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The effect of mustard greens (Brassica rapa l.) ethanol extract on blood glucose and malondialdehyde levels of hyperglycemic Wistar rats

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ABSTRACT

Purpose: This study aims to determine the effect of mustard greens ethanol extract (*Brassica rapa* L.) in reducing blood glucose and malondialdehyde levels in hyperglycemic Wistar rats.

Methods: The hyperglycemic Wistar rats were divided into 5 groups. Normal groups, positive control group (given glibenclamide) and treatment group were given orally with mustard extract with a dose of dose of 10mg/KgBW, 15 mg/KgBW, and 20 mg/KgBW.

Results and Discussion: The ethanol extract of green mustard with a dose of 10, 15, and 20 mg/KgBW reduced 49.60 mg/dl; 74.00 mg/dl; 101.20 mg/dl blood glucose levels, respectively and 6.31 nmol/ml; 6.43 nmol/ml; 7.14 nmol/ml malondialdehyde levels. The phenol groups contained in the extract was hypothesized to be the key for blood glucose levels reduction by the mechanism of capturing or neutralizing the excess free radical in hyperglycemic Wistar rats.

Conclusion: One Way ANOVA analysis and Post Hoc Study showed that ethyl acetate fraction of mustard greens extract dose of 10, 15, and 20 mg/KgBW were able to significantly reduce blood glucose and malondialdehyde levels in hyperglycemic Wistar rats ($p<0.05$).

Keywords: Hyperglycemia, Mustard greens, Blood glucose, Malondialdehyde, Brassica lapa l.

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INTRODUCTION

Hyperglycemia is a condition where the glucose levels in the blood plasma exceed its normal limits. A fasting serum glucose level of higher than 110 mg/dL or 2-hour postprandial blood glucose of 140 and mg/dl is considered as hyperglycemia.[1] Hyperglycemia when left untreated for years, can cause various complications and death. The state of hyperglycemia is one of the basic diagnoses of diabetes mellitus (DM).[2] Diabetes mellitus is a highly prevalent disease in Indonesia, the latest data in 2015 by the Endocrinology Society stated that the number of diabetic patients in Indonesia had reached 9.1 million people. Until this article was written, Indonesia was the fifth for the highest number of diabetic patients in the world. Since 2000, the number of patients with diabetes mellitus in Indonesia has increased. The World Health Organization (WHO) predicted that by 2030, people with diabetes mellitus would reach 21.3 million people.[3] The latest epidemiology study had put type-2 DM as an epidemic in Indonesia. Nearly 80% of DM is caused by the patient's lifestyle. The lifestyles of the world community, especially in Indonesia, in recent years have shown to be transformed from a traditional and nutritious one to a fast-food lifestyle that is low in nutritional value (junk food). The impact of these unhealthy lifestyles changes is the emergence of various diseases, one of which is DM, which is a disease characterized by hyperglycemia, that caused by abnormalities in insulin secretion or disorders. This condition can increase the reactive oxygen species (ROS) compounds through enzymatic process namely oxidation and phosphorylation reactions (ox-phos) as well as ADPH-oxidase through a non-enzymatic process by forming glucooxidant and glycation.[4]

The state of hyperglycemia and release of excess fatty acids will constitute for triglycerides formation in the liver. The auto-oxidation process in hyperglycemia and glycation results in the release of electrons. This release of electrons triggers the formation of free radicals. Increased production of free radicals produces oxidative stress.[5] Oxidative stress is when the free radicals in the form of reactive molecules are produced through a biochemical reaction of a normal cell that damage the cell membrane and cause various damages to the body. Oxidative stress is one component of the tissue damage mechanism in humans. Oxidative stress can be indicated by the increase in serum and tissue malondialdehyde (MDA). Increased MDA is a marker of an increase in lipid peroxidation reactions. MDA is formed from lipid peroxidation in cell membranes, namely free radical reaction (hydroxyl radical) with polyunsaturated fatty acids (PUFA).[6] Therefore, hyperglycemia will increase

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oxidative stress, which will worsen the health of the patients. Therefore, it is of the utmost importance that antihyperglycemic medications are properly used to treat this condition.

In its treatment, people use traditional medicines more often. (please specify if the treatment refer specifically for DM or for general diseases) The use of traditional medicine by the community is considered to be 'safer' than using synthetic drugs. Experience also proves that not all synthetic drugs are able to overcome various health problems optimally. One of the medicinal plants that can be used as an antidiabetic drug is mustard green. Mustard green (*Brassica rapa L.*) is a species from the Brassicaceae family which play a major role in vegetable production and consumption throughout the world. Mustard greens have been cultivated for centuries throughout Europe which eventually spread to central and eastern Asia.[7] Plant parts such as roots, leaves, and seeds have been used in traditional medicine, generally for the treatment of several diseases such as diabetes.[8]

Based on Mahmudah's research in 2011,[9] mustard greens contain alkaloids, terpenes, tannins, saponins, and glycosides. Alkaloids have been shown to have the ability to regenerate damaged pancreatic β cells. Terpen serves as an antidiabetic because terpenes are the main component of essential oils while saponin functions to increase glucose homeostasis by increasing insulin sensitivity. Corrugated mustard green leaves contain biologically active compounds such as flavonoids including isorhamnetin, kaempferol and quercetin glycosides, phenylpropanoid derivatives, indole alkaloids, and glucoside sterols.[10,11] Several studies have suggested that polyphenols and flavonoids have beneficial effects, especially in diabetes.[12] The use of mustard greens as an anti-diabetic drug is still rare. Generally, people use mustard green to make a home-cooked dish.

There has been no scientific study of the effects of mustard greens ethanol extract to decrease blood glucose levels and reduce oxidative stress which is characterized by a decrease in MDA levels. Therefore, this study will examine the effects of mustard greens ethanol extract (Brassica rapa L.) on blood sugar and malondialdehyde levels in Wistar Hyperglycemia rats.

MATERIALS AND METHODS

Materials

The materials used were mustard greens (Brassica rapa L.) obtained from Sumber Village, Sanankulon District, Blitar Regency. Glibenclamide (BPOM) as the standard anti-diabetes drug, alloxan monohydrate for induction of diabetes in rats, 70% ethanol, FeCl3, Mg powder, concentrated HCl, 1% HCl, Mayer reagent, anhydrous acetic acid, concentrated sulfuric acid, chloroform, male Wistar rats, standard feed, alloxan, ethylenediaminetetraacetic acid (EDTA) solution, 15% trichloroacetic acid (TCA) solution, 0.37% Thiobarbituric acid (TBA) solution in 0.25N HCl, anesthetic, and distilled water.

Equipment

The equipment used in this study include: UV-VIS spectrophotometer, Gas chromatography-mass spectrometry (GC-MS), spatula, blender, glass jar, glass tools, funnel, rotary vacuum evaporator, analytical balance, 100μL micropipette, water bath, centrifugation tool, test tube (tube centrifugation), syringe, EDTA tube, filter paper, aluminum foil, drop pipette, pricking tool, mouse cage, gloves, and mask.

METHODS

Extraction of Mustard Greens

A total of 1000 g of mustard greens powder (Brassica rapa L.) were extracted by maceration using 96% ethanol as solvent until all the powder was immersed in the solvent. Soaking was done for ± 48 hours repeatedly until a clear filtrate is obtained. The clear filtrate was then run with a thin layer chromatography plate to confirm complete extraction. The ethanol extract was filtered and separated from the solvent using a rotary vacuum evaporator until a thick extract was produced which from now on will be called as ethanol extract of thick mustard green (Brassica rapa L.). The thick ethanol extract was fractionated using water, n-hexane, and ethyl acetate. The fractionated products were then evaporated, and dosage form was made for the initial test of the most effective dose to reduce blood glucose levels in hyperglycemic Wistar rats.

Analysis of Ethyl Acetate Fraction of Mustard Green Extract

- Phytochemical screening

Green mustard ethyl acetate fraction added with reagents: Willstatter, FeCl3, Wagner, diluted HCl, and Liberman-Burchard.[13] The color changes that occurred were recorded before and after the addition of color reagents.

- GC-MS analysis

The mustard green extract ethyl acetate fraction was identified using the GC-MS tool at the Police Forensic Laboratory of Denpasar, Bali using the appropriate work parameters. The spectrum

obtained was compared with the spectrum in the Wiley (W10N14.L) / NIST (NIST14.L) database at the Police Forensic Laboratory, Denpasar, Bali.

Laboratory-Animals Treatment

After a one-week adaptation process, the samples were grouped. Based on Federer's formula (1977),[14] the rats were randomly assigned into 5 groups consisting of 5 rats each, which will be divided into two control groups and three treatment groups. For the control group, the Wistar rats were not given any treatment; the positive control group diabetic rats were induced by alloxan and given glibenclamide as the standard anti-diabetes drug. For the three treatment groups, diabetes in each of them was induced by alloxan and ethanol extract of mustard green were administered at a dose of 10.0 mg/KgBW for group I, 15.0 mg/KgBW for group II, and 20.0 mg/KgBW for group III.

Before inducing diabetes, all Wistar rats were fasted for 16-18 hours (drinking water was still given sufficiently) and examined for their blood glucose levels using a blood glucose test. After fasting, the O_3, O_5, O_7, and O_9 groups were made into hyperglycemia by administering a single dose of 125 mg/KgBW alloxan. After injection, rats were fed and given fluids as per usual.

Alloxan was given for 3 days. All Wistar rats were examined for their blood glucose levels, if there was an increase in rat blood glucose levels to 125 mg/dL or higher, the mice could be considered to be hyperglycemia and were ready to be tested for MDA levels and treated with mustard greens extract.

Wistar rats were randomly grouped, the P0 group was only given distilled water as the negative control, and the P1 group was given glibenclamide as a positive control. P2, P3, and P4 group were given mustard green extract with a dose of 10.0 mg/KgBW; 15.0 mg/KgBW; 20.0 mg/KgBW. The extract was given for 30 days. At the end of the phase, blood glucose and MDA levels were measured.

Analysis of Blood Sugar and MDA Levels of the Wistar Rats

Measurement of blood glucose levels was obtained from venous blood of mice using the GLUKO method (glucose test device) in mg/dL after the Wistar rats fasted for 10-12 hours units.[15] (Srinivasan, 2007) Measurement of MDA levels was performed using the TBA reactive substance (TBARS) method.

Statistical Analysis

The results of the research data were analyzed statistically using ANOVA method with Statistical Product and Services Solution (SPSS) program for Windows software with a 95% confidence interval (CI).

RESULTS AND DISCUSSION

Mustard Green Extraction

The results of the chemical products obtained from the green mustard powder were 8.69%, i.e. from the 1000 gr powder, 86.93 gr of thick ethanol extract were produced. The reaction products were calculated to determine the amount of extract produced by the mustard green powder.

The thick ethanol extract was fractionated using water, n-hexane, and ethyl acetate. The fractionated results were then evaporated and dosage form made to determine the most effective dose to reduce blood glucose levels in hyperglycemic Wistar rats. (already stated in the methods section. Hence, unnecessary. Consider to remove this statement) The results of the preliminary test showed that the ethyl fraction decreased at a higher than other fractions. This was seen from a decrease in blood glucose levels in ethyl acetate, ethanol, and n-hexane fractions of 55 mg/dl, 38 mg/dl, and 26 mg/dl, respectively. From these results, the ethyl acetate fraction was selected for further testing, because the ethyl acetate fraction has the highest potential in reducing blood glucose levels.

Results of Analysis of Mustard Green Ethyl Acetate Fraction

Phytochemical screening performed on ethyl acetate fraction includes alkaloids, flavonoids, terpenoids, steroids, saponins, and phenolics. Phytochemical screening results show that ethyl acetate fraction does not contain steroid compounds, but contains alkaloids, flavonoids, terpenoids, saponins and phenolic compounds (table 1).

Mass spectrometer identification obtained chromatogram with 6 peaks in the fraction. The chromatogram results can be seen in figure 1, and the identification of alleged compounds based on GC-MS spectrometer database can be seen in Table 2.

The mass spectrum of each peak is then identified by comparing the mass spectrum in the database so that the compounds contained in the fraction can be predicted. Estimates of compounds based on databases can be seen in table 2.

Blood sugar and MDA Levels Results

Data on the decrease of blood glucose levels from day 0 to day 14 can be seen in table 3. The results showed that administration of various doses of mustard green ethyl acetate fraction could reduce

Table 1 Phytochemical screening of Mustard Green Ethyl Acetate Fraction

Tests	Reagents	Outcome
Flavonoid	Willstatter	+
Phenolic	$FeCl_3$	+
Alkaloid	Wagner	+
Terpenoid	Lieberman Burchard	+
Steroid	Lieberman Burchard	-
Saponin	Hot aquadest + HCl	+

Notes: (+) detected, (-) not detected

Table 2 Identification of the six possible compounds

Peak	Tr	% area	M+	Compounds
1	4.41	23.90	100	Vinyl propionate
2	4.52	2.85	73	Butyl formate
3	13.60	2.29	150	2-Methoxy-4-vinylphenol
4	17.90	1.71	194	13-oxadispiro[5.0.5.1]tridecan-1-one
5	19.68	19.68	178	Methyl iso-eugenol 1
6	20.14	20.14	166	Phenol, 3-isopropoxy-5-methyl-

Table 3 Average blood glucose levels

Time (day)	Average blood glucose levels (mg/dL) and standard deviation				
	P_0	P_1	P_2	P_3	P_4
0	84.8±2.86	100.2±4.09	85.6±16.77	83.6±7.64	91±9.25
(*Pre-test*)					
3	94±3.67	209.6±10.90	153.2±12.79	178.4±12.20	211.2±18.93
7	97.6±3.43	124.6±12.78	122±10.61	132.8±8.23	133.2±17.20
(*Post-test*)					
14	109.6±24.15	97.8±3.77	103.6±4.56	104.4±10.81	110±11.81

Description:
P_0: Normal
P_1: Positive control (Glibenclamide)
P_2: 10 mg/KgBW ethyl acetate fraction of mustard green ethanol extract group
P_3: 15 mg/KgBW ethyl acetate fraction of mustard green ethanol extract group
P_4: 20 mg/KgBW ethyl acetate fraction of mustard green ethanol extract group

Table 4 Mean MDA levels

Time (days)	Mean MDA levels (nmol/ml) and standard deviation				
	P_0	P_1	P_2	P_3	P_4
(*Pre-test*)					
3	0.88±0.15	9.28±0.50	9.15±0.87	8.93±0.53	9.04±0.56
(*Post-test*)					
14	1.01±0.12	2.32±0.30	2.90±0.46	2.50±0.42	1.90±0.27

Description:
P_0: Normal
P_1: Positive control (Glibenclamide)
P_2: 10 mg/KgBW ethyl acetate fraction of mustard green ethanol extract group
P_3: 15 mg/KgBW ethyl acetate fraction of mustard green ethanol extract group
P_4: 20 mg/KgBW ethyl acetate fraction of mustard green ethanol extract group

blood glucose levels in hyperglycemic Wistar rats. Based on table 3, the higher the dose of the extract, the higher the ability of the extract to lower the blood glucose levels. The 20 mg/KgBW mustard green ethyl acetate fraction has almost the same ability as the glibenclamide (positive control) in reducing blood glucose levels.

Test for normality and homogeneity of glucose levels reduction shows that the data ware normally distributed and homogeneous. While the One Way ANOVA test shows statistically significant results. Least significant difference statistical analysis showed that there were significant differences between negative control group on positive control, 10mg/KgBW treatment group, 15 mg/KgBW treatment group, and 20mg/KgBW treatment group, which showed that the treatment with ethyl acetate fraction of mustard green ethanol extract could reduce blood glucose levels in hyperglycemic Wistar rats. Whereas no significant difference between the positive control and treatment dose of 20 mg/KgBW group, which can be interpreted both have a similar effect to decrease blood glucose levels.

The mechanism of action of the mustard green ethyl acetate fraction in reducing blood glucose levels is due to the compounds contained in the extract. Some compounds are potentially active as antihyperglycemic and antioxidants, namely the phenol compounds. Phenol compounds are widely used as antioxidants.[16] (Stuckey,1986)Phenol works as an antioxidant which inhibits the formation of free radicals and protects cells from oxidation. Phenol has a cardioprotective effect, which is a very powerful antioxidant. 2-methoxy-4-vinyl phenol, methyl isoeugenol 1 and 3-isopropoxy-5-methyl-phenol compounds are thought to be the active compounds that contribute to antioxidant and antihyperglycemic activity in the mustard green ethyl acetate fraction. According to Ravikumar's research in 2012,[17] the 2-Methoxy-4-vinyl phenol compound is a phenolic group that has antioxidant, antimicrobial, and anti-inflammatory activities.

The mean MDA levels are presented in Table 4. While MDA blood profile profiles from various doses of mustard green ethyl acetate fraction are presented in Figure 3.

Table 4 shows that the highest decrease in MDA blood levels of hyperglycemic Wistar rats occurred in the administration of 20 mg/Kg BW extract. Decreased MDA levels are affected by the antioxidant compounds in the fractions that can supply electrons to the radical compounds. Decreasing serum MDA levels can also prevent a decrease in membrane fluidity and cell damage.

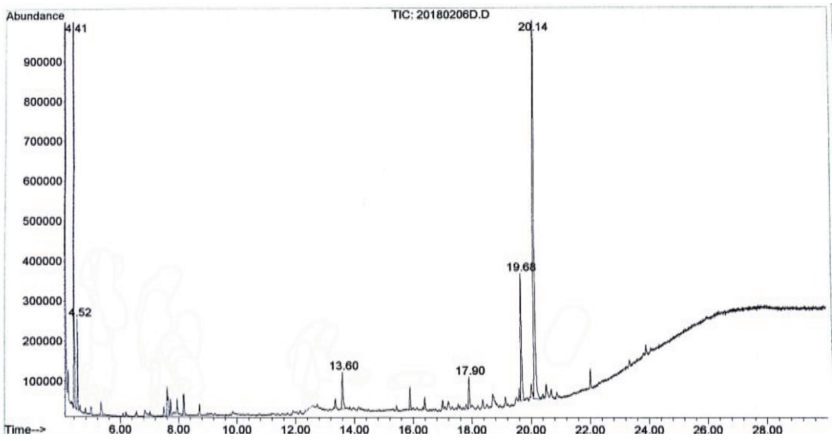

Figure 1 Chromatogram of ethyl acetate fraction of mustard greens

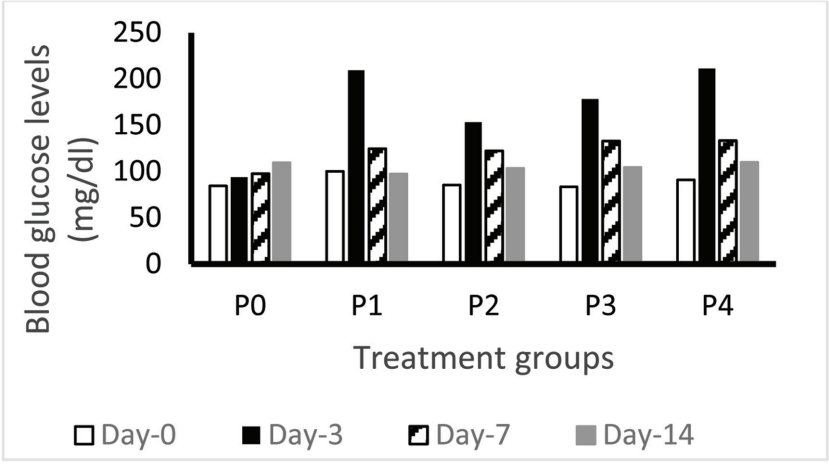

Figure 2 Blood glucose levels profile

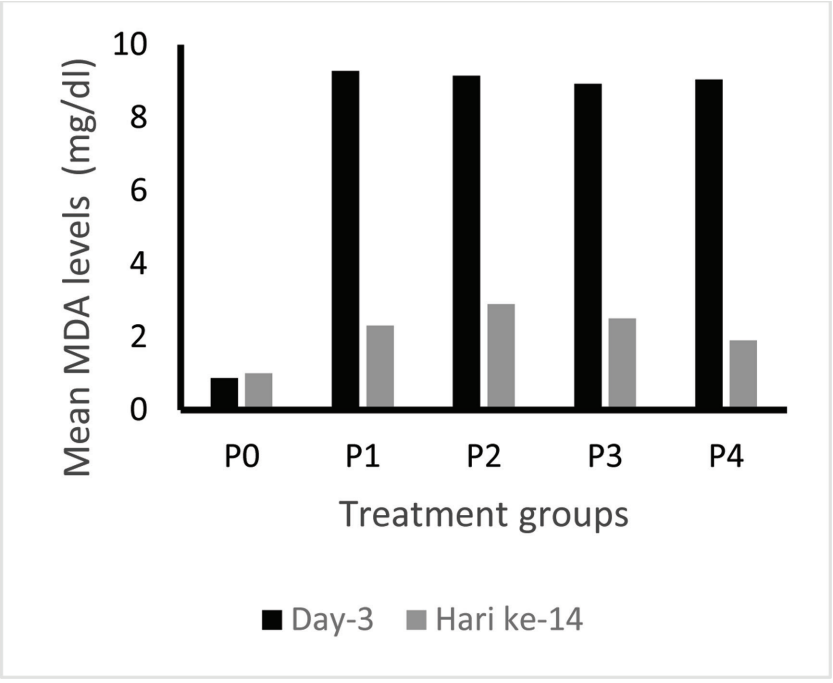

Figure 3 MDA levels profile after treatment

Least significant difference statistical analysis in Appendix 16 shows that there is a significant difference between negative control on positive control, treatment group dose of 10mg/KgBW, treatment of 15 mg/KgBW, and 20 mg/KgBW which shows that the treatment of ethyl acetate fraction of mustard green extract can reduce MDA levels in hyperglycemia Wistar rats. While the treatment dose of 10 mg/KgBW and the treatment dose of 15 mg/KgBW and positive control of the treatment dose of 15mg/KgBW and 20 mg/KgBW, both have no significant differences and can be interpreted as having the same effect on decreasing MDA levels.

CONCLUSION

Administration of ethanol extract of mustard green (Brassica rapa L.) ethyl acetate fraction at a dose of 20 mg/KgBW can significantly reduce blood sugar levels in hyperglycemic Wistar rats ($p < 0.05$). Administration of ethanol extract of mustard green (Brassica rapa L.) ethyl acetate fraction at a dose of 10 mg/KgBW, 15 mg/KgBB, and 20 mg/KgBB can significantly reduce MDA levels in hyperglycemic Wistar rats ($p < 0.05$). Phenol group compounds contained in the ethyl acetate fraction of mustard greens extract (Brassica rapa L.) function as a powerful antioxidant to reduce blood glucose and MDA levels in hyperglycemic Wistar rats.

RECOMMENDATIONS

Further researches on the isolation and identification of active compounds in green mustard green (Brassica rapa L.) ethanol extract which can reduce blood sugar levels in hyperglycemic Wistar rats and acute toxicity test of mustard green ethanol extract are needed. In addition, further research using other anti-inflammatory parameters such as IL-6, TNF-α, and 8-OHDG is needed.

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DISCLOSURE

The author reports no conflicts of interest in this work.

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