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Research Article

The Inhibition Effect of Moringa (Moringa oleifera L.) Leaves and Red Ginger Rhizome (Zingiber officinale Rosc.) Pt. "P" Ethanol Extract on Enhancement Serum Creatinine Levels of Male Wistar Rat Dyslipidemia Models

Efek Inhibisi Ramuan Ekstrak Etanol Daun Kelor (*Moringa oleifera* L.) dan Rimpang Jahe Merah (*Zingiber officinale* Rosc.) terhadap Peningkatan Kadar Kreatinin Serum Tikus Wistar Jantan Model Dislipidemia

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Abstract

Elevation of serum creatinine levels is a biomarker of decreased kidney function. Dyslipidemia can decrease kidney function. Long-term consuming statins can increase creatinine levels. Moringa leaves (Moringa oleifera L.) and red ginger rhizomes (Zingiber officinale Rosc.) have antioxidant effects. Aim of study is to determine the inhibitory effect on increasing serum creatinine levels of concoction Moringa Leaves and Red Ginger Rhizome ethanol extract (CML-RGRE). True laboratory experimental study with completely randomized design was conducted on 30 male Wistar rat dyslipidemia models, induced by high-fat feeding (HFF)-Propylthiouracil (PTU) 0.01% for 21 days. Subjects were divided into six groups (n=5), Group I, II, III were treated CML-RGRE 135, 202.5, 270 mg/kg BW, IV dyslipidemia control, V Rosuvastatin 1.8 mg/kg BW, and VI fenofibrate 5.4 mg/kg BW, all still given HFF-PTU for 21 days. Serum creatinine levels (mg/dL) were measured 3 times, after acclimatization, created dyslipidemia models, and treatment using spectrophotometer, colorimetric kinetic methods. Data were analysed with paired t-test, ANOVA test, and Fischer's LSD, $\alpha = 0.05$. All doses of CML-RGRE have effectively inhibited the increase serum creatinine levels ($p < 0.01$). Conclusion, Moringa Leaves and Red Ginger Rhizome ethanol extract effectively inhibited the increased serum creatinine levels of animal dyslipidemia models.

***Keywords**: Moringa oleifera L. leaves; Zingiber officinale Rosc. Rhizome; creatinine*

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Abstrak

Peningkatan kadar Kreatinin serum merupakan penanda biokimia penurunan fungsi ginjal. Dislipidemia dapat menjadi penyebab penurunan fungsi ginjal. Konsumsi statin jangka panjang terbukti menurunkan kadar kolesterol, tetapi dapat meningkatkan kadar kreatinin. Daun kelor (*Moringa oleifera* L.) dan rimpang jahe merah (*Zingiber officinale* Rosc.) memiliki efek antioksidan. Tujuan penelitian ingin mengetahui efek inhibisi ekstrak etanol ramuan daun kelor dan rimpang jahe merah (REDK-RJM) terhadap peningkatan kadar kreatinin serum. Penelitian Eksperimental Laboratorium Sungguhan dengan Rancangan Acak Lengkap terhadap 30 ekor tikus Wistar jantan model dislipidemia, diinduksi Pakan Tinggi Lemak (PTL) dan Propiltiourasil (PTU) 0,01%. Subjek dikelompokkan menjadi 6 kelompok (n=5), tetap diberi PTL dan PTU 21 hari masa perlakuan. Kelompok I, II, III diberi REDK-RJM 135, 202,5 dan 270 mg/kgBB; IV kontrol dislipidemia tanpa perlakuan tambahan; V kontrol pembanding rosuvastatin 1,8 mg/kgBB; VI fenofibrate 5,4 mg/kgBB. Kadar kreatinin serum (mg/dL) diukur dengan spektrofotometer, metode kinetik kolorimetri, pasca 7 hari aklimatisasi, pasca 21 hari diinduksi PTL, dan pasca 21 hari perlakuan. Analisis data penurunan kadar Kreatinin dengan ANOVA, dilanjutkan dengan Fischer's LSD, $\alpha = 0,05$. Semua dosis REDK-RJM dapat menginhibisi peningkatan kadar kreatinin serum ($p<0,01$). Ekstrak Etanol Ramuan Daun Kelor dan Rimpang Jahe Merah efektif menginhibisi peningkatan kadar kreatinin serum hewan model dislipidemia.

Kata kunci: daun *Moringa oleifera* L.; rimpang *Zingiber officinale* Rosc.; kreatinin

Introduction

Serum creatinine is a biomarker of kidney function. An elevation of serum creatinine levels can be used as an indicator of decreasing kidney function. Creatinine is the product of creatine metabolism in muscle tissue, especially skeletal muscle. The process of creatine synthesis takes place in the liver and is then stored in muscle tissue as an energy reserve in the form of the Creatine Phosphate (CP), a small amount of creatine will be converted into creatinine in the muscles and then excreted by the kidneys with urine. Creatinine is the result of CP catalysis by Creatine Kinase (CK) enzyme in the process of synthesizing Adenosine Triphosphate (ATP) from Adenosine Diphosphate (ADP) in muscle tissue. Serum creatinine levels depend on muscle mass and kidney function to excrete creatinine. Normal serum creatinine levels of men are relatively higher than women because men's muscle mass is greater than women. Serum creatinine levels will increase when creatinine excretion by the kidneys decreases so that serum creatinine levels can be one of the markers of kidney function decreasing. Kidney function decreasing can be caused by various conditions, including *Diabetes mellitus* (DM), dyslipidemia, glomerulonephritis, pyelonephritis, acute tubular necrosis, urinary tract obstruction, renal blood flow decreases, diabetic nephropathy, nephritis, rhabdomyolysis. Dyslipidemia can cause kidney damage but on the other hand, chronic kidney disease (CKD) can be the cause of secondary.[1]

Dyslipidemia is a condition of increased lipid serum levels, Total-Cholesterol (Total-C), Low-Density Lipoprotein Cholesterol (LDL-C), or/and Triglycerides (TG) with/without decreased of High-Density Lipoprotein Cholesterol (HDL-C) levels.[2] RISKESDAS 2018 reports the prevalence of dyslipidemia in the aged 15 years population is 27,9% with triglyceride levels

above 150 mg/dL, 28,8% with total cholesterol levels above 200 mg/dL, 24,4% with HDL levels less than 40 mg/dL, and 72,8% with LDL levels above 100 mg/dL.[3] Dyslipidemia and insulin resistance condition can cause Cardiorenal Metabolic Syndrome. Cardiorenal Metabolic Syndrome (CRMs) is a complex disease in which both the heart and kidney are simultaneously affected, and their deleterious outcomes are reinforced in a feedback cycle, with accelerated progression**.** Although the coexistence of kidney and heart failure in the same individual carries an extremely rueful prognosis.[4] Dyslipidemia will increase the risk of kidney function decreasing by 1,65%.[5,6] Decreasing kidney function can increase the risk of secondary dyslipidemia in patients with CKD due to decreased HDL formation and increased triglyceride levels in the blood.[1]

Statins and fabric acid are drugs that are often used to treat dyslipidemia.[5] One of the statin drugs is rosuvastatin, which works effectively by inhibiting the HMG-CoA reductase enzyme so that it can reduce LDL levels.[7] One of the fabric acid drugs is fenofibrate, which works by increasing K-HDL levels.[8,9] Studies comparing the effects of atorvastatin with rosuvastatin on kidney function in diabetics have shown that there is a decrease in estimated Glomerular Filtration Rate (eGFR) with rosuvastatin 5-20 mg per day for more than 12 months.[10] Fenofibrate is also contraindicated for patients with severe renal impairment because it can increase creatinine levels progressively.[11]

Indonesia is a country with a diversity of spice plants. The use of herbal plants as traditional medicine is relatively increasing. RISKESDAS 2018 reports that the use of herbal medicines in Indonesia has relatively increased by 44.3% compared to 2010.[3] Red Ginger or *Zingiber officinale* Rosc. were contained non-volatile compounds such as phenolics, alkaloids, saponins, tannins, flavonoids, triterpenoids, steroids, vitamin C, and beta carotene. The major volatile compound of red ginger rhizomes was oleoresin, which contained gingerol, shogaol, resin, and flavonoid compounds[12,13] which have antihyperlipidemic, anti-inflammatory, and antioxidant effects.[14,15] The antioxidant effect of flavonoids can function as a renoprotection and antihyperlipidemic.[16,17] This study uses a human dose conversion of 3 x 500 mg/day as stated on the package.

The purpose of this study is to determine the safety level of consumption of concoction red ginger rhizome extract (*Zingiber officinale* Rosc.) and Moringa leaf (*Moringa oleifera* L.) products from PT. "P" on serum creatinine levels and its comparison with rosuvastatin and fenofibrate.

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Methods

Experimental Research Real Laboratory with Completely Randomized Design (CRD) on rats (*Rattus norvegicus*) Wistar Male dyslipidemia model carried out in Experimental Animal Laboratory, in Faculty of Medicine, Maranatha Christian University Bandung, in the period December 2020 to December 2021.[18] This study used herbs with ethanol extract of Moringa leaves (*Moringa oleifera* L.) and red ginger rhizomes (*Zingiber officinale* Rosc.) which were purchased from PT "P" online. The concoction of Moringa leaf and Red Ginger Rhizome extract (CML-RGRE) were made in 3 doses, 135, 202.5, and 270 mg/kg BW, and prepared rosuvastatin 1.8 mg/kg BW and fenofibrate 5.4 mg/kg BW as a comparison control.

The research subjects were 30 male Wistar rats aged 11-12 weeks, weighing 200-225g which were grouped into six groups, consisting of five rats in each group, obtained from Biopharma. The procedure of this study was that all subjects have acclimatized for seven days to the new environment and were given standard feeding. The first blood samples have been taken after adaptation as a normal or baseline value. Followed by the induction of high-fat feeding (HFF) and Propylthiouracil (PTU) 0.01% mixed into their drink for 21 days.

The second blood samples drawn have been carried out after induction to evaluate whether there were increase in creatinine levels due to dyslipidemia. The first and second blood samples were taken from the retro-orbital plexus. Followed by giving treatment according to six groups of rats for 21 days and still given HFF and 0.01% PTU. Groups I, II, and III were given the CML-RGRE treatment at doses of 135 mg/kg BW/day, 202.5 mg/kg BW/day, and 270 mg/kg BW/day. The rats in Group IV as hyperlipidemia control group were only given HFF and 0.01% PTU. The studied animals in comparison control group V were given rosuvastatin 1.8 mg/kg BW/day; meanwhile, animals in group VI were given fenofibrate 5.4 mg/kg BW/day. Carboxymethyl Cellulose (CMC) 1% as an herbal solvent, rosuvastatin, and fenofibrate will be probed on rats. The third blood sample was carried out after the treatment had been completed, followed by termination. The parameters measured were creatinine levels (mg/dL), which were measured after acclimatization, after HFF and 0.01% PTU induction, and after treatment have been completed. The serum samples have drawn from 3 mL of blood from the retro-orbital plexus of rats. Blood samples were collected in centrifuge tubes and centrifuged at 3000 rpm for 5 minutes. The serum on the upper surface of each sample has separated into each Eppendorf tube and placed in a refrigerator at -8^{o}C. Creatinine levels were measured using an automatic chemistry autoanalyzer.

The data of creatinine levels in all subjects between the post-acclimatization and post-high-fat feeding (HFF) and Propylthiouracil (PTU) 0.01% induction for 21 days were tested by paired t-test, $\alpha = 0.05$. The data on mean creatinine levels between the post-acclimatization groups

Research Article

were statistically tested with the Shapiro-Wilk normality test to determine whether the study data were normally distributed. The data is normally distributed if $p \geq 0.05$. The research data were analysed using ANOVA was the percentage increase or decrease in creatinine levels between treatment groups, if $p < 0.05$ was obtained, which means at least a pair of groups were significantly different, followed by the Fischer's LSD (Least Significant Difference) mean difference test. The results were stated to be significantly different if $p < 0.05$ and very significant if $p < 0.01$. The implementation of this research has been approved by the Medical Ethics Commission, Faculty of Medicine, Maranatha Christian University, Bandung number 044/KEP/III/2021.

Results

A true experimental laboratory analytical study with a completely randomized design was used to examine the effect of the concoction Moringa Leaves and Red Ginger Rhizome extract (CML-RGRE) on serum creatinine levels of male Wistar rats dyslipidemia model. The average creatinine levels between the treatment groups after 7 days acclimatization, after HFF and 0.01% PTU induction for 21 days, and after giving the CML-RGRE herb treatment and still being given HFF and 0.01% PTU for 21 days are described in Table 1.

Table 1 The Mean Creatinine Level Measurement Results

Groups	Creatinine Levels		
	Before HFF Induction (mg/dL)	After HFF Induction (mg/dL)	After Treatment (mg/dL)
I	0.30 ± 0.00	0.35 ± 0.05	0.34 ± 0.03
II	0.40 ± 0.25	0.42 ± 0.03	0.36 ± 0.02
III	0.35 ± 0.12	0.29 ± 0.05	0.27 ± 0.02
IV	0.19 ± 0.08	0.33 ± 0.09	0.47 ± 0.02
V	0.36 ± 0.16	0.27 ± 0.03	0.39 ± 0.06
VI	0.35 ± 0.03	0.45 ± 0.02	0.63 ± 0.15

Description:
Group I: the studied animals that were given HFF + 0.01% PTU + CML-RGRE at a dose of 135 mg/kg BW/day
Group II: the studied animals that were given HFF + 0.01% PTU + CML-RGRE at a dose of 202.5 mg/kg BW/day
Group III: the studied animals that were given HFF + 0.01 PTU + CML-RGRE at a dose of 270 mg/kg BW/day
Group IV (control): the studied animals that were given high-fat feeding (HFF) + 0.01% PTU
Group V (comparison control 1): the studied animals that were given HFF + 0.01% PTU + Rosuvastatin 1.8mg/kg BW/day
Group VI (comparison control 2): the studied animals that were given HFF + 0.01% PTU + Fenofibrate 5.4 mg/kg BW/day

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Table 1 shows the mean creatinine level of male Wistar rats in the group before HFF and 0.01% PTU induction, after HFF and 0.01% PTU induction, and after treatment were 0.19-0.40; 0.27-0.45; 0.27-0.63 mg/dL. Before and after HFF and 0.1% PTU, the group induction did not find a significant increase in creatinine levels between groups because the study time was not long enough so the condition of dyslipidemia has not increased creatinine levels. The groups before and after treatment found an increase in creatinine levels in groups IV, V, and VI; while groups I, II, and III using CML-RGRE have not experienced an increase and instead experienced a decrease in creatinine levels. Elevated creatinine levels were tested by paired t-test. This paired t-test was conducted to see whether there was a significant difference between before and after HFF and PTU 0.01 for 21 days. The paired t-test was to see whether the condition of dyslipidemia has increased creatinine levels significantly or not. The results of paired t-test for the increase in serum creatinine between before and after HFF-PTU 0.01% induction for 21 days are described in Table 2.

Table 2 showed no difference in serum creatinine levels before and after HFF and 0.01% PTU induction with p-value = 0.198 ($p \geq 0.05$). There has not been a significant increase in creatinine levels after HFF and 0.01% PTU induction for 21 days due to fewer times, so dyslipidemia has not caused chronic kidney disease. The results of the paired t-test for total cholesterol levels before and after HFF and 0.01% PTU induction obtained *p*-value = 0.000 ($p < 0.01$). The paired t-test for triglyceride levels in the group before and after HFF and 0.01% PTU induction showed $p = 0.000$ ($p < 0.01$), so it can be concluded that rats were in dyslipidemia condition. Followed by treatment with CML-RGRE, rosuvastatin, and fenofibrate, the percentage increase or decrease in creatinine levels will be calculated.

The data from the measurement of creatinine levels between groups had tested with the Shapiro-Wilk test to determine the data distribution. The results of the Shapiro-Wilk normality test are described in Table 3. The average percentage increase or decrease in creatinine levels after treatment is described in Table 4.

Table 2 The Paired t-Test Serum Creatinine Level

Groups	Before Induction ± SD	After Induction ± SD	*p-value**
Creatinine Serum Level	0.33 ± 0.14	0.35 ± 0.08	0.19

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Table 4 showed that CML-RGRE doses 1, 2, and 3 were able to inhibit the increase in creatinine when compared to controls. CML-RGRE dose 2 which has the greatest inhibitory effect on increasing creatinine levels compared with the other two doses. The greatest increase in creatinine occurred in group IV rats that were only given HFF and 0.01% PTU.

The results of the one-way ANOVA test in Table 4 showed *p*-value = 0.003 ($p < 0.01$), there was a very significant difference in the percentage of creatinine levels at least in a pair of treatment groups. The data were analysed using the Fischer LSD (Least Significant Difference) test to find out which groups were different, as described in Table 5.

Table 3 Shapiro-Wilk Test Initial Creatinine Levels Between Treatment Groups

Groups	Saphiro-Wilk		
	Statistic	**df**	***p-value***
I	0.821	5	0.119
II	0.878	5	0.302
III	0.896	5	0.391
IV	0.934	5	0.626
V	0.977	5	0.917
VI	0.933	5	0.620

Description:
Group I : the studied animals that were given HFF + 0.01% PTU + CML-RGRE at a dose of 135 mg/kg BW/day.
Group II: the studied animals that were given HFF + 0.01% PTU + CML-RGRE at a dose of 202.5 mg/kg BW/day.
Group III: the studied animals that were given HFF + 0.01 PTU + CML-RGRE at a dose of 270 mg/kg BW/day.
Group IV (control): the studied animals that were given high-fat feeding (HFF) + 0.01% PTU.
Group V (comparison control 1): the studied animals that were given HFF + 0.01% PTU + Rosuvastatin 1.8mg/kg BW/day.
Group VI (comparison control 2): the studied animals that were given HFF + 0.01% PTU + Fenofibrate 5.4 mg/kg BW/day.

Table 4 The Percentage Increase/Decrease in Creatinine Level

Groups	Percentage Increase or Decrease Creatinine Levels (%)	ANOVA
I	Decrease (1.22 ± 6,76) %	0.003
II	Decrease (9.98 ± 12.07) %	
III	Decrease (1.87 ± 20.23) %	
IV	Increase (49.90 ± 44.73) %	
V	Increase (46.68 ± 27.22) %	
VI	Increase (39.39 ± 33.96) %	

Description:
Group I: the studied animals that were given HFF + 0.01% PTU + CML-RGRE at a dose of 135 mg/kg BW/day.
Group II: the studied animals that were given HFF + 0.01% PTU + CML-RGRE at a dose of 202.5 mg/kg BW/day.
Group III: the studied animals that were given HFF + 0.01 PTU + CML-RGRE at a dose of 270 mg/kg BW/day.
Group IV (control): the studied animals that were given high-fat feeding (HFF) + 0.01% PTU.
Group V (comparison control 1): the studied animals that were given HFF + 0.01% PTU + Rosuvastatin 1.8mg/kg BW/day.
Group VI (comparison control 2): the studied animals that were given HFF + 0.01% PTU + Fenofibrate 5.4 mg/kg BW/day.

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The results of the Fischer LSD test in Table 5 show a very significant difference in mean creatinine between the CML-RGRE doses 1, 2, and 3 groups compared to the control group with *p*-value = 0.007; 0.002; and 0.006 ($p < 0.01$). It can be concluded that CML-RGRE doses 1, 2, and 3 can inhibit the increase in creatinine levels. The group that used rosuvastatin 1.8 mg/kg BW/day and fenofibrate 5.4 mg/kg BW/day as a comparison control compared to the control showed a non-significant difference in mean creatinine levels with the control group with p = 0.85 and p = 0. 55 (p 0.05). The groups that were given doses of 1, 2, and 3 of CML-RGRE were compared to the comparison control of rosuvastatin were showed significant differences with *p*-value = 0.01; 0.02; and 0.01 ($p < 0.05$). The CML-RGRE -RJM dose 1 and 3 groups were compared to the comparison control fenofibrate, which showed significant differences with *p* values = 0.02 and 0.02 ($p < 0.05$). The dose group of 2 CML-RGRE was compared with the comparison control, fenofibrate showed a very significant difference with *p*-value = 0.009 ($p < 0.01$). The CML-RGRE groups at doses 1, 2, and 3, all groups were showed no significant difference, so increasing the dose of CML-RGRE did not have a very different effect.

Table 5 The Fisher LSD Test Results Percentage of Increased Creatinine Levels

Groups (n=6)	The Increase of Creatinine Levels (mg/dL)					
	CML-RGRE 1	CML-RGRE 2	CML-RGRE 3	C	CC-1	CC-2
CML-RGRE 1		TB (0.61)	TB (0.97)	** (0.007)	* (0.01)	* (0.02)
CML-RGRE 2			**TB** (0.64)	**** (0.002)	**** (0.02)	****(0.009)
CML-RGRE 3				**** (0.006)	* (0.01)	**** (0.02)
C					**TB** (0.85)	**TB** (0.55)
CC-1						**TB** (0.67)
CC-2						

Description:
Group I: the studied animals that were given HFF + CML-RGRE at a dose of 135 mg/kg BW/day.
Group II: the studied animals that were given HFF + CML-RGRE at a dose of 202.5 mg/kg BW/day.
Group III: the studied animals that were given HFF + CML-RGRE at a dose of 270 mg/kg BW/day.
Group IV (control or C): the studied animals that were given HFF.
Group V (comparison control 1or CC-1): the studied animals that were given HFF + Rosuvastatin 1.8mg/kg BW/day.
Group VI (comparison control 2 or CC-2): the studied animals that were given HFF + Fenofibrate 5.4 mg/kg BW/day.

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Discussion

The results of the study comparing CML-RGRE doses 1, 2, and 3 compared to controls found that CML-RGRE doses 1, 2, and 3 effectively inhibited the increase in creatinine levels by -1.22%, -9.98%, and -1,87%. This can happen because the 2 herbs used have an antioxidant effect with ascorbic acid, flavonoid, phenolic, and carotenoid content which work by preventing the generation of ROS and directly capturing ROS.[16,19] Another mechanism of action is changing free radicals. reactive free radicals become relatively stable reactive free radicals by giving hydrogen atoms to reactive free radicals to inhibit oxidation so that they can function as renoprotection.[20] Doses 1, 2, and 3 when compared, the results were not significantly different, so the increase in dose did not make a significant difference. An increase in the dose of CML-RGRE tends to cause a decrease in the effectiveness of the inhibition of increased creatinine levels, although there is no significant difference when compared. This happened because CML-RGRE doses 1, 2, and 3 containing antioxidants had experienced saturation which caused a ceiling effect.[21] The result of doses 1, 2, and 3 when compared with the comparison control of rosuvastatin, there were significant differences, so it can be concluded that doses 1, 2, and 3 CML-RGRE can have an inhibitory effect on increasing creatinine levels. Doses 1 and 3 of CML-RGRE were compared with the comparison control of fenofibrate, there were significant differences, while doses of 2 compared to the comparison control of fenofibrate there were significantly different. The comparison control compared with control obtained results that were not significantly different. It can be simulant that rosuvastatin and fenofibrate do not inhibit the increase in serum creatinine levels.

This study found that rosuvastatin and fenofibrate could not inhibit the increase in creatinine levels compared to CML-RGRE. This study comparing atorvastatin with rosuvastatin on kidney function in diabetic people shows that there will be a decrease in eGFR (estimated Glomerular Filtration rate) when taking rosuvastatin 5-20 mg per day for more than 12 months.[10] Fenofibrate also has contraindications for patients with severe renal impairment because it can increase creatinine levels progressively.

The results were compared with previous studies regarding the effect of Moringa leaves on the serum creatinine levels of male Wistar rats induced by formalin and the effect of adding Moringa leaves flour to the creatinine levels of Wistar rats. This study compared to two previous studies, which yielded better results than the previous two studies, where the results were not significantly different from the p-values in the previous study, namely 0.085 and 0.118 (p 0.05). It happened because this study used two types of herbs that have antioxidant effects. Moringa leaves (*Moringa oleifera* L.) contain ascorbic acid, flavonoids, phenolics, carotenoids, tannins, saponins, alkaloids, triterpenoids, and polyphenols (epigallocatechin gallate (EGCG). These ingredients are useful as antioxidants, anti-inflammatory, antihyperlipidemic, antibiotic,

antihypertensive, diuretic, antidiabetic, and antibacterial. Antioxidants can work by preventing the degeneration of ROS and directly capturing ROS so that they can reduce oxidative stress which can cause kidney damage.[16,19] In addition, antioxidants can convert reactive free radicals into reactive free radicals that are more stable by donating hydrogen atoms to reactive free radicals so that they can inhibit oxidation.[20] Antihyperlipidemic in Moringa leaves works by inhibiting the activity of the enzyme Acyl-CoA cholesterol acyltransferase (ACAT) and 3-hydroxy-3-methyl-glutaryl CoA reductase on HepG2. Saponins can also act as antihyperlipidemia by binding bile salts and forming compounds that cannot be absorbed to lower total cholesterol levels.[24] Flavonoids are useful as an anti-inflammatory by inhibiting cyclooxygenase and lipoxygenase activities which play a role in converting arachidonic acid into cytokines such as prostaglandins and leukotrienes, which can cause inflammation in tissues.[25,26] Phenolates and tannins have antioxidant effects, which can protect cell structures by neutralizing the oxidation reaction of free radicals.[14] Beta carotene as an antioxidant function to prevent the effects of free radicals and peroxidation that can damage lipid membranes.[20]

Red ginger rhizome (*Zingiber officinale* Rosc.) contains flavonoids, shogaol, resin, and gingerol.[23] Antioxidants in red ginger aim to inhibit the fibrosis process by inhibiting ACE to decrease the formation of angiotensin II. The TGF-receptor regulator is angiotensin II. TGF-β is a pro-oxidant that easily turns into an oxidant and increases the fibrosis process. As angiotensin II synthesis decreases, TGF-β synthesis decreases, oxidant formation decreases, and cell damage also decreases. The administration of both types of herbal ingredients containing high levels of antioxidants is expected to reduce the progression of renal fibrosis so that they can act as renoprotection.[17] Antioxidants from flavonoids can directly capture superoxide and peroxide nitrite. Flavonoids can increase the bioavailability of nitric oxide (NO) and inhibit the formation of peroxide nitrite.[19]

Puspitaningrum *et al*. in 2018 gave Moringa leaves ethanol extract (*Moringa oleifera* L.) doses of 2.5%, 5%, 10%, and 20% for one month to male Wistar rats that had been induced with formalin for 21 days. It was proven that Moringa leaf extract did not increase creatinine levels and only a dose of 2.5% can decrease creatinine levels significantly ($p < 0.05$).[27]

Dosom *et al*. in 2018 gave Moringa leaf flour (*Moringa oleifera* L.) to Wistar rats for 30 days and it was proven that Moringa leaf flour did not increase levels of creatinine significantly ($p < 0.05$).[27] The conclusion of the two previous research, the administration Moringa leaves extract ethanol is not toxic and safe for the kidneys.[27,28]

Duppa *et al*. in 2020 gave extract ethanol red ginger (*Zingiber officinale* Rosc) rhizomes dose 150 mg/kg BW and 300 mg/kg BW for 10 days to Wistar rats that was induced with paracetamol 500 mg/kg BW on the 4th day and 300 mg/kg BW on 5th day. The administration of

extracts ethanol of red ginger dose 150 mg/kg BW and 300 mg/kg BW orally after toxic doses of paracetamol induction prove that red ginger can decrease the levels of AST, ALT, urea, and creatinine significantly ($p < 0.05$). The effectiveness on decrease creatinine levels of extract ethanol red ginger was not significantly different with *Curcuma xanthorrhoea* ($p > 0.05$). So based on the result of previous research have proven that red ginger rhizomes are safe for kidney and can inhibit the increase in creatinine levels. Research examining the relationship between rosuvastatin use and renal toxicity found that rosuvastatin can cause kidney tubular and muscle damage, resulting in rhabdomyolysis. The use of rosuvastatin up to a dose of 20 mg is still relatively safe for the kidney function, if it is increased to 40-80 mg it can cause a decrease in kidney function.[30]

This study found that at all doses of treatment 135 mg/kg BW/day, 202.5 mg/kg BW/day, and 270 mg/kg BW/day of the concoction Moringa leaves, and Red Ginger Rhizome ethanol extract (CML-RGRE) could inhibit the increase serum creatinine levels very significantly ($p < 0.01$), especially dose 202.5 mg/kg BW/day has the greatest potency. This proven is by following the research hypothesis. Increasing the dose of CML-RGRE to 270 mg/kg BW/day reduced the potency of CML-RGRE in lowering creatinine levels due to a ceiling effect.

Conclusion

The conclusion drawn from this study was that the concoction Moringa leaves, and Red Ginger Rhizome ethanol extract of PT "P" effectively inhibited the increase of serum creatinine levels of animal dyslipidemia models.

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Conflict of Interest

The authors declared no conflict of interest.

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