World Organisation for Animal Health (OIE) and were granted approval by the Research Ethics Committee at the Faculty of Medicine, Tadulako University, with approval number 2576/UN.28.1.30./K/2019. Male Wistar rats, weighing between 200 and 250 grams, were introduced to designated local animal cages. These rats underwent a 14-day acclimatization period. The criteria for rat selection included an age of approximately three months, a body weight falling within the 200-250 g range, white fur, male gender, and the demonstration of active behavior.

The experimental subjects were divided into six groups: normal control (group I), negative control (group II), positive control receiving branded VCO (group III), and test groups receiving enzymatic VCO at doses of 0.2 mL/kg BW (group IV), 0.4 mL/kg BW (group V), and 0.8 mL/kg BW (group VI).

Cholesterol levels in each group (group II-VI) were measured on the 14th day after the high-fat diet was administered during the two weeks of acclimatization period (Salim et al., 2018; Nurmasitoh, 2015). The rats with cholesterol levels exceeding 200 mg/dL were subsequently induced with STZ. To induce the blood glucose, 1 mL of STZ solution was injected peritoneally just after the acclimatization period ended. Three days after STZ induction, the blood glucose was measured. According to the literature, Wistar rats are classified as diabetic if their blood sugar levels exceed 135 mg/dL (Hidayaturrahmah et al., 2020), and they are categorized as hypercholesterolemic if their cholesterol levels exceed 200 mg/dL (Supriatna et al., 2018). Rats meeting these two criteria were subsequently selected for the treatment. The treatment was administered daily for 14 days (type of treatment given was based on the group). The urea and creatinine levels were measured in blood samples taken from the tail, starting from the initiation of the treatment and continued until day 35, which included day 0, 21, 28, and 35.

**Data Analysis**

The data are presented as mean ± standard deviation (SD). All data underwent a normality test and homogeneity test. In cases where the data exhibited a normal and homogeneous distribution, data analysis was performed using a One-Way ANOVA. However, if the data did not follow a normal distribution and were not homogenous, non-parametric statistics were employed, specifically the Kruskal-Wallis test, followed by the Mann-Whitney test to assess the differences between treatments, with the assistance of statistical analysis software, SPSS (SPSS for Windows, Version 16.0. Chicago, SPSS Inc.).
RESULT AND DISCUSSION

Bromelain identification

Bromelain is a proteolytic enzyme found in pineapples, particularly in the stem and flesh of the fruit. It plays a significant role in the production of Virgin Coconut Oil (VCO). In the context of VCO production, bromelain is used as a protein-cleaving agent, aiding in the separation of protein components from the extracted coconut oil (Harimurti et al., 2020, 2022). This is a crucial step in the VCO extraction process because the resulting coconut oil needs to be protein-free to ensure its high quality. Bromelain helps ensure that the extra virgin coconut oil is free from protein contamination, resulting in a clearer and higher-quality coconut oil (Hamzah et al., 2021; Hamdan et al., 2022; Ng et al., 2019; Natalia et al., 2019). In the VCO production process, we conducted a qualitative study to identify the presence of bromelain in both pineapple juice and the VCO obtained through the enzymatic approach (Table 1). The presence of bromelain in both components suggests that bromelain may play a role in expediting the production of VCO.

Table 1. Qualitative identification of bromelain enzyme in pineapple juice

<table>
<thead>
<tr>
<th>Test</th>
<th>Observation</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pineapple hump juice test</td>
<td>Black precipitate brown color</td>
<td>(+)</td>
</tr>
<tr>
<td>Enzymatic VCO test</td>
<td>Brownish color with a little black precipitate</td>
<td>(+)</td>
</tr>
</tbody>
</table>

Description: (+) means the presence of bromelain

Enzymatic VCO effect on serum urea level

All test animals underwent a baseline check for urea and creatinine levels before being subjected to a high-fat diet and streptozotocin induction. With the exception of the normal control group, the five rat groups were fed a high-fat diet for 14 days. The high-fat diet aimed to elevate the free fatty acid content in plasma cells, subsequently leading to a decrease in insulin sensitivity. After 14 days, the average cholesterol level in male white rats exceeded 200 mg/dL. Subsequently, the male white rats developed hypercholesterolemia and were intraperitoneally injected with streptozotocin at a dose of 40 mg/kg BW. Streptozotocin exerts a cytotoxic effect, potentially damaging pancreatic beta cells. It specifically causes DNA damage in pancreatic beta cells through the formation of NO, hydroxyl radicals, and hydrogen peroxide, all of which are potent free radicals that quickly harm cell tissues.

On day 0, which indicates that the urea levels across all groups were within the normal range. This is because on day 0, the test animals had not received any induction aside from standard feeding. This aligns with the literature, which indicates that the normal urea levels in rats fall within the range of 15-21.8 mg/dL (Tandi et al., 2022). The results of the Kruskal-Wallis statistical test on day 0 demonstrated that all treatment groups did not exhibit significant differences, with a p-value of 0.937 (p value > 0.05). This implies that the urea levels at the study’s outset were consistent with normal levels.

Urea level measurements on day 21 following the induction of a high-fat diet and streptozotocin showed an increase in urea levels in male white rats. This elevation in urea levels was a consequence of the high-fat diet and streptozotocin induction. The results of the Kruskal-Wallis statistical test indicated significant differences among all treatment groups on day 21, with a p-value of 0.022 (p < 0.05), signifying that the high-cholesterol diet and streptozotocin induction had a noticeable effect. Subsequently, Mann-Whitney tests were conducted to discern the differences between all treatment groups. The Mann-Whitney test results revealed significant distinctions between the negative control, positive control, and various enzymatic VCO dosage groups (0.2 mL/kg BW, 0.4 mL/kg BW, and 0.8 mL/kg BW), in comparison to the normal control. This suggests that the animals in the treatment groups exhibited an increase in urea levels due to the induction of a high-fat diet and streptozotocin. In this observation point, animal group with 0.8 mL/kg BW enzymatic VCO has the lowest average serum urea level among the treated groups but the value was not significant (Table 2).
Measurement of urea levels on the 28th day after administration of enzymatic Virgin Coconut Oil (VCO) for 14 days, the urea levels of male white rats showed a slight decrease. This is because the treatment group has been induced with Enzymatic Virgin Coconut Oil (VCO). The results of the Kruskal Wallis statistical test were significantly different with $p<0.05$, which indicated that there were significant differences in all treatment groups on day 28, so it was continued with the Mann Whitney test to see the differences between all treatment groups. Mann Whitney further test results showed that the doses of Enzymatic Virgin Coconut Oil (VCO) 0.2 mL/kg BW, 0.4 mL/kg BW, and 0.8 mL/kg BW were not significantly different from the positive control but significantly different from negative control. This indicates that these three doses had an impact on reducing urea levels but didn't fully restore urea levels to those observed in the normal control. This suggests that the active components within Virgin Coconut Oil (VCO) were not completely absorbed enzymatically, thus not achieving the maximum effect (Puspita et al., 2023).

The reduction in blood urea levels by Virgin Coconut Oil (VCO) can be attributed to several potential mechanisms related to the active components present in pure coconut oil. VCO, containing medium-chain triglycerides (MCTs), may enhance fat metabolism, thereby reducing the accumulation of fats and triglycerides in the body, alleviating kidney stress and subsequently lowering urea levels (Wang et al., 2018; Zicker et al., 2019). Additionally, VCO exhibits anti-inflammatory and antioxidant properties, which can help mitigate kidney inflammation, maintain renal health, and ultimately reduce blood urea levels (Sinaga et al., 2019). Furthermore, VCO's potential to improve insulin sensitivity, notably through MCTs, may aid in regulating excessive urea production in the liver (Thomas et al., 2019). Moreover, certain VCO components, such as lauric acid, may exert direct effects on metabolic processes associated with urea reduction.

The measurement results on the 35th day revealed that within each group, the dose variations of enzymatic Virgin Coconut Oil (VCO) at 0.2 mL/kg BW, 0.4 mL/kg BW, and 0.8 mL/kg BW had an impact on reducing urea levels. Among these doses, the enzymatic Virgin Coconut Oil (VCO) dose of 0.8 mL/kg BW proved to be the most effective. This dose was chosen because it was the one closest to the normal and positive control values, yielding the most substantial reduction in urea levels.

Although not significantly different between treatment groups, the decrease in serum urea levels on day 35 follows a decreasing pattern with the increase in VCO dosage. This indicates that VCO treatment is dose-dependent. This is consistent with the results reported in previous studies where VCO therapy yielded dose-dependent results, both in reducing serum metabolic levels and enzymatic activity (de Moura e Dias et al., 2018; Rahim et al., 2017).

**Enzymatic VCO effect on serum creatinine level**

This study was also conducted to determine the initial creatinine levels before treatment. On day 0 the mean creatinine levels were obtained for the normal group, negative group, positive group, Enzymatic Virgin Coconut Oil (VCO) 0.2 mL/kg BW, 0.4 mL/kg BW, and 0.8 mL/kg BW are 0.42±0.12, 0.36±0.06, 0.41±0.05, 0.31±0.05, 0.34±0.06, and 0.32±0.08 mg/dL, respectively, which...
indicates that the initial creatinine levels of male white rats are in the normal range. This is in accordance with the literature which states that the normal level of rat creatinine is 0.2–0.8 mg/dL (Huseyin et al., 2022). The results of the Kruskal Wallis statistical test on day 0 showed that all treatment groups were not significantly different with p value = 0.098 (p value > 0.05). This means that creatinine levels at the beginning of the study were homogeneous.

<table>
<thead>
<tr>
<th>Days to-</th>
<th>Normal Control</th>
<th>Negative Control</th>
<th>Positive Control</th>
<th>Enzymatic VCO 0.2 mL/kg BW</th>
<th>Enzymatic VCO 0.4 mL/kg BW</th>
<th>Enzymatic VCO 0.8 mL/kg BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.42±0.12</td>
<td>0.36±0.06</td>
<td>0.41±0.05</td>
<td>0.31±0.05</td>
<td>0.34±0.06</td>
<td>0.32±0.08</td>
</tr>
<tr>
<td>21</td>
<td>0.41±0.05</td>
<td>1.29±0.18</td>
<td>1.36±0.15</td>
<td>1.31±0.10</td>
<td>1.40±0.04</td>
<td>1.34±0.14</td>
</tr>
<tr>
<td>28</td>
<td>0.38±0.10</td>
<td>1.60±0.21</td>
<td>1.02±0.04</td>
<td>1.11±0.10</td>
<td>1.07±0.08</td>
<td>1.04±0.19</td>
</tr>
<tr>
<td>35</td>
<td>0.37±0.07</td>
<td>1.65±0.20</td>
<td>0.34±0.07</td>
<td>0.45±0.10</td>
<td>0.40±0.11</td>
<td>0.36±0.06</td>
</tr>
</tbody>
</table>

Note: (*, p<0.05) shows a significant differences to normal group. (a, p<0.05) shows significant differences to negative control.

Measurement of creatinine levels on the 21st day shows significant differences in all treatment groups and negative group to normal control (Table 3), which means that there was an effect on feeding high-fat and high-fat diets. Mann Whitney test results further showed that the normal control was significantly different from all treatment groups. This was because the test animals in the treatment group were had increased creatinine levels (Cullaro et al., 2018).

Measurement of creatinine levels on the 28th day shows significant differences in all treatment groups on day 28, so that it was continued with the Mann Whitney test to see the differences between all treatment groups. The results of the Mann Whitney test showed that normal control was significantly different from all treatment groups, and negative control was also significantly different from all treatment groups. Positive control was not significantly different with all test group, but significantly different from normal and negative controls. These findings suggest that the three dosage levels have influenced a reduction in creatinine levels but were unable to fully normalize creatinine levels due to inadequate suspension of enzymatic Virgin Coconut Oil (VCO) at that dose. This resulted in imperfect absorption of the active components, preventing the attainment of maximum effects (Lie et al., 2019).

The measurement results on the 35th day demonstrated that all test group produced effects in decreasing creatinine levels. Among these doses, the enzymatic Virgin Coconut Oil (VCO) at 0.8 mL/kg BW proved to be the most effective, as it led to a reduction in creatinine levels close to those observed in the normal control and positive control groups.

The decline in both urea and creatinine levels can be attributed to the presence of antioxidants in Virgin Coconut Oil (VCO) supplements, leading to an increase in glutathione peroxidase levels and a decrease in lipid peroxidation characterized by reduced MDA levels. Medium Chain Fatty Acids (MCFAs) like lauric acid, palmitic acid, and capric acid in Virgin Coconut Oil (VCO) can enhance insulin secretion by increasing intracellular calcium release within cell plasma membranes. Elevated insulin secretion, in turn, reduces the production of free radicals, thereby mitigating cell damage. Furthermore, the presence of vitamin E in Virgin Coconut Oil (VCO) serves to neutralize accumulated free radicals in diabetes, effectively inhibiting cell damage (Supriatna, 2018).

CONCLUSION

In summary, this study concludes that Virgin Coconut Oil (VCO) has the potential to reduce urea and creatinine levels in hypercholesterolemic-diabetic male white rats. Notably, the administration of
Virgin Coconut Oil (VCO) at a dosage of 0.8 mL/kg BW proved to be effective in reducing urea and creatinine levels, resulting in values of 17.40 mg/dL and 0.36 mg/dL, respectively.

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