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Research Article

The Hypolipidemic Effect of Mountain Papaya and Bitter Melon Fruit Ethanolic Extract in Diabetic Rats

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ABSTRACT

Traditional medicine has been developed rapidly throughout the world to treat hyperlipidemia. However, the use of a single compound in hyperlipidemia treatment usually have low efficacy. Therefore, a combination of ingredients is bound to have more synergistic impact in therapy. This research aimed to examine the hypolipidemic potential of mountain papaya (MPE) and bitter melon fruit ethanolic extract (BME) in alloxan-induced rats. Forty rats divided into eight groups were used in this study. Groups are divided into normal control, negative control, positive control, as well as MPE and BME groups which divided into single doses and three combination doses. Induction of 150 mg/kg alloxan intraperitoneally were performed to generate a model of diabetes and hyperlipidemia. The treatment was carried out for four weeks of the experiment. The single and combination doses of both extracts sufficiently exhibited hypolipidemic activity ($p < 0.05$). The levels of lipid profiles total such as cholesterol, triacylglycerides, low-density lipoprotein, high-density lipoprotein, and very high-density lipoprotein were decreased after MPE and BME administration ($p < 0.05$). The combination of MPE and BME also has hypolipidemic action equivalent to simvastatin. The single and combined doses of mountain papaya, as well as bitter melon fruit ethanolic extracts, have the potential to improve the biochemical (lipid profile) modifications of alloxan-induced.

INTRODUCTION

Diabetes mellitus is a metabolic disorder identified by hyperglycemia because of deficiencies in insulin secretion and/or insulin activity (Laela et al. 2021). In 2017, the International Diabetes Federation (IDF) assessed the number of diabetes cases reached 425 million and predicted that this value would be rise to 629 million by 2045 (Cho et al. 2018). According to numerous studies, diabetes mellitus tends to increase lipid levels, blood pressure, and leads to other complications (Alwardat et al. 2018; Susanti et al. 2018). Dyslipidemia is identified by elevated total blood cholesterol levels, low-density lipoprotein cholesterol, and decreased levels of high-density lipoprotein cholesterol. The condition of hyperlipidemia is the primary cause of cardiovascular disorders

(Duraipandiyan et al. 2016). Numerous studies on diabetic therapy showed that controlling lipid levels help to prevent cardiovascular system complications (Donate-Correa et al. 2020).

In hyperlipidemia therapy, several synthetic drugs, including statin groups and fibrates, have been used. However, these drugs have been discovered to have several side effects like myopathy, hepatic dysfuction, rhabdomyolysis, and peripheral neuropathy (Ramkumar et al. 2016). Therefore, there is a challenge to discover non-detrimental alternatives for the theraphy. Indonesia is abundant of prospective plants especially medicinal plants (Susanti et al. 2018). These plants are used as sources of active secondary metabolites use for complementary or alternative treatments (Kamel et al. 2017). Several advancements in oral hyperlipidemia therapy from natural metabolites have been made. However, the use of a single compound in hyperlipidemia treatment is usually has low efficacy. Therefore, a combination of ingredients is bound to have a more synergistic impact in therapy (Sasongko et al. 2020).

Plants with flavonoid have hypolipidemic activity related to inhibition of HMG-CoA reductase and hence mevalonate production (Ziaee et al. 2009; Ling et al. 2020). Mountain papaya (*Vasconcellea pubescens*) and bitter melon (*Momordica charantia*) are two Indonesian plants known to exhibit this activity (Elangovan et al. 2019; Gao et al. 2019).

According to previous study, ten chemicals were detected in the fruits and active fractions of mountain papaya, tentatively identified as hydroxycinnamic acid glycosides and nine as quercetin glycoside derivatives (Simirgiotis et al. 2009). Furthermore, the fruit's extract contains additional metabolites, including tannins, triterpenoids, as well as polyphenols, and these compounds exhibit anti-diabetic properties (Sasongko et al. 2020; Sasongko et al. 2021). Meanwhile, bitter melon is known to contain phytochemicals including proteins, polysaccharides, flavonoids, triterpenes, saponins, ascorbic acid, and steroids (Jia et al. 2017). Several studies shown this fruit has hypoglycemic and hypolipidemic properties. Moreover, the potential mechanism of this action is possibly through induction of insulin release from the remaining pancreatic beta cells (Mahwish et al. 2017; Raish 2017). This study, therefore, purposed to investigate the hypolipidemic efficacy of a mixture of mountain papaya and bitter melon fruit ethanolic extracts.

MATERIALS AND METHODS

Materials

Mountain papaya and bitter melon were collected from Dieng, Wonosobo, and traditional market at Surakarta, Central Java, Indonesia respectively. Wistar rats (*Rattus norvegicus*) were obtained from Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia. The fruits were subjected to taxonomic identification at the Faculty of Mathematics and Sciences, Universitas Sebelas Maret, Indonesia (No. 207/UN27.9.6.4/Lab/2019).

Fruit Extract Preparation

The fresh fruit samples were washed under running water, sliced into small pieces, and oven-dried separately at 50 °C. This was followed by pulverization using a mechanical grinder and extraction using the maceration method in 70% ethanol (1:5) for 3 x 24 hours (Sugiyarto et al. 2018). The extract's yield was then evaporated by a rotary evaporator at a temperature below 60 °C until thick extracts were obtained from the mountain papaya (MPE) and bitter melon (BME).

Phytochemical Screening

Flavonoid

One gram of the extract was added to each separate reaction tube. Then, 3 drops of HCl 2N, a few milligrams of magnesium powder, and 1 milliliter of amyl alcohol were added to each test tube, and they were agitated until homogenous. The reaction is positive if a yellow to crimson solution is produced (Santoso et al. 2018).

Saponin

One gram of extract was added to each separate reaction tube. Then added aquadest in each tube and shaken strongly. The presence of saponins is shown by the foam formation (Weli et al. 2018).

Alkaloid

One gram of extract was added to each separate reaction tube. This test was carried out using four reaction tubes, as well as five drops of chloroform to each tube. Tube 1 served as a control, while a drop or two Mayer's reagent, Dragendorff's reagent, and Wagner's reagent were added to tubes 2, 3, and 4, respectively. The formation of white, orange, and brown precipitates indicated a positive result for tubes 2, 3, and 4, respectively (Santoso et al. 2018).

Tannin

This test was performed by filling 2 separate reaction tubes with 1 gram of MPE and BME, then added 2 to 3 drops of 1% $FeCl_3$, as well as 1% gelatin solution to each tube. The formation of a white precipitate shows the presence of tannins (Santoso et al. 2018).

Phenolic compounds

This test was performed by filling 2 separate reaction tubes with 1 gram of MPE and BME, then adding 2 to 3 drops of 1% $FeCl_3$ to each tube. The formation of a black precipitate shows the presence of phenolic compounds (Santoso et al. 2018).

Animal Preparation

This study received approval for all animal handling protocols from the Ethics Committee of Moewardi Hospital, Surakarta, Indonesia (No: 1046/III/

HREC/2019). Male Wistar rats (aged 10–12 weeks) weighing 150 to 180 grams were obtained from the Integrated Laboratory, Universitas Sebelas Maret Surakarta, Indonesia, and were fed with corn seed, as well as water ad libitum, and allowed to be acclimatized to the laboratory conditions for a week before the experiment.

In Vivo Experimental

Hyperglicemia induction in the rats was done using 150 mg/kg intraperitoneally alloxan monohydrate (Mourya et al. 2017). Alloxan is a toxic glucose analog that can be used for rapid induction of diabetes with hyperlipidemia as the side effect (Erejuwa et al. 2016). Forty rats were divided into eight groups: three control groups and five test groups. The normal control group (I) was not given any treatment, the positive control group (II) was orally administered 0.9 mg/kg of simvastatin suspension, and the negative control group III was orally administered a suspension of 0.25% sodium carboxymethyl cellulose (CMC-Na). Meanwhile, the test groups were orally administered a combination of MPE-BME at the ratios of 50% : 50% (IV), 25% : 75% (V), and 75% : 25% (VI), as well as 100% MPE (VII), and 100% BME (VIII) at the dosage of 173.900 mg/kg, and 378.170 mg/kg, respectively. The extract dosage was based on the total flavonoid activity (quercetin equivalent), 121.334 ± 3.404 mg/100 g ethanol extract, and 55.795 ± 1.601 mg /100 g ethanol extract, for MPE and BME, respectively (Sasongko et al. 2020). Furthermore, the lipid profile measurements were performed 28 days after the treatment, using the Biochemistry Semi Analyzer by Biosystem (BTS350) at a wavelength of 500 nm.

Determination of Lipid Profile

The rat was anesthetized with ketamine hydrochloride (50 mg/kg BW) intraperitoneally. The 2 mL of the blood was collected with cardiac puncture method. The blood was centrifuged for 10 minutes at 4000 rpm and 4 °C to separate and collect the serum for biochemical study. Subsequently, the lipid profile, comprising the total cholesterol (TC), triacylglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), very high-density lipoprotein (VLDL), atherogenic index (AI), atherogenic coefficient (AC), and cardiovascular risk index (CRI), was determined using the Biosystems instrument commercial kits (Biosystems, Spain). Meanwhile, the atherogenic index, atherogenic coefficient, and cardiovascular risk index were calculated using the following formulae, respectively (Kinosian et al. 1994; Azizian et al. 2018):

Atherogenic Index (AI) = (Total Cholesterol – HDL Cholesterol) / HDL Cholesterol

Atherogenic Coefficient (AC) = LDL Cholesterol / HDL Cholesterol

Cardiovascular Risk Index (CRI) = Total Cholesterol / HDL Cholesterol

Statistical Analysis

The data were subjected to a one-way analysis of variance (ANOVA), followed by a Tukey HSD post hoc test to analyze the significant differences between groups at $p < 0.05$. Subsequently, all data were proved as mean ± standard error of the mean (SEM).

RESULTS AND DISCUSSION

Results

Phytochemical qualitative analysis

The results of this study showed a slightly lower percentage yield of 21.1% for the BME, compared to the MPE of 26.43% (Table 1). This was calculated using a 100% weight comparison between extract and dried fruit. Meanwhile, the qualitative phytochemical analysis of MPE-BME indicated the presence of alkaloids, flavonoids, saponin, polyphenols, and tannins. There have been numerous previous studies that demonstrated qualitatively (Sugiyarto et al. 2018).

Table 1. The percentage yields of MPE and BME extraction.

Extract sample	Dried fruit (gram)	Extract (gram)	Yield (%)
MPE	454	119.97	26.43
BME	479	100.62	21.01

Note: MPE = mountain papaya extract, BME = bitter melon extract.

The effect of MPE and BME treatment on body weight

Figure 1 shows the administrative effect of MPE and BME on body weight profile. Based on the statistical analysis at the 28th day, all the dose of MPE and BME treatments significantly influenced the body weight of alloxan-induced diabetic rats besides MPE: BME (75% : 25%) compared with negative control. Statistically, intraperitoneal administration of a single alloxan dose led to a significant ($p < 0.05$) reduction of the rats' body weight, com-

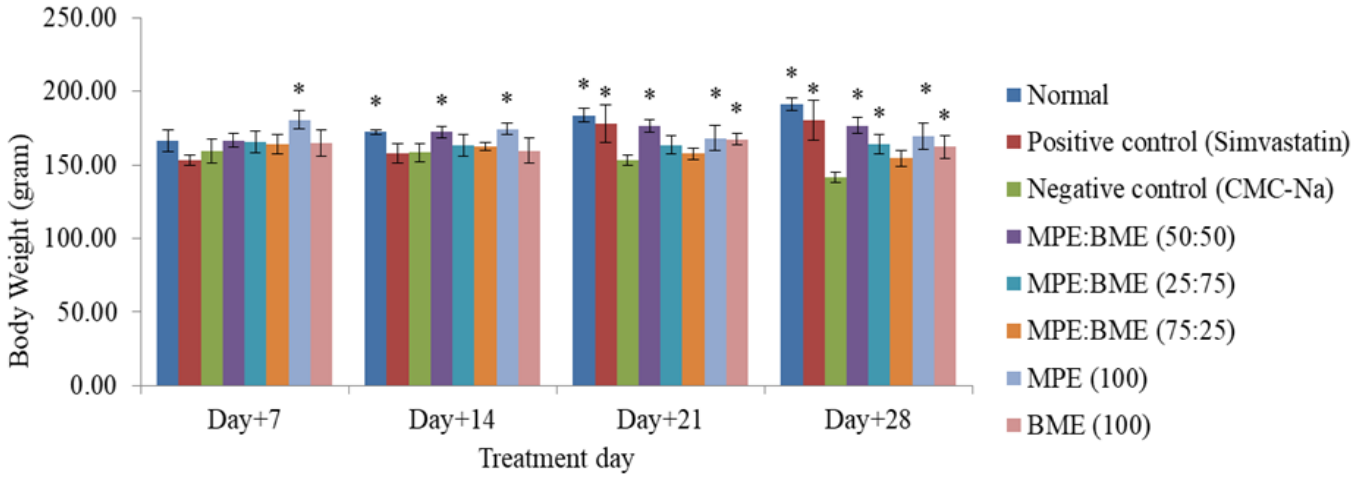

Figure 1. The effect administration of MPE and BME on body weight profile. The data are represented as means ± SEM (n = 5). *$p < 0.05$ compared with the negative control group. MPE = mountain papaya and BME = bitter melon extract.

Table 2. The ability of MPE and BME to improve lipid profiles in diabetic rats after 28 days of testing.

Groups	Lipid profiles							
	TC ± SEM (mg/dL)	TG ± SEM (mg/dL)	HDL ± SEM (mg/dL)	LDL ± SEM (mg/dL)	VLDL ± SEM (mg/dL)	AI ± SEM	AC ± SEM	CRI ± SEM
Normal	90.137 ± 5.17*	89.93 ± 0.9*	30.5 ± 0.2*	28.57 ± 2.3*	31.067 ± 1.86*	1.95 ± 0.12*	0.936 ± 0.21*	2.955 ± 0.18*
Negative control (CMC-Na)	255.467 ± 3.37	223.51 ± 3.8	23.64 ± 0.3	210.2 ± 3.9	21.627 ± 1.3	9.81 ± 0.59	8.892 ± 0.53	10.806 ± 0.65
Positive control (Simvastatin)	98. 379 ± 3.72*	108.13 ± 6.2*	30.25 ± 0.3*	50.14 ± 3.8*	17.989 ± 1.08*	2.25 ± 0.14*	1.657 ± 0.12*	3.252 ± 0.2*
MPE (100)	99.746 ± 4.93*	99.28 ± 3.3*	30.17 ± 0.55*	53.73 ± 2.1*	15.846 ± 0.95*	2.31 ± 0.14*	1.781 ± 0.18*	3.306 ± 0.18*
BME (100)	98.69 ± 3.04*	94.14 ± 4.6*	30.36 ± 0.51*	49.5 ± 2.4*	18.83 ± 1.13*	2.25 ± 0.15*	1.63 ± 0.14*	3.250 ± 0.21*
MPE:BME (50:50)	100.703 ± 1.88*	106.91 ± 5.7*	31.31 ± 0.31*	48.01 ±0.8* #	21.383 ± 1.28	2.216 ± 0.13*	1.533 ± 0.13*	3.216 ± 0.19*
MPE:BME (25:75)	99.043 ± 1.44*	106.25 ± 6.1*	30.85 ± 0.36*	46.94 ± 1.9* #	21.253 ± 1.28	2.21 ± 0.13*	1.522 ± 0.14*	3.210 ± 0.22*
MPE:BME (75:25)	102.042 ± 4.8*	101.72 ± 9.5*	30.11 ± 0.21*	51.59 ± 3.9*	20.342 ± 1.22	2.38 ± 0.14*	1.713 ± 0.11*	3.388 ± 0.25*

Note: The data are represented as means ± SEM (n=5). *$p < 0.05$ compared with the negative control group; # $p < 0.05$ compared with single extract group . MPE = mountain papaya extract; BME = bitter melon extract; TC = total cholesterol; TG = triglycerides; HDL = high - density lipoprotein; LDL = low - density lipoprotein; VLDL = very high - density lipoprotein; AI = atherogenic index; AC = atherogenic coefficient; CRI = cardiovascular risk index.

pared to the normal control. Meanwhile, in each week of observations, it was shown that there were several groups that decreased and increased in terms of body weight, especially in the normal group and simvastatin positive control group.

The effect of MPE and BME treatments on lipid profile of diabetic rats

Table 2 showed the rats' lipid profiles after oral administration of MPE and BME. The hyperlipidemia group (negative control) has a significantly higher ($p < 0.05$) difference in serum lipids after 28 days, compared to the normal group. Furthermore, the treatment with 0.9 mg/kg simvastatin caused a significant decrease in the hyperlipidemic profile of the diabetic rats ($p < 0.05$). According to Table 2, all the treatment groups exhibited hypolipidemia ($p < 0.05$). The lipid profile evaluation on the 28th day showed that the single and combined extracts did not differ significantly from the positive control ($p > 0.05$). This showed that the administration of single or combined extracts have similar effect as simvastatin. Therefore, the single and combination doses of MPE and BME have the similar capacity to improve the lipid profiles ($p > 0.05$).

Discussion

In this study, mountain papaya and bitter melon were extracted to evaluate their hypolipidemic effect. These extracts were shown that they contained saponins, flavonoids, alkaloids, and tannins in this research. According to another study, bitter melon contained anthraquinones, glucosinolates (Joseph & Jini 2013), carbohydrates, glycosides, proteins, and amino acids (Shukla & Kashaw 2018; Li et al. 2020). In addition, the phytochemical analysis of mountain papaya ethanolic extract showed the presence of flavonoids, tannins, and phenolic compounds (Simirgiotis et al. 2009; Laily et al. 2012; Sasongko et al. 2018). Figure 1 showed that alloxan induced weight loss in the diabetic rat model. Alloxan caused diabetes in rats by damaging the insulin-secreting cells of the pancreas, leading to hyperglycemia and hyperlipidemia (Shatynska et al. 2020; Atanu et al. 2021). According to the American Diabetes Association (American Diabetes Association 2007), polyphagia and polydipsia, as well as loss in body weight, were significant signs of diabetes mellitus, and these symptoms were exhibited by the diabetic groups in this study. This is possibly associated with structural protein atrophy and muscle wasting (Frier et al. 2008). The diabetic rats group showed a reduction of the body weight each week. However, the hyperlipidemic rats given MPE and BME kept their body weight the same, while the rats given simvastatin gained weight.

Alloxan injection causes diabetes mellitus, which is often found in unstable lipid profile conditions (TG, TC, HDL, LDL, and VLDL). Similar to one previous study, uncontrolled hyperglycemia leaded to hyperlipidemia (Rahimi-Madiseh et al. 2017). Alloxan contributes to insulin secretion loss, resulting in elevated plasma glucose levels, by killing Langerhans islets β-cells

(Roghani & Aghaie 2007). A study by Akbari et al (2013) showed the inappropriate activity of lipolytic hormones on adipose tissues was possibly attributed to alloxan-induced hyperlipidemia. In vivo, fatty acid mobilization from adipose tissue's triglyceride stores is regulated by hormone-sensitive lipase. According to (Cignarelli et al. 2019), insulin regulates the mobilization of lipids in the body from adipose tissue. Therefore, in the absence of insulin, hormone-sensitive lipase is activated, leading to a rise in serum lipid levels (Okazaki et al. 2002).

Generally, lipid disorders are related to diabetes and this leads to cardiovascular conditions, including elevated levels of TC, TG, LDL, and reduced HDL (Elangovan et al. 2019). Similar to the report by Sasongko et al. (2020), alloxan-induced diabetes triggered a considerable rise in serum MDA and a significant decreased in antioxidant enzymes in this study. As a distinctive characteristic of oxidative stress, lipid peroxidation (LPO) plays significant role in the development of diabetes mellitus, disrupting components of the cell membrane, necrosis, inflammation, and serves as a buffer against oxidative stress. Increased TC, TG, LDL, VLDL, and HDL levels, as well as decreasing HDL levels, are typically associated with diabetes, which lead to cardiovascular disease (Elangovan et al. 2019). This explains why untreated diabetic rats (negative control) have higher amounts of TC, TG, LDL, and VLDL but lower levels of HDL. For 28 days, MPE and BME extracts were given and significantly lowered TC, TG, LDL, and VLDL levels while increasing HDL levels. In the MPE and BME combination-treated group of rats, similar effect was seen. Bitter melon, a health-promoting vegetable, is traditionally used for medical nutrition therapy to cure diabetes, but to reap maximum health claims, vigilant control of its substances in the diet is crucial as part of curative action for effective diabetes management. On the other hand, the lipid profile in the negative control group was found to be higher. This might be related to insulin insufficiency in a hyperglycemic condition, which could lead to hormone-sensitive lipase-mediated free fatty acid release from adipose tissue (Goldberg 2001).

CONCLUSION

This study concluded that single and combined doses of mountain papaya, as well as bitter melon fruit ethanolic extracts, have the potential to improve the biochemical (lipid profile) modifications of alloxan-induced. In addition, the MPE and BME combination provided similar effective activity as simvastatin against hypolipidemia. The combination of both extracts had no synergistic effects on most lipid profiles, with the exception of LDL levels, which improved when compared to single extracts. Therefore, the ameliorative role of both extracts towards alloxan-induced hyperlipidemia in the rats' livers is possibly linked to the phenolic compounds present.

AUTHORS CONTRIBUTION

The authors contribution: H.S designed the research, wrote the manuscript

and supervised all the process; R.G.L collected and analyzed data especially for lipid profile; R.D.I conducted the extraction process and phytochemical screening; R.D.W conducted phytochemical screening and analyzed the data; S.M conducted the extraction process, analyzed the data and wrote the manuscript.

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CONFLICT OF INTEREST

All authors declare no conflict of interest.

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