

RESEARCH STUDY

OPEN ACCESS

## Red Rice Bran Extract Intervention Ability to Improve Lipid Profile and Malondialdehyde Levels in Type 2 Diabetes Mellitus Model Rats

### *Intervensi Ekstrak Bekatul Beras Merah untuk Memperbaiki Profil Lipid dan Kadar Malondialdehid pada Tikus Model Diabetes Melitus Tipe 2*

Isniati Dwijayanti<sup>1\*</sup>, Brian Wasita<sup>2</sup>, Ida Nurwati<sup>3</sup>

<sup>1</sup>Master Program of Nutrition Sciences, School of Postgraduate, Universitas Sebelas Maret, Surakarta, Indonesia

<sup>2</sup>Department of Anatomical Pathology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia

<sup>3</sup>Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia

#### ARTICLE INFO

Received: 09-03-2022

Accepted: 23-05-2022

Published online: 24-11-2022

#### \*Correspondent:

Isniati Dwijayanti

[Isniatidwijayanti@gmail.com](mailto:Isniatidwijayanti@gmail.com)



DOI:

10.20473/amnt.v6i4.2022.404-413

Available online at:

<https://e-journal.unair.ac.id/AMNT>

#### Keywords:

Rice bran, Hyperlipidemias,

Malondialdehyde, Diabetes Mellitus

#### ABSTRACT

**Background:** Diabetes mellitus accompanied by oxidative stress can cause cardiovascular complications. Red rice bran extract contains antioxidants that have the potential to prevent oxidative stress and improve hyperlipidemia in patients with type 2 diabetes mellitus.

**Objectives:** Analyzing the effect of red rice bran extract on lipid profile and malondialdehyde levels in a diabetes mellitus rat model.

**Methods:** Pretest-Posttest Control Group Design. Thirty-five male Wistar Albino rats were divided into 5 groups, namely, negative control, positive control given acarbose as much as 1.8 mg/200gr/day, and 3 treatment groups given red rice bran extract, 165, 330, 660 mg/kg/day for 21 days, respectively.

**Results:** There was a change in lipid profile and MDA levels ( $p < 0.05$ ) after the treatment of bran extract with doses of 165, 330, and 660 mg/kg BW/day. Red rice bran extract at a dose of 660 mg/kg BW/day can be an alternative to acarbose in reducing cholesterol, LDL, and MDA levels, as well as elevating HDL levels in type 2 diabetes mellitus rats.

**Conclusions:** Red rice bran extract can significantly improve lipid profile and malondialdehyde levels in the type 2 diabetes mellitus rat model. Red rice bran extract at a dose of 660 mg/kg BW/day might be used as an alternative to acarbose in improving lipid profiles and MDA levels.

#### ABSTRAK

**Latar Belakang:** Diabetes melitus yang disertai dengan stres oksidatif dapat menyebabkan terjadinya komplikasi kardiovaskular. Ekstrak bekatul beras merah mengandung antioksidan yang dapat berpotensi mencegah terjadinya stress oksidatif dan memperbaiki kondisi hiperlipidemia pada penderita diabetes melitus.

**Tujuan:** Menganalisis pengaruh ekstrak bekatul beras merah terhadap profil lipid dan kadar malondialdehid pada tikus model diabetes melitus.

**Metode:** Pretest-Posttest Control Group Design. Tiga puluh lima tikus jantan Wistar Albino dibagi menjadi 5 kelompok perlakuan yaitu, kontrol negatif, kontrol positif yang diberi obat acarbose sebanyak 1,8 mg/200gr/hari dan 3 kelompok perlakuan dengan pemberian ekstrak bekatul beras merah masing-masing 165, 330, 660 mg/kgBB/hari selama 21 hari.

**Hasil:** Terjadi perubahan profil lipid dan kadar MDA ( $p < 0,05$ ) setelah pemberian ekstrak bekatul dosis 165, 330, 660 mg/kgBB/hari. Ekstrak bekatul beras merah dosis 660 mg/kgBB/hari dapat menjadi alternatif pengganti obat acarbose dalam menurunkan kadar kolesterol, LDL dan MDA serta meningkatkan kadar HDL pada tikus model diabetes melitus tipe 2.

**Kesimpulan:** Ekstrak bekatul beras merah secara signifikan dapat memperbaiki profil lipid dan kadar malondialdehid pada tikus model diabetes melitus tipe 2. Ekstrak bekatul beras merah dosis 660 mg/kgBB/hari dapat menjadi alternatif pengganti obat acarbose dalam memperbaiki profil lipid dan kadar MDA.

**Kata Kunci:** Bekatul beras, Hiperlipidemia, Malondialdehid, Diabetes Melitus

## INTRODUCTION

Diabetes mellitus accompanied by oxidative stress can cause micro and macrovascular complications<sup>1</sup>. One of the most common complications, also being the main cause of death in DM patients, is cardiovascular complications with a prevalence of 70%<sup>2</sup>.

Cardiovascular abnormalities in DM patients are caused by dyslipidemia, which is characterized by changes in lipid profiles. Profile changes that occur are caused by impaired insulin resistance which causes glucose in DM patients to not be converted into energy. This condition causes the energy produced in DM patients to be obtained from the breakdown of fat through the lipolysis process. The result of the lipolysis process is free fatty acids in the blood which will be carried to the liver to be converted into cholesterol (hypercholesterolemia) and triglycerides (hypertriglyceridemia)<sup>3</sup>.

On the other hand, the presence of excess free fatty acids or hyperlipidemic conditions can also cause ROS overproduction which can lead to mitochondrial DNA damage and malfunction of pancreatic cells, which will have an impact on the emergence of oxidative stress in diabetics<sup>4</sup>. The overproduction of ROS that occurs will also stimulate the oxidation of LDL (LDLox) which cannot be recognized by LDL receptors, so in the end, it will cause cardiovascular complications in DM patients<sup>5</sup>.

One of the ways to control diabetes dyslipidemia can be done with non-pharmacological and pharmacological therapies<sup>6</sup>. Pharmacological therapies include the administration of acarbose. However, acarbose generates side effects, namely abdominal pain, diarrhea, nausea, and vomiting<sup>7</sup>. Acarbose is also considered to be not effective to improve lipid profiles and reduce the formation of oxidative stresses<sup>8,9</sup>. There need to be alternatives that can simultaneously treat hyperlipidemia and diabetes without producing side effects<sup>10</sup>.

Red rice bran is a natural food ingredient that has the potential to be a therapy for diabetes mellitus and its complications. Rice bran contains antioxidant compounds consisting of vitamin E (tocopherols and tocotrienols),  $\gamma$ -oryzanol, phenolic compounds, and anthocyanins<sup>11</sup>. The antioxidant content of rice bran can potentially prevent oxidative stress formation by breaking the lipid peroxidation chain and improving hyperlipidemic conditions in DM patients by inhibiting HMG-CoA reductase<sup>12,13</sup>. In vitro studies also found that the antioxidant content, especially anthocyanins found in red bran, exerts a superior function compared to acarbose drug, brown rice bran and purple rice bran in treating DM<sup>14</sup>. In vivo studies on experimental animals also revealed that the administration of white and black rice bran extract could improve blood glucose levels in people with diabetes mellitus<sup>15,16</sup>.

The red rice bran used in this study comes from Magelang Regency, Central Java. Red rice bran extract in this study had an antioxidant activity of 60.14%, vitamin E content of 112.04 mg/100g, flavonoid content of 456.4 mg/100g and anthocyanin content of 340.24 mg/100g<sup>17,18</sup>. Red rice bran from Magelang has the advantage of higher anthocyanin content when compared to red rice bran from Texas and Bali<sup>18</sup>. These differences are caused by the variety, location of cultivated land and different extraction methods<sup>17</sup>. Red rice bran also contains phenolic compounds, flavonoid compounds, and higher total proanthocyanidins than brown and purple rice bran<sup>14</sup>.

Rice bran in Indonesia has not been used properly and is only used as waste. Meanwhile, red rice bran extract (RRBE) contains antioxidants that have the potential to treat people with DM dyslipidemia. To our knowledge, there has been no study on the effect of RRBE administration in vivo study of diabetes mellitus rats. This study aims to analyze the effect of RRBE intervention on lipid profile and malondialdehyde (MDA) levels of type 2 DM rats.

## METHODS

This study uses a laboratory experimental model with the type of Pretest-Posttest Control Group Design. The study was conducted in October-November at the Central Laboratory of Food and Nutrition Studies, Gajah Mada University using five treatment groups.

The materials used in this study were 96% ethanol, bran obtained from the milling process of red rice of the Inpari 24 variety which was grown organically in Magelang Regency, Central Java. The characteristics of the bran used have a smooth texture and have a taste that is dominated by bland, slightly bitter and sweet flavors<sup>19</sup>. Wistar rats, comfeed standard feed, acarbose, streptozotocin (STZ), nicotinamide (NA), thiobarbituric acid (TBA) reagent, Trichloroacetic Acid (TCA), glacial acetic acid, cholesterol oxidase-p-aminophenozone reagent (CHOD-PAP), and glycerol phosphate oxidase-p-amino-phenazone (GPO-PAP) reagent. The equipment used in this study was 60 mesh size, Whatman no. 1 filter paper, oven, shaker, rotary evaporator, a set of rat breeding cages, gastric probe, gloves, analytical scales, animal scales, and spectrophotometer (SP-300: Optima Japan).

The experimental design is shown in Table 1. The intervention was carried out for 21 days with an additional seven days of adaptation<sup>20,21</sup>. This research has passed the Ethical Clearance (No.62/UN27.06.6.1/KEP/EC/2021) from the Health Research Ethics Commission, Faculty of Medicine, Sebelas Maret University.

**Table 1.** Experimental design

Treatment Group	Intervention
K-	Diabetes Mellitus Rats + Comfeed Standard Feed
K+	Diabetes Mellitus Rats + Acarbose 1.8 mg/200gr/day
P1 Group	Diabetes Mellitus Rats + Red rice bran extract (165 mg/kg BW/day)
P2 Group	Diabetes Mellitus Rats + Red rice bran extract (330 mg/kg BW/day)
P3 Group	Diabetes Mellitus Rats + Red rice bran extract (660 mg/kg BW/day)

### Dosage Determination

The dose of acarbose commonly used by adult patients is 100 mg/kg BW/day<sup>22</sup>. The dose was converted from humans to white rats and obtained a dose of 1.8 mg/200gr/day. The dose of RRBE in this study was determined based on the anthocyanin requirement of adult humans of 12.5 mg/day and the study of giving anthocyanins of 10-20 mg could affect blood glucose, superoxide dismutase, malondialdehyde, blood antioxidant status, and inhibition of pancreatic cell damage<sup>23,24</sup>. The daily dose in humans of 12.5 mg was used as the median dose in this study and then converted to a rat dose and adjusted to the content contained in the RRBE with the result of 330 mg extract/kgBW/day. The first dose (165 mg extract/kgBW/day) was obtained from the second dose (330 mg extract/kgBW/day) and the third dose (660 mg extract/kgBW/day) was obtained by doubling the second dose.

### Bran Ethanol Extract

The red rice bran in this study came from the milling process of red rice with the type of Inpari 21 variety and the same planting location, namely Magelang Regency. This is so that the rice obtained is homogeneous.

The red rice bran obtained was then filtered using a mesh size of 60 to have the same size of bran, then the bran that had been extracted was then heated using an oven at 105°C for five minutes. Then, the process of maceration of the ethanol solution was carried out in a ratio of 1 to 6 with constant stirring for seven days using a shaker at 150 rpm. Furthermore, the process of filtering and concentration were done using a rotary evaporator with a temperature of 30°C<sup>25,26</sup>.

### Experimental Animal Induction and Treatment

Thirty-five male albino Wistar rats were divided into five treatment groups, with each group consisting of seven rats that followed the provisions of the Institutional Animal Care & Use Committee<sup>27</sup>. The inclusion criteria in this study were rats aged eight weeks, having a body weight between 150-200 g, fasting glucose levels  $\geq$  150 mg/dL, healthy condition with normal activities and behavior. The exclusion criteria were rats that had diarrhea, rats that appeared to be unhealthy, and rats that died during treatment.

The rats were adapted for seven days in a special room with a maintenance room temperature (27-29°C), a humidity of 70-90%, with a bright light cycle and a dark light cycle every 12 hours. This is a purpose to adjust the lifestyle and prevent the rats from experiencing stress. During the adaptation, rats were fed a standard Comfeed fed AD II (Carbohydrate 51%, Protein 15%, Fiber 6%, Fat 7%).

The adapted rats were induced intraperitoneally using a high dose of 230 mg/kgBW/day nicotinamide (NA) (Sigma-Aldrich 72340) first, after 15 minutes the rats were induced using a high dose of 65 mg/kgBW/day streptozotocin (STZ) (Nacalai Tesque 32238-91). After 5 days post-induction of STZ-NA, the rats fasted for eight hours before taking blood through

the retro-orbital flexus. Rats with hyperglycemic conditions with fasting blood glucose levels  $>$ 150 mg/dL and changes in lipid profile were used as study samples<sup>28-30</sup>.

Administration of STZ in this study causes damage to pancreatic cells which can cause insulin receptor disturbances in experimental animals. While offering NA serves to protect pancreatic cells from the toxic effects of STZ<sup>31,32</sup>.

### Lipid Profile

The measured lipid profile was taken from the blood serum of experimental animals. The blood sample was taken through the orbital sinus after STZ NA induction and after 21 days of intervention. The obtained rat blood was placed in the serum separator tube (SST), followed by inversion of the tubes 4-5 times. The tube was incorporated into a centrifuge and rotated for 20 minutes at 3000 rpm to obtain the blood serum. Examination of triglyceride levels using the glycerol phosphate oxidase-p-amino-phenazone (GPO-PAP) method. Examination of total cholesterol, HDL, and LDL using the cholesterol oxidase-p-aminophenazone (CHOD-PAP) method. Serum concentrations of total cholesterol, HDL, and LDL were determined using a total cholesterol assay kit, LDL assay kit, HDL assay kit and triglycerides assay kit (Human).

### MDA

The MDA measurement was performed from the blood serum of experimental animals. The blood sample was taken through the orbital sinus after STZ NA induction and after 21 days of intervention, and processed to obtain the serum through centrifugation. Serum MDA levels were examined by a spectrophotometric method using the kit and protocol from the Elabsience Malondialdehyde (MDA) Assay Kit (E-BC-K025).

### Data Analysis

The hypothesis in this study is that there is a dose effect (165, 330, or 660 mg/kgBW/day) of the ethanolic extract of red bran. On the levels of total cholesterol, triglycerides, HDL, LDL and MDA in rats model T2DM. The data obtained were processed using SPSS version 17. Before carrying out the analysis, the data were tested for normality using the Shapiro-Wilk test with a p value  $>$  0.05. Then the data were analyzed using a paired t-test to analyze differences in lipid profile and MDA levels before and after treatment in all groups and an Analysis of Variance (ANOVA) test was performed to analyze the differences between the five treatment groups, followed by Post Hoc Tukey HSD. All values are reported on an average basis and  $\pm$  standard deviation. P value  $<$  0.05 was considered a statistical significance level.

## RESULTS AND DISCUSSION

### The Effect of RRBE on Total Cholesterol Levels

Table 2 represents the total cholesterol levels before and after the intervention, which decrease

significantly in the K+, P1, P2, and P3 groups. The greatest decrease in cholesterol was found in the P3 treatment group (-85.37 mg/dL). The results of the ANOVA test in this study showed that there were significant differences in total cholesterol levels between each treatment group before and after the intervention.

Table 2 also shows that the average difference in cholesterol levels in the group receiving RRBE (P1, P2, P3), when compared to the negative group, was

statistically significant. This indicates that the RRBE was able to reduce the cholesterol levels in DM model rats and has the potential to treat DM patients who have elevated cholesterol levels. Table 2 also expresses that the positive group, when compared with the P3 group, was not statistically significant, thus the administration of RRBE with a dose of 660 mg/kg BW/day could be an alternative to acarbose in lowering cholesterol levels in T2DM rats.

**Table 2.** Average cholesterol levels after treatment for 21 days

Group	Average Cholesterol Level (mg/dL)		Δ Cholesterol	p <sup>1</sup>
	D1	D21		
K-	190.12±3.99	192.68±3.89	2.56±0.10	0.001*
K+	186.99±2.17	109.56±4.98	-77.43±2.81 <sup>a</sup>	0.001*
P1	188.94±3.48	138.73±6.35	-50.21±2.87 <sup>b</sup>	0.001*
P2	193.05±2.48	118.89±4.51	-74.16±2.03 <sup>c</sup>	0.001*
P3	190.80±3.56	105.43±2.18	-85.37±1.38 <sup>a</sup>	0.001*
p <sup>2</sup>	0.020*	0.001*		

D1: Before intervention (post STZ-NA induction); D21: After intervention; K-: Negative control group; K+: positive control group given acarbose 1.8 mg/200gr/day; P1: RRBE 155 mg/kgBW; P2: RRBE 330 mg/kgBW; P3: RRBE 660 mg/kgBW; Δ Cholesterol: the difference in the mean cholesterol levels after induction and after administration of the extract for 21 days. \*) significant (P<0.05); P<sup>1</sup>) test paired t-test; P<sup>2</sup>) one way ANOVA test; <sup>a,b,c</sup>) Superscripts with different letters, show significant differences (Post Hoc Tukey HSD).

The antioxidant content of rice bran is being studied intensively because it can show several beneficial biological activities both in vitro and in vivo which can potentially be used as supportive therapy in overcoming diabetes mellitus conditions while reducing the emergence of complications<sup>33,34</sup>. RRBE contains antioxidant compounds consisting of vitamin E (tocopherols and tocotrienols), γ-oryzanol, phenolic compounds, and anthocyanins<sup>11</sup>.

Based on the results of statistical analysis, the administration of RRBE in groups P1, P2, and P3 could reduce total cholesterol levels in T2DM rats and group P3 with a dose of 660 mg/dL being the best dose of RRBE in reducing total cholesterol levels compared to other groups. This is in line with in vivo and in vitro studies which found that the level of reduction in cholesterol levels was in line with the amount of bran extract given<sup>20,35</sup>. The decrease in cholesterol levels was higher in the P3 group probably due to the higher antioxidant content when compared to other doses.

The content of antioxidants, especially vitamin E (tocotrienol), γ-oryzanol and phenolic compounds in rice bran can function in regulating the synthesis of endogenous cholesterol by inhibiting the activity of HMG-CoA reductase<sup>13,36,37</sup>. HMG-CoA reductase is an enzyme that occurs during the process of converting HMG-CoA into mevalonic acid which is an important step in cholesterol biosynthesis<sup>38</sup>. Inhibition of HMG-CoA reductase will result in isoprene not being formed and squalene formation not occurring so that cholesterol synthesis is inhibited. This inhibited cholesterol will result in decreased cholesterol synthesis<sup>37</sup>. The process of inhibiting HMG-CoA reductase can reduce cholesterol levels in animal and human studies<sup>38</sup>.

Red rice bran also contains phenolic compounds consisting of protocatechuic acid,

chlorogenic acid, vanillic acid, hydroxybenzoic acid, coumaric acid, and ferulic acid<sup>13</sup>. Phenol compounds found in rice bran, especially chlorogenic acid, can reduce cholesterol levels by increasing glucose uptake in skeletal muscle cells through the activation of AMP-activated protein kinase (AMPK) which can reduce fatty acid synthesis in liver cells<sup>13,39</sup>. This decrease will increase the uptake of cholesterol in the blood to be resynthesized which will cause a decrease in cholesterol levels in the blood<sup>37,40</sup>.

The results of other studies also showed that oryzanol contained in rice bran was able to prevent an increase in cholesterol levels. Oryzanol in the body plays a role in the mechanism of cholesterol absorption in the intestine by forming complex compounds with insoluble cholesterol. This causes the solubility of cholesterol in the body to be reduced in bile acid solutions. Excess cholesterol contained in the body will eventually be excreted with feces. This causes cholesterol levels in the body to be reduced<sup>41</sup>. In addition, the content of oryzanol and tocotrienol in rice bran can lower cholesterol levels in the blood by inducing the release of the cholesterol 7-α-hydroxylase (CYP7A1) enzyme which plays a role in maintaining the stability of cholesterol levels in the body by converting it into bile acids<sup>42</sup>.

The results of the analysis also showed a decrease in total cholesterol levels in the high P3 group when compared to the administration of acarbose in this study. This is probably due to the flavonoid content, especially anthocyanins and proanthocyanidins in the P3 group can inhibit the activity of α-glucosidase and α-amylase better than high-dose acarbose drugs, according to previous in vitro studies<sup>14</sup>. Inhibition of α-amylase and α-glucosidase activity can inhibit carbohydrate absorption, resulting in decreased postprandial glucose levels and increased insulin

secretion<sup>41</sup>. Increased insulin secretion will prevent increased lipolysis and impaired lipogenesis in the liver which causes cholesterol levels in the body not to increase<sup>43</sup>.

### The Effect of RRBE on Triglyceride Levels

Table 3 shows that the triglyceride levels before and after intervention experienced a significant decrease in the K+, P1, P2, and P3 groups. The greatest decrease in triglycerides was found in the P3 treatment group (-52.39 mg/dL). The results of the ANOVA test in this study showed that there were significant differences

in triglyceride levels between each treatment groups after the intervention.

Table 3 also shows that the average difference in triglyceride levels in the group receiving RRBE (P1, P2, P3) when compared to the negative group, was statistically significant. This shows that RRBE can reduce triglyceride levels in DM model rats and has the potential to treat DM, which has increased triglyceride levels. Table 3 also denotes that the group given RRBE (P1, P2, P3), when compared with the positive group, has a statistically significant result. This result indicates that the administration of RRBE has been able to replace acarbose in reducing triglyceride levels in T2DM rats.

**Table 3.** Average triglyceride levels after treatment for 21 days

Group	Average Triglyceride Level (mg/dL)		Δ Triglyceride	P <sup>1</sup>
	D1	D21		
K-	128.52±2.87	130.87±2.51	2.35±-0.36	0.001*
K+	127.31±2.02	79.72±3.26	-47.59±1.24	0.001*
P1	126.00±2.70	106.90±3.29	-19.10±0.59 <sup>a</sup>	0.001*
P2	122.99±5.22	86.04±3.04	-36.95±-2.18 <sup>b</sup>	0.001*
P3	127.51±2.11	75.12±2.01	-52.39±-0.10 <sup>c</sup>	0.001*
P <sup>2</sup>	0.677	0.001*		

D1: Before intervention (post STZ-NA induction); D21: After intervention; K-: Negative control group; K+: positive control group given acarbose 1.8 mg/200gr/day; P1: RRBE 155 mg/kgBW; P2: RRBE 330 mg/kgBW; P3: RRBE 660 mg/kgBW; Δ Triglyceride: the difference in the mean triglyceride after induction and after administration of the extract for 21 days. \*) significant (P<0.05); P<sup>1</sup>) test paired t-test; P<sup>2</sup>) one way ANOVA test; <sup>a,b,c</sup>) Superscripts with different letters, show significant differences (Post Hoc Tukey HSD).

Several studies recently suggested that the decrease and increase in triglyceride levels were positively correlated with cholesterol levels.<sup>41</sup> This is in line with the results of statistical analysis in this study, namely the administration of RRBE can reduce cholesterol and triglyceride levels in all groups receiving RRBE and P3 groups with a dose of 660 mg/dL being the best dose of RRBE in this study.

Triglycerides are a form of fat that is absorbed by the intestine after hydrolysis which then enters the plasma in two forms, namely as chylomicrons (derived from intestinal absorption after ingestion of fat) and as very low density lipoprotein (VLDL) which is formed by the liver<sup>44</sup>.

The content of phenolic compounds (ferulic acid) and flavonoids (anthocyanins) found in rice bran can improve insulin resistance in people with diabetes mellitus<sup>45,46</sup>. Improvement of insulin resistance can suppress the production of free fatty acids in the body. blood circulation by inhibiting fatty acid synthase (FAS), which is an enzyme that is very important in fat metabolism. Inhibited FAS can directly reduce the formation of fatty acids, thereby reducing the formation of triglycerides and VLDL which will prevent the occurrence of hypertriglyceridemia, postprandial hyperlipidemia and lipoprotein lipase resistance in people with diabetes mellitus<sup>46,47</sup>.

The antioxidant content in rice bran which has the effect of inhibiting HMG-CoA reductase can also reduce triglyceride levels in plasma. The decrease in plasma triglyceride levels occurs due to an increase in the speed of LDL catabolism, thereby reducing LDL storage in plasma which affects the decrease in triglyceride levels<sup>48</sup>.

### Effect of RRBE on HDL Levels

Table 4 show that HDL levels before and after intervention experienced a significant increase in the acarbose (K+) group and the RRBE group (P1, P2, P3) groups. The greatest increase in HDL was in the P3 treatment group (50.89 mg/dL). The results of the ANOVA test in this study showed that there were significant differences in HDL levels between each treatment group before and after the intervention

Table 4 also shows that the average difference in HDL levels in the group receiving RRBE (P1, P2, P3), when compared to the negative group, was statistically significant. The results mean that RRBE can increase HDL levels in DM model rats, thus having the potential to treat DM with increased HDL levels. Table 4 also described that the P3 group when compared with the positive group was statistically insignificant. Hence, the administration of RRBE at a dose of 660 mg/kg BW/day could be an alternative to acarbose in increasing HDL levels in T2DM rats.



**Table 4.** Average HDL levels after treatment for 21 days

Group	Average HDL Level (mg/dL)		Δ HDL	P <sup>1</sup>
	D1	D21		
K-	21.18±1.64	20.38±1.45	-0.80±0.19	0.001*
K+	21.47±1.92	71.01±1.94	49.54±0.02 <sup>a</sup>	0.001*
P1	21.28±1.56	49.16±3.22	27.88±1.66 <sup>b</sup>	0.001*
P2	20.60±1.56	66.91±2.29	46.31±0.73 <sup>c</sup>	0.001*
P3	21.38±1.61	72.27±3.05	50.89±1.44 <sup>a</sup>	0.001*
P <sup>2</sup>	0.876	0.001*		

D1: Before intervention (post STZ-NA induction); D21: After intervention; K-: Negative control group; K+: positive control group was given acarbose 1.8 mg/200gr/day; P1: RRBE 155 mg/kgBW; P2: RRBE 330 mg/kgBW; P3: RRBE 660 mg/kgBW; Δ HDL: the difference in the mean HDL after induction and after administration of the extract for 21 days. \*) significant (P<0.05); P<sup>1</sup>) test paired t-test; P<sup>2</sup>) one way ANOVA test; <sup>a,b,c</sup>) Superscripts with different letters, show significant differences (Post Hoc Tukey HSD).

Based on the results of statistical analysis, giving RRBE to groups P1, P2, and P3 could increase HDL levels in T2DM rats. This increase in HDL is in line with in vivo study which states that bran supplementation can increase HDL levels in experimental animals<sup>40</sup>.

The P3 group with a dose of 660 mg/dL is the best dose of RRBE in increasing total HDL levels compared to other groups. The higher the increase in HDL levels in the body, the greater its capacity to transport cholesterol and prevent blockages in the blood vessels<sup>49</sup>.

Flavonoids in rice bran also specifically anthocyanins and proanthocyanidins function to increase HDL by activating peroxisome proliferator-activated receptor alpha (PPARα) in the liver<sup>52</sup>. PPARα functions as a regulator of lipid metabolism which plays an important role in inducing the absorption of glucose and fatty acids by liver cells<sup>53</sup>. Activation of PPARα in the liver can increase the amount of apolipoprotein A-1 which helps the formation of HDL and increases HDL levels in the blood<sup>54</sup>.

#### Effect of RRBE on LDL Levels

Table 5 represents the LDL levels before and after the intervention, which decrease significantly in the K+, P1, P2, and P3 groups. The greatest reduction in LDL was found in the P3 treatment group (-44.24 mg/dL). The results of the ANOVA test in this study showed that there were significant differences in LDL levels between each treatment group before and after the intervention.

Table 5 indicates that the average difference in LDL levels in the group receiving RRBE (P1, P2, P3), when compared to the negative group, was statistically significant. This result implies that RRBE can depress the LDL levels in DM model rats and has the potential for DM therapy with increased LDL levels. Table 5 also demonstrates that the positive group when compared with the P3 group was not statistically significant. Therefore, the administration of RRBE at a dose of 660 mg/kg BW/day could be an alternative to acarbose in reducing LDL levels in T2DM rats.

**Table 5.** Average LDL levels after treatment for 21 days

Group	Average LDL Level (mg/dL)		Δ LDL	P <sup>1</sup>
	D1	D21		
K-	76.82±1.74	78.03±2.47	1.21±0.73	0.006*
K+	76.62±1.82	35.71±1.98	-40.91±0.16 <sup>a</sup>	0.001*
P1	74.54±1.18	54.65±1.77	-19.89±0.59 <sup>b</sup>	0.001*
P2	76.32±3.36	41.12±2.17	-35.20±1.19 <sup>c</sup>	0.001*
P3	76.82±1.50	32.58±1.64	-44.24±0.14 <sup>a</sup>	0.001*
P <sup>2</sup>	0.221	0.001*		

D1: Before intervention (post STZ-NA induction); D21: After intervention; K-: Negative control group; K+: positive control group was given acarbose 1.8 mg/200gr/day; P1: RRBE 155 mg/kgBW; P2: RRBE 330 mg/kgBW; P3: RRBE 660 mg/kgBW; Δ LDL: the difference in the mean LDL after induction and after administration of the extract for 21 days. \*) significant (P<0.05); P<sup>1</sup>) test paired t-test; P<sup>2</sup>) one way ANOVA test; <sup>a,b,c</sup>) Superscripts with different letters, show significant differences (Post Hoc Tukey HSD).

Low Density Lipoprotein (LDL) is a lipoprotein molecule consisting of a combination of protein and fat synthesized by the liver containing 45% cholesterol, which can affect the incidence of heart disease<sup>54</sup>. Reducing LDL levels in T2DM rats is in line with in vitro studies of bran in humans which stated that the antioxidant content in bran can reduce LDL levels through the mechanism of inhibiting LDL oxidation<sup>41</sup>.

The LDL oxidation process occurs due to damage to plasma lipoproteins caused by fat oxidation

in people with diabetes mellitus. This oxidation process in LDL will increase LDL cholesterol levels in the blood and cause blood viscosity to become thicker and cause the risk of blockage of blood vessels (atherosclerosis) to be higher<sup>35,37</sup>.

The antioxidant content of rice bran, especially vitamin E (112.04 mg/100g) which is quite high in this study can inhibit the oxidation process through the peroxyl radical scavenging process which is included in

peroxidation or through reactions with lipid peroxyl radicals<sup>41</sup>.

The antioxidant content in rice bran, especially phenolic compounds and oryzanol, can reduce LDL levels by inhibiting HMG-CoA reductase<sup>37,50</sup>. Inhibition of HMG-CoA reductase will result in cholesterol resistance which can reduce the formation of very low density lipoprotein (VLDL) in the liver. This inhibition of VLDL synthesis will automatically cause a decrease in the amount of LDL in the blood<sup>37</sup>.

The results of the analysis in this study also showed that the P3 group with a dose of 660 mg/dL was the best dose of RRBE in reducing LDL levels compared to other groups with lower RRBE doses. This is in line with several studies which state that the consumption of rice bran is in line with a decrease in LDL levels in experimental animals<sup>42</sup>.

### Effect of RRBE on MDA Levels

Table 6 denotes the MDA levels before and after the intervention, which decrease significantly in the K+, P1, P2, and P3 groups. The greatest MDA levels reduction was found in the P3 treatment group (-5.95 nmol/mL). The MDA levels alleviation differs slightly when compared to the positive control group (-5.73 nmol/mL) and the P2 group (-4.84 nmol/mL). The results of the ANOVA test in this study showed that there were significant differences in MDA levels between each treatment group before and after the intervention.

Table 6 describes that the average difference in MDA levels in the negative group, in comparison to the group receiving RRBE (P1, P2, P3), was statistically significant. This conveys that RRBE can suppress the MDA levels in DM model rats and has the potential for DM therapy. Table 6 also exhibits that the P3 group when compared with the positive group was statistically insignificant. Therefore RRBE at a dose of 660 mg/kg BW/day could be an alternative to acarbose in reducing MDA levels in T2DM rats.

**Table 6.** Average MDA levels after treatment for 21 days

Group	Average MDA Level (mg/dL)		Δ MDA	P <sup>1</sup>
	D1	D21		
K-	8.75±0.49	9.42±0.35	0.67±-0.14	0.001*
K+	8.69±0.33	2.96±0.30	-5.73±-0.03 <sup>a</sup>	0.001*
P1	8.80±0.36	6.23±0.39	-2.57±0.03 <sup>b</sup>	0.001*
P2	8.91±0.53	4.07±0.42	-4.84±-0.11 <sup>c</sup>	0.001*
P3	8.58±0.43	2.63±0.35	-5.95±-0.80 <sup>a</sup>	0.001*
P <sup>2</sup>	0.704	0.001*		

D1: Before intervention (post STZ-NA induction); D21: After intervention; K-: Negative control group; K+: positive control group was given acarbose 1.8 mg/200gr/day; P1: RRBE 155 mg/kgBW; P2: RRBE 330 mg/kgBW; P3: RRBE 660 mg/kgBW; Δ MDA: the difference in the mean MDA after induction and after administration of the extract for 21 days. \*) significant (P<0.05); P<sup>1</sup>) test paired t-test; P<sup>2</sup>) one way ANOVA test; <sup>a,b,c</sup>) Superscripts with different letters, show significant differences (Post Hoc Tukey HSD).

Conditions of hyperglycemia and hyperlipidemia in patients with diabetes mellitus will cause overproduction of ROS, which in turn can cause damage to mitochondrial DNA and malfunction of pancreatic cells, all of which will have an impact on the emergence of oxidative stress and decreased antioxidant capacity in patients with diabetes mellitus<sup>15,55</sup>. Measurement of MDA levels in this study aims to determine the incidence of oxidative stress that occurs in people with diabetes mellitus. MDA is an indicator of the presence of free radical compounds in the body<sup>56</sup>.

Based on the results of statistical analysis, the administration of RRBE in groups P1, P2, and P3 could reduce MDA levels in T2DM rats. The decrease in MDA levels in the P3 group in this study had the best effectiveness and the decrease was not much different when compared to the P2 and P+ groups with acarbose administration. This is in line with a study that states that giving anthocyanins as much as 10-20 mg can reduce malondialdehyde levels in vivo studies on hyperglycemic rat models<sup>23,24</sup>. The P3 and P2 groups in this study contained anthocyanin levels of 12.5 and 25 mg.

The decrease in blood MDA levels that occurs proves that the antioxidant content of RRBE administration can inhibit excess free radical activity in rat models of diabetes mellitus. Lipid peroxide is a reaction that occurs because free radicals bind to lipids through several stages of initiation, propagation and termination with the final result in the form of MDA<sup>57</sup>.

Lipid peroxide that occurs in rats can be inhibited by the antioxidant content found in rice bran. Phenolic compounds found in rice bran are radical scavengers that will inhibit lipid peroxide at the initiation stage that occurs in rats after STZ-NA induction so that MDA is not produced<sup>57</sup>. Meanwhile, anthocyanin compounds in rice bran act as SOD enzymes which will inhibit ROS activity so that they cannot bind to lipids in forming lipid peroxide reactions so that MDA is not produced<sup>58</sup>.

The content of phenolic compounds, vitamin E (tocopherols and tocotrienols) and anthocyanins in rice bran can reduce MDA levels by protecting cells from free radical damage by adding one free electron to a free radical or accepting an unstable electron so that it becomes more stable<sup>59-62</sup>. The addition can block the oxidation reaction at the initiation or propagation stage so that MDA is not produced<sup>15</sup>.

The administration of RRBE in this study has the potential to be an alternative treatment for diabetics who experience dyslipidemia, and can be a reference material for further study as well. The current study was limited to biochemical examination of lipid profiles and MDA from blood samples only. The effect of giving RRBE on tissue damage in animals was not yet performed, thus it is not known whether RRBE can repair tissue damage.

### CONCLUSIONS

Red rice bran extract can improve lipid profile and MDA levels in the T2DM rat model significantly. RRBE with a dose of 660 mg/kg BW/day can be used as an alternative to acarbose to improve lipid profiles and MDA levels. Further study is needed to analyze the effect of RRBE on tissue damage that plays an important role in the occurrence of complications caused by vascular disorders in people with diabetes mellitus, such as liver, pancreas and kidney tissue.

### ACKNOWLEDGMENTS

The author would like to thank the laboratory assistant Center for Food and Nutrition Studies, Gadjah Mada University who has helped during the research process.

### CONFLICT OF INTEREST

All authors have no conflict of interest in this article.

### REFERENCES

1. Jain, A. B. & Jain, V. A. Vitamin E, its Beneficial Role in Diabetes Mellitus (DM) and its Complications. *J. Clin. Diagnostic Res.* **6**, 1624–1628 (2012).
2. Michael, O. A., Olarinde, O. O., Tunji, O. Y. & Ayodeji, K. B. Prevalence, Variants and Determinants of Electrocardiographic Abnormalities Amongst Elderly Nigerians with Type 2 Diabetes. *Int. Res. J. Med. Med. Sci.* **4**, 324–328 (2013).
3. Effendi, A. T. *Nutrigenomik Resistensi Insulin Prediabetes*. (IPB Press, 2013).
4. Oyenihi, A. B., Ayeleso, A. O., Mukwevho, E. & Masola, B. Antioxidant Strategies in the Management of Diabetic Neuropathy. *Biomed Res. Int.* (2015).
5. Bajaj, S. & Khan, A. Antioxidants and Diabetes. *Indian J. Endocrinol. Metab.* **16**, (2012).
6. Ozder, A. Lipid Profile Abnormalities Seen in T2DM Patients in Primary Healthcare In Turkey: A cross-sectional study. *Lipids Health Dis.* **13**, 1–6 (2014).
7. DeFronzo, R. A., Ferrannini, E., Groop, L., Henry, R. R., Herman, W. H., Holst, J. J. & Weiss, R. I. Type 2 Diabetes Mellitus. *Nat. Rev. Dis. Prim.* **1**, 1–23 (2015).
8. Standl, E., Theodorakis, M. J., Erbach, M., Schnell, O. & Tuomilehto, J. On The Potential of Acarbose to Reduce Cardiovascular Disease. *Cardiovasc. Diabetol.* **13**, 1–12 (2014).
9. DiNicolantonio, J. J., Bhutani, J. & O’Keefe, J. H. Acarbose: Safe and Effective for Lowering Postprandial Hyperglycaemia and Improving Cardiovascular Outcomes. *Open Hear.* **2**, (2015).
10. Komal, C., Bhushan, C. & Gauri, B. Effects of Rice Bran Oil and Gamma Oryzanol on Antioxidant Defense in Streptozotocin Nicotinamide Induced Diabetic Rats. *Int. J. Eng. Sci. Res. Technol.* **4**, 458–471 (2015).
11. Dwi, P. M. & Fitriyono, A. Analisis Kadar Tokoferol,  $\gamma$ -Oryzanol Dan B-Karoten Serta Aktivitas Antioksidan Minyak Bekatul Kasar. *J. Nutr. Coll.* **2**, 350–357 (2013).
12. Tuncel, N. B., Yilmaz, N., Kocabiyyik, H. & Uygur, A. The Effect of Infrared Stabilized Rice Bran Substitution on Physicochemical and Sensory Properties of Pan Breads: Part I. *J. Cereal Sci.* **59**, 155–161 (2014).
13. Tantipaiboonwong, P., Pinthaa, K., Chaiwangyena, W., Chewonarib, T., Pangitc, K., Chumphukama, O. & Suttajita, M. Anti-hyperglycaemic and Anti-Hyperlipidaemic Effects of Black and Red Rice in Streptozotocin-Induced Diabetic Rats. *ScienceAsia* **43**, 281–288 (2017).
14. Boue, S. M., Daigle, K. W., Chen, M. H., Cao, H. & Heiman, M. L. Antidiabetic Potential of Purple and Red Rice (*Oryza Sativa* L.) bran extracts. *J. Agric. Food Chem.* **64**, 5345–5353 (2016).
15. Fuadah, N. Pengaruh Terapi Ekstrak Etanol 96% Bekatul (Rice Bran) Terhadap Kadar Glukosa Darah dan MDA (Malondialdehyde) Pada Hati Mencit (*Mus Musculus*) Diabetes Mellitus. (Universitas Islam Negeri Maulana Malik Ibrahim, 2019).
16. Dwinanti, S. N. Kemampuan Ekstrak Etanol Bekatul Beras Hitam dalam Menurunkan Kadar Glukosa Darah pada Tikus Nefropati Diabetes. *UIN Maulana Malik Ibrahim* **39**, 1–15 (2014).
17. Dwijayanti, I., Wasita, B. & Nurwati, I. The Analysis of Tocopherol Contents and Antioxidant Activity Of Ethanol Extract of Red Rice Bran. in *Prosiding The 2 nd International Nursing and Health Sciences Symposium (INHSS) 28* (2022).
18. Nurrohima, D., Wasita, B., & Susilawati, T.N. The Levels of Flavonoid and Anthocyanin in The Ethanol Extract of Red Rice Bran. in *Prosiding The 2 nd International Nursing and Health Sciences Symposium (INHSS) 7* (2022).
19. Sharif, M. K., Butt, M. S., Anjum, F. M. & Khan, S. H. Rice Bran: A Novel Functional Ingredient. *Crit. Rev. Food Sci. Nutr.* **54**, 807–816 (2014).
20. Kurniawati, D. C. Pemberian Ekstrak Bekatul Beras Merah (*Oryza Nivara*) Sama Efektif Dengan Simvastatin Dalam Memperbaiki Kadar Kolesterol Total dan Trigliserida Pada Tikus Wistar Jantan (*Rattus Norvegicus*) Dislipidemia. (Universitas Udayana Denpasar, 2017).
21. Hasim, N, M. A., Setyono, A., Qomiliah, E. N. & Faridah, D. N. Ekstrak Angkak dan Bekatul untuk Mencegah Peroksidasi Lipid Tikus Spague-Dawley Hiperglikemik. *Jurnal Aplikasi Teknologi Pangan.* **9**, 62–70 (2020).
22. Pradiningsih, A., Pandanwangi Tw, S. & Aribowo, A. Pengaruh Ekstrak Daun Kembang Bulan



- (*Tithonia Diversifolia* A.Gray) Terhadap Kadar Glukosa Darah Tikus Putih Wistar Yang Diinduksi Oleh Alokan. *J. Holist. Heal. Sci.* **1**, 133–144 (2018).
23. Herawati, E. R. N. Pengaruh Konsumsi Ekstrak Antosianin Ubi Jalar Ungu (*Ipomoea Batatas* L.) Terhadap Glukosa Darah, Status Antioksidan Darah, dan Gambaran Histopatologis Pankreas Tikus Hiperglikemia Induksi Alokan. (Universitas Gadjah Mada, 2013).
  24. Mahanani, W. Efektivitas Ekstrak Antosianin Beras Merah (*Oryza Sativa* L.) dan Kedelai Hitam (*Glycine Max* (L) Merr.). (Universitas Gadjah Mada, 2016).
  25. Rai, I. W. & Arnata, I. W. Stabilitas Aktivitas Antioksidan Ekstrak Bekatul Beras Merah Terhadap Oksidator dan Pemanasan Pada Berbagai pH. *J. Teknol. dan Ind. Pangan* **25**, 193–199 (2014).
  26. Rahmanisa, S., Kurniawaty, E., Mustofa, S. & Hayatillah, N. Pengaruh Pemberian Ekstrak Etanol 96% Bekatul Beras Merah Terhadap Jumlah dan Viabilitas Spermatozoa Tikus Putih (*Rattus norvegicus*) Galur Sprague dawley yang Diinduksi Asap Rokok Kretek. *J. Unila* 1–9 (2018).
  27. Institutional Animal Care And Use Committee (IACUC). *Institutional Animal Care and Use Committee Guidebook*. (Office of Laboratory Animal Welfare, 2002).
  28. Purbowati, P., Johan, A. & Kisdjamiatun. Pengaruh Jamur Tiram Putih (*Pleurotus Ostreatus*) Terhadap Kadar Glukosa Darah, Profil Lipid dan Kadar MDA Pada Tikus (*Rattus Norvegicus*) Diabetes Melitus. *The Indones. J. Nutr.* **4**, 131–137 (2016).
  29. Ghasemi, A., Khalifi, S. & Jedi, S. Streptozotocin-nicotinamide-induced Rat Model of Type 2 Diabetes (review). *Acta Physiol. Hung.* **101**, 408–420 (2014).
  30. Palupi, F. D., Waskita, B. & Nuhriawangsa, A. M. P. Pengaruh Dosis dan Lama Waktu Pemberian Ekstrak Etanol Pegagan (*Centella asiatica*) Terhadap Kadar Gula Darah dan Derajat Insulinitis Tikus Model Diabetes Melitus Tipe 2. *Media Gizi Mikro Indones.* **10**, 111–124 (2019).
  31. Hascek, W. ., Rousseaux, C. ., Wallig, M. ., Bolon, B. & Ochoa, R. *Hascek and Rousseaux's Handbook of Toxicologic Patholog.* (Academic Press, 2013).
  32. Szkudelski, T. Streptozotocin-nicotinamide-induced Diabetes In The Rat. Characteristics Of The Experimental Model. *Exp. Biol. Med.* **237**, 481–490 (2012).
  33. Friedman, M. Rice brans, rice bran oils, and rice hulls: Composition, Food and Industrial Uses, and Bioactivities in Humans, Animals, and Cells. *J. Agric. Food Chem.* **61**, 10626–10641 (2013).
  34. Islam, M. S., Matsuki, N., Nagasaka, R., Ohara, K., Hosoya, T., Ozaki, H. & Hori, M. Rice Bran Antioxidants in Health and Wellness. *Wheat and Rice in Disease Prevention and Health* (2014).
  35. Kurnia, D., Lisniawati, N. A. & Dinata, D. I. Uji Pengikatan Kolesterol Oleh Ekstrak Metanol Bekatul Beras Ketan Hitam Secara in Vitro. *J. Kim. Ris.* **4**, 74 (2019).
  36. Devarajan, S., Chatterjee, B., Urata, H., Zhang, B., Ali, A., Singh, R., & Ganapathy, S. A Blend of Sesame and Rice Bran Oils Lowers Hyperglycemia and Improves The Lipids. *Am. J. Med.* **129**, 731–739 (2016).
  37. Prasetya, I. R. I. Pengaruh Pemberian Selai Kacang Tanah Dengan Substitusi Bekatul Terhadap Kadar Kolesterol LDL dan HDL Tikus Hiperkolesterolemia. *J. Nutr. Coll.* **4**, 171–179 (2015).
  38. Baskaran, G., Pattiram, P. D. & Yunus, S. M. HMG-CoA Reductase Inhibitory Activity and Phytocomponent Investigation of Basella Alba Leaf Extract As A Treatment for Hypercholesterolemia. *Drug design, development and therapy.* **9**, 509 (2015).
  39. Ong, K. W., Hsu, A. & Tan, B. K. H. Chlorogenic Acid Stimulates Glucose Transport in Skeletal Muscle via AMPK Activation: A Contributor to The Beneficial Effects of Coffee on Diabetes. *PLoS One* **7**, (2012).
  40. Manalu, W., Suprayogi, A., & Astuti, D. A. Perbaikan Parameter Lipid Darah Mencit Hiperkolesterolemia dengan Suplemen Pangan Bekatul. *Mkb* **45**, 1–9 (2013).
  41. Jufri, N. N., Wirjatmadi, B., & Adriani, M. Combined Food ( Bekatul dan Lemak ) Menurunkan Kadar Kolesterol Total , Trigliserida , dan LDL pada Tikus Galur Wistar. *Jurnal Kedokteran Brawijaya.* **28**, 208–212 (2015).
  42. Jolfaie, N. R., Rouhani, M. H., Surkan, P. J., Siassi, F. & Azadbakht, L. Rice Bran Oil Decreases Total and LDL Cholesterol in Humans: A Systematic Review and Meta-Analysis of Randomized Controlled Clinical Trials. *Horm. Metab. Res.* **48**, 417–426 (2016).
  43. Sutrisno, R. D. Pengaruh Kombinasi Ekstrak Etanol Daun *Mangifera indica* L. var arumanis dan Perasan Buah *Abelmoschus esculentus* L. Moench terhadap Kadar Kolesterol Total pada *Rattus norvegicus* Diabetes. (Universitas Muhammadiyah Malang, 2018).
  44. Gani, N., Momuat, L. I. & Pitoi, M. M. Plasma Lipid Profile of Hypercholesterolemic Wistar Mice on Giving Red Gedi (*Abelmoschus manihot* L.). *J. Mipa Unsrat* **2**, 44–49 (2013).
  45. Zhao, G., Zhang, R., Huang, F., Dong, L., Liu, L., Jia, X. & Zhang, M. Hydrolyzed Bound Phenolics from Rice Bran Alleviate Hyperlipidemia and Improve Gut Microbiota Dysbiosis in High-Fat-Diet Fed Mice. *Nutrients* **6**, 1277 (2022).
  46. Mutia, S., Fauziah & Thomy, Z. Pengaruh Pemberian Ekstrak Etanol Daun Andong (*Cordyline Fruticosa* (L.) A Chev) Terhadap Kadar Kolesterol Total dan Trigliserida Darah Tikus Putih (*Rattus Norvegicus*) Hiperkolesterolemia. *J. Bioleuser* **2**, 29–35 (2018).
  47. Monterio, M. Pengaruh Pemberian Ekstrak Labu Kuning Per Oral (*Cucurbita Moschata* Duchenes ) Terhadap Kadar Trigliserida Tikus Jantan (*Rattus Norvegicus* Strain Wistar) Model Diabetes

- Mellitus Tipe 2. *Gastronomía ecuatoriana y turismo local*. (Universitas Brawijaya Malang, 2013).
48. Wahyuningrum, I. & Zubaidah, E. Pengaruh Angkak Dengan Penambahan Bekatul Terhadap Penurunan Profil Lipid Tikus Wistar Jantan Hiperkolesterolemia. *JPA*. **4**, 127–136 (2016).
49. Zewinger, S., Speer, T., Kleber, M. E., Scharnagl, H., Woitas, R., Lepper, P. M. & Fliser, D. HDL Cholesterol Is Not Associated with Lower Mortality in Patients with Kidney Dysfunction. *J. Am. Soc. Nephrol.* **25**, 1073–1082 (2014).
50. Bumrungpert, A., Chongsuwat, R., Phosat, C. & Butacnum, A. Rice Bran Oil Containing Gamma-Oryzanol Improves Lipid Profiles and Antioxidant Status in Hyperlipidemic Subjects: A Randomized Double-Blind Controlled Trial. *J. Altern. Complement. Med.* **25**, 353–358 (2019).
51. Nurhuda, A. Pengaruh Pemberian Ekstrak Etanol Daun Andong (*Cordyline fruticosa* (L.) A Chev) terhadap Kadar Kolesterol Total dan Trigliserida Darah Tikus Putih. (Universitas Brawijaya Malang, 2019).
52. Amrizal, S. N., Zakaria, F. R., Chasanah, E. & Suliantari, S. Intervensi Tahu Ungu Mampu Memperbaiki Profil Lipid Darah Subjek Penderita Diabetes Melitus Tipe-2. *J. Gizi dan Pangan* **12**, 225–230 (2017).
53. Al-Ishaq, R. K., Abotaleb, M., Kubatka, P., Kajo, K. & Büsselberg, D. Flavonoids and Their Anti-Diabetic Effects: Cellular Mechanisms and Effects to Improve Blood Sugar Levels. *Biomolecules* **9**, (2019).
54. Triana, E., Yulinery, T. & Nurhidayat, N. Analisis Citrinin, Lovastatin dan Pigmen pada Angkak Hasil Fermentasi Beras IR 42 dengan *Monascus Purpureus* Hasil Mutagenesis Etidium Bromida. in *Prosiding Seminar Nasional Penelitian dan PKM: Sains, Teknologi dan Ilmu Kesehatan* **4**, 459–466 (2014).
55. Prawitasari, D. S. Diabetes Melitus dan Antioksidan. *KELUWIH J. Kesehat. dan Kedokt.* **1**, 48–1. (2019).
56. Anggraeni, S., Setyaningrum, T. & Listiawan, Y. Perbedaan Kadar Malondialdehid (MDA) sebagai Petanda Stres Oksidatif pada Berbagai Derajat Akne Vulgaris. *Berk. Ilmu Kesehat. Kulit dan Kelamin – Period. Dermatology Venereol.* **29**, 36–43 (2017).
57. Winarti, G. R. Pengaruh Pemberian Ekstrak Daun Sukun (*Artocarpus altilis* F.) Terhadap Penurunan Kadar Malondialdehid (MDA) Pada Tikus (*Rattus norvegicus*) Model Diabetes Melitus. (Universitas Sebelas Maret Surakarta, 2013).
58. Li, D., Zhang, Y., Liu, Y., Sun, R. & Xia, M. Purified Anthocyanin Supplementation Reduces Dyslipidemia, Enhances Antioxidant Capacity, and Prevents Insulin Resistance in Diabetic Patients. *J. Nutr.* **145**, 742–748 (2015).
59. Anggun, R. P. Pengaruh Pemberian Yoghurt Susu Kambing dengan Fortifikasi Tepung Bekatul Beras Merah terhadap Kadar Glukosa Darah dan Trigliserida pada Tikus Wistar Model Diabetes Melitus Induksi Streptozotocin. (Universitas Brawijaya, 2019).
60. Luh, R. N., Komang, A. & Wiwik, S. R. Efek Ekstrak Etanol Biji Jagung (*Zea Mays*) Terhadap Profil Lipid Tikus Wistar Dengan Diet Tinggi Lemak. *J. Kim.* **2**, 151–156 (2017).
61. Diah, K., Hapsari, A. T., Sulistyoningrum, E. & Prastowo, A. Pengaruh Propolis Terhadap Profil Lipid Plasma Tikus Model Hiperkolesterolemia. *J. Gizi Klin. Indones.* **8**, 106 (2012).
62. Ghorbani, A. Mechanisms of Antidiabetic Effects of Flavonoid Rutin. *Biomed. Pharmacother.* **96**, 305–312 (2017).