

## Effect of *arum manis* mango peel extract on cholesterol and triglyceride levels in dyslipidemic Sprague-Dawley rats

Taufik Saputra, Haidar Satya Naufal, Astika Widy Utomo, Nyoman Suci Widyastiti, Muhammad Farhan Kurniawan, Arfianty Nur Azizah



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### Authors' affiliations:

Faculty of Medicine, Universitas  
 Diponegoro, Semarang, Indonesia

### Corresponding author:

Taufik Saputra  
 Faculty of Medicine, Universitas  
 Diponegoro, Jalan Prof. Sudarto No.  
 13, Tembalang, Tembalang, Semarang  
 50275, Central Java, Indonesia  
 Tel/Fax: +62-24-76928010/  
 +62-24-76928011  
 E-mail: [taufiksaputra99@gmail.com](mailto:taufiksaputra99@gmail.com)

### ABSTRACT

**BACKGROUND** Dyslipidemia is characterized by an increase in low-density lipoprotein (LDL) and triglyceride (TG) levels and a decrease in high-density lipoprotein (HDL). Cholestyramine as an antidyslipidemia has several side effects, so an alternative is needed. Pectin is a natural substance with a mechanism of action similar to that of cholestyramine. Mango peel is one of the sources of pectin, containing 10–15% of this substance. This study aimed to prove the effect of *arum manis* mango (*Mangifera indica* L.) peel extract on LDL, HDL, and TG levels in dyslipidemic Sprague-Dawley rats.

**METHODS** 25 Sprague-Dawley rats were divided into 5 groups. All groups were given high-fat diet for the first 18 days, followed by standard feed (negative control group), cholestyramine (Sequest®) 80 mg/200 g body weight (standard treatment group), and mango peel extract (M-90 [90 mg/day], M-180 [180 mg/day], and M-360 [360 mg/day] groups) for the next 15 days. LDL and HDL levels were analyzed using the cholesterol oxidase-phenyl aminopyrazolone method and TG level using the glycerol-3-phosphate-oxidase-phenol-aminophenazone method.

**RESULTS** The M-360 group reduced the LDL level ( $p = 0.015$ ), while the standard treatment group increased the HDL level ( $p = 0.042$ ). Although significant TG level changes were found in the negative control, standard treatment, and M-360 groups ( $p = 0.042$ ), the mean differences of LDL, HDL, and TG levels between groups were not significantly different ( $p = 0.245, 0.328, \text{ and } 0.454$ , respectively).

**CONCLUSIONS** *M. indica* peel extract reduced LDL and TG levels at 360 mg/day.

**KEYWORDS** cholestyramine, high-density lipoprotein, low-density lipoprotein, mango, pectin, triglyceride

Dyslipidemia is a lipid metabolism disorder characterized by an increase in total cholesterol, low-density lipoprotein (LDL) and triglyceride (TG) levels and a decrease in high-density lipoprotein (HDL) level.<sup>1</sup> The management of dyslipidemia is mainly through lifestyle modification by adopting a low-fat diet and regular physical activity. If non-medical treatment is deemed inadequate, antidyslipidemic medicines, such as cholestyramine, which acts as a bile acid sequestrant, are used.<sup>2,3</sup> However, this medication has several adverse effects, including bloating and

constipation. An alternative therapy with a similar mechanism of action is needed.<sup>4</sup> Pectin has been reported to effectively decrease cholesterol levels.<sup>5</sup>

Mango peel is a potential pectin source, comprising approximately 10–15% pectin.<sup>6</sup> Thicker peels tend to have a higher pectin level.<sup>7</sup> While mango pulp is commonly consumed, mango peel is often discarded and remains underutilized. Previous studies regarding orange, apple, and guava peel extracts have been conducted, although no research regarding the effect of pectin in *arum manis* mango (*Mangifera indica* L.)

peel extract on LDL, HDL, and TG levels has been reported. This study determined the effects of *M. indica* peel extract on LDL, HDL, and TG levels in dyslipidemic Sprague-Dawley rats.

## METHODS

### Design and subject

This study used a pre- and post-test randomized controlled group design. Twenty-five healthy Sprague-Dawley male white rats (age: 12–16 months; weight: 200–350 g) were obtained from the Laboratory of Experimental Animals at the Faculty of Medicine, Universitas Diponegoro. This study was conducted from September to November 2020. The dependent variables were LDL, HDL, and TG levels of the dyslipidemic Sprague-Dawley rats, and the independent variable was *M. indica* peel extract at stratified doses of 90, 180, and 360 mg/day.

### Experimental design

One week prior to treatment, the rats were provided standard rat feed and water *ad libitum*. Twenty-five rats were randomly assigned to five groups that were fed a high-fat diet for 18 days, followed by standard feed (negative control group), cholestyramine (Sequest®, Novell Pharmaceutical Laboratories, Indonesia; 80 mg/200 g body weight [standard treatment group]), or mango peel extract (M-90 [90 mg/day], M-180 [180 mg/day], and M-360 [360 mg/day] groups) for the subsequent 15 days. The treatment was administered via oral gavage.

### Mango peel extract production

The mango peel extract (Baki Fruit Garden, Sukoharjo, Indonesia) was made by drying, washing, mashing, sieving, and adding 500 ml of citric acid to the mango peel. Next, the mango peel flour was extracted at 90–95°C for 180 min, according to a previous study.<sup>8</sup> After the extraction, the filtrate was cooled and precipitated for 24 hours with 96% ethanol. The precipitate was separated from the solution and placed in an oven at 50°C for 24 hours. The mango peel extract powder was dissolved in aquadest (Agarindo Biological Company, Indonesia) for oral administration to the rats. This method produced a galacturonic content of 74.16%, surpassing the threshold of 35%. The galacturonic content measured the purity of the obtained pectin. Higher galacturonic content indicated higher quality and purity.<sup>9</sup>

### Biochemical assays

Blood was collected from the rats at two timepoints: on Day 18 after the induction of a high-fat diet and on Day 33 after treatment. The amount of blood collected was 1% of the body weight of each rat. The blood was obtained from the retro-orbital plexus of the rats. The LDL and HDL levels were measured using the cholesterol oxidase-phenyl aminopyrazolone method. The TG level was measured using the glycerol-3-phosphate-oxidase-phenol-aminophenazone method.<sup>9</sup>

### Statistical analysis

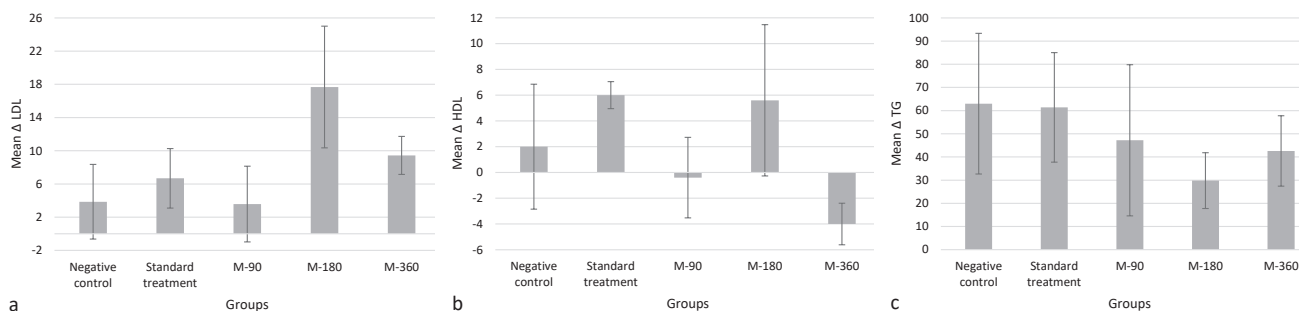
The LDL, HDL, and TG levels at different timepoints were compared using the paired *t*-tests for normally distributed data and the Wilcoxon test for data with abnormal distribution. A one-way analysis of variance test was used to compare the mean difference of LDL, HDL, and TG levels before and after treatment ( $\Delta$ LDL,  $\Delta$ HDL, and  $\Delta$ TG) between the groups. When the data had an abnormal distribution, the Kruskal–Wallis test was used. The SPSS software version 26 (IBM Corp., USA) was used to analyze the data, with a cut-off point of  $p < 0.05$ .

### Ethics

This study was approved by the Health Research Ethics Commission, Faculty of Medicine, Universitas Diponegoro (approval numbers 70/EC/H/FK-UNDIP/VII/2020 and 75/EC/H/FK-UNDIP/VII/2020). All experimental animals were treated according to the Declaration of Helsinki. After treatment, the rats were anesthetized using ether and sacrificed by dislocating the atlanto-occipital joint.

## RESULTS

The LDL level was decreased after treatments in all groups, though the difference was significant only in the M-360 group (from 34.32 mg/dl to 24.88 mg/dl,  $p = 0.015$ ) (Figure 1a). The leveled dose of mango extract did not cause a significant change in the LDL, HDL, and TG levels between the groups ( $p = 0.245, 0.102, \text{ and } 0.454$ , respectively). The HDL level was increased in the negative control, standard treatment, and M-180 groups after treatments, though the increase was only significant in the standard treatment group (from 35.20 mg/dl to 41.20 mg/dl,  $p = 0.042$ ) (Figure 1b). The TG level was decreased after treatments in all groups,



**Figure 1.** Changes in cholesterol levels after 15 days of treatments. Value are expressed in terms of mean difference ( $\Delta$ ) in LDL (a), HDL (b), and TG (c) levels. Standard treatment was given 80 mg/200 gBW cholestyramine (Sequest®). The treatments were administered for the subsequent 15 days. M-90=mango peel extract at 90 mg/day; M-180=mango peel extract at 180 mg/day; M-360=mango peel extract at 360 mg/day. One-way analysis of variance:  $p = 0.015$  for LDL changes,  $p = 0.042$  for HDL changes; Kruskal–Walis test:  $p = 0.043$  for TG changes

with a significant reduction in the negative control (from 106.80 mg/dl to 43.80 mg/dl,  $p = 0.043$ ), standard treatment (from 105.60 mg/dl to 44.20 mg/dl,  $p = 0.043$ ), and M-360 groups (from 92.80 mg/dl to 45.00 mg/dl,  $p = 0.043$ ) (Figure 1c).

## DISCUSSION

In this study, the LDL level was increased in rats fed a high-fat diet, indicating that the acclimatization and high-fat diet for the first 18 days were successful, where the level of normal LDL cholesterol in rats was 2–27 mg/dl. The LDL level after treatment was significantly decreased only in the M-360 group. This may be due to the suboptimal feeding method using tube feeding.

Mango peel extract is difficult to dissolve in room-temperature water, often leading to clogs in the feeding tube, which may affect the dose of mango peel extract ultimately administered to the rat. In addition, the decrease indicated the presence of pectin in mango peels. Pectin can bind bile acids, which are the final products of cholesterol metabolism, resulting in increased excretion of bile acids. Pectin is a water-soluble fiber that can form a thick layer on the intestinal wall, inhibiting the reabsorption of bile acids and cholesterol in the intestine. The subsequent conversion of cholesterol to bile acids increases, decreasing free cholesterol in the liver. To compensate for cholesterol levels in the liver, LDL receptors in the liver increase LDL uptake, resulting in a decrease in serum LDL concentration.<sup>10,11</sup>

In the standard treatment group, the LDL level did not change significantly after administering cholestyramine. This may be due to an insufficient

dose of cholestyramine. The cholestyramine dose used in this study was obtained by converting the human dose to the rat dose. Increasing the dose may increase the effectiveness of cholestyramine for reducing LDL level.<sup>12</sup>

The HDL level is typically >35 mg/dl in rats.<sup>11</sup> In a recent study, a high-fat diet reduced the HDL level in rats.<sup>13</sup> In the current study, the HDL level was <35 mg/dl in all rats fed a high-fat diet except for the standard treatment and M-360 groups. This may be because the HDL level in the M-360 group was higher than those in the other groups prior to the high-fat diet. However, this could not be confirmed as the HDL level was not assessed before the administration of the high-fat diet. In this study, the HDL level was not significantly increased after the administration of mango peel extract. These results correspond with those of previous studies regarding the effect of pectin from guava on cholesterol levels<sup>14</sup> and may be due to the cholesterol-lowering mechanisms of pectin, which increases cholesterol excretion.<sup>10</sup>

In this study, the rat TG levels remained normal (26–145 mg/dl) after the high-fat and standard diets. This may be because the duration of the high-fat diet was relatively short. A previous study reported an increased TG level after 22 weeks of a high-fat diet.<sup>15</sup> In addition, the rats were fed through a feeding tube, which was not optimal. The mixture of pork oil and egg yolk often clogs the feeding tube. However, the TG levels significantly decreased in the standard treatment, negative control, and M-360 groups, indicating that the mango peel extract can significantly reduce TG level. This finding is in accordance with those of previous studies that tested the effects of

pectin from guava, orange, peanut, and pomegranate on TG level in adult male Sprague-Dawley rats.<sup>16,17</sup>

The leveled dose of mango extract was not significantly effective in lowering TG levels between the groups in this study, suggesting that administering various doses of mango peel extract and cholestyramine did not lead to significant changes in TG level. This result contradicts those of previous studies and may be attributed to the natural fluctuation of TG level or the influence of several factors, including stress. Rats tend to experience stress when fed via oral gavage, leading to increased cortisol and blood sugar levels, and prolonged stress may lead to increased TG level.<sup>18</sup> In addition, pectin, as a bile acid sequestrant, can bind to and increase the excretion of bile acids in the feces, decreasing the availability of bile acids in the intestine and preventing cholesterol absorption. Decreased cholesterol absorption in the intestine results in decreased TG synthesis.<sup>8</sup>

This study had several limitations. First, it was conducted only in rats. The high-fat diet was only administered for 18 days before the administration of the drug and mango peel extract; therefore, it was unclear whether the treatment effects were due to the drug and extract or due to the cessation of the high-fat diet. Therefore, a high-fat diet should be administered throughout the study period, and various doses of medication and extract are needed to determine the optimal benefits of the pectin contained in the peel extract in future studies. In addition, mango peel extract requires special preparation for its dissolution in room-temperature water. The pectin levels, pharmacodynamics, pharmacokinetics, therapeutic doses, therapeutic windows, and adverse effects of the mango peel extract must be determined.

In conclusion, the administration of 360 mg/day of *M. indica* peel extract reduced the LDL and TG levels in dyslipidemic Sprague-Dawley rats. However, the HDL level did not change, and the mean LDL, HDL, and TG levels were not significantly different after mango peel treatment.

#### Conflict of Interest

The authors affirm no conflict of interest in this study.

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## REFERENCES

- Shenoy C, Shenoy MM, Rao GK. Dyslipidemia in dermatological disorders. *N Am J Med Sci*. 2015;7(10):421–8.
- Gitawati R, Widowati L, Suharyanto F. [The use of jamu in patients with hyperlipidemia based on data from the medical record, in some health care facilities, in Indonesia]. *Jurnal Kefarmasian Indonesia*. 2015;5(1):41–8. Indonesian.
- Nishida S, Horinouchi A, Higashimura Y, Akahori R, Matsumoto K. Cholestyramine, a bile acid sequestrant, increases cecal short chain fatty acids and intestinal immunoglobulin a in mice. *Biol Pharm Bull*. 2020;43(3):565–8.
- Alexander C, Guard BC, Suchodolski JS, Swanson KS. Cholestyramine decreases apparent total tract macronutrient digestibility and alters fecal characteristics and metabolites of healthy adult dogs. *J Anim Sci*. 2019;97(3):1020–6.
- Chen Y, Xu C, Huang R, Song J, Li D, Xia M. Butyrate from pectin fermentation inhibits intestinal cholesterol absorption and attenuates atherosclerosis in apolipoprotein E-deficient mice. *J Nutr Biochem*. 2018;56:175–82.
- Minzanova ST, Mironov VF, Arkhipova DM, Khabibullina AV, Mironova LG, Zakirova YM, et al. Biological activity and pharmacological application of pectic polysaccharides: a review. *Polymers (Basel)*. 2018;10(12):1407.
- Suleria HA, Barrow C. Bioactive compounds from plant origin: extraction, applications, and potential health benefits. 1st ed. New York: Apple Academic Press; 2019.
- Nurman Z, Masrul M, Sastri S. [Effect of apple (*Malus sylvestris* Mill) pectin on LDL cholesterol levels in hypercholesterolemia male white rats (*Rattus Novergicus*)]. *Jurnal Kesehatan Andalas*. 2017;6(3):679–84. Indonesian.
- Nurmila, Nurhaeni, Ridhay A. [Extraction and characterization of pectin from *Harumanis* mango peels with various temperatures and times]. *KOVALEN: Jurnal Riset Kimia*. 2019;5(1):58–67. Indonesian.
- Hu W, Cassard AM, Ciocan D. Pectin in metabolic liver disease. *Nutrients*. 2022;15(1):157.
- Indra LP, Tjahjono K, Setyawati A. [The effect of mangosteen pericarp extract (*Garcinia mangostana* L.) and simvastatin on LDL cholesterol level in Sprague-Dawley rats serum with high lipid diets]. *Jurnal Media Medika Muda*. 2014. Indonesian.
- Heriansyah T. [The effect of various durations of high-fat diet administration on the lipid profile of male white rats (*Rattus novergicus wistar strain*)]. *Jurnal Kedokteran Syiah Kuala*. 2013;13(3):144–50. Indonesian.
- Nurhidajah, Astuti R, Nurrahman. Black rice potential in HDL and LDL profile in Sprague Dawley rat with high cholesterol diet. *IOP Conf Ser: Earth Environ Sci*. 2019;292:012019.
- Tsalissavrina I, Wahono D, Handayani D. [The influence of high-carbohydrate diet administration in comparison with high-fat diet toward triglyceride and HDL level in blood on *Rattus novergicus strain wistar*]. *Junal Kedokteran Brawijaya*. 2006;22(2):80–9. Indonesian.
- Nurmawati T. [The correlation of weight and blood cholesterol levels of white rat (*Rattus Norvegicus*) with high-fat diet]. *Jurnal Ners dan Kebidanan*. 2016;3(3):202–6. Indonesian.
- Vigne JL, Lairon D, Borel P, Portugal H, Pauli AM, Hauton JC, et al. Effect of pectin, wheat bran and cellulose on serum lipids and lipoproteins in rats fed on a low- or high-fat diet. *Br J Nutr*. 1987;58(3):405–13.
- Elhardallou SB, Babiker WA, Sulieman AM, Gobouri AA. Effect of diet supplementation with food industry by-products on diabetic rats. *Food Nutr Sci*. 2015;06(10):875–82.
- Garbarino S, Magnavita N. Work stress and metabolic syndrome in police officers. A prospective study. *PLoS One*. 2015;10(12):e0144318.