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Hypoglycemic and Hypolipidemic Effects Red Rosella Flower Steeping on Diabetic Rats

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ABSTRACT
Diabetes mellitus is a metabolic disease characterized by hyperglycemia and can cause dyslipidemia. One of the herbal plants that is thought to antidiabetic and hypolipidemic effects is the red rosella flower. This study aims to identify the capacity of hypoglycemic and hypolipidemic of red rosella flower steeping on diabetic rats. This study was experimental research with pre and post-test control design. The samples were male rats, divided into two groups, consisting of a negative control group (alloxan 130 mg/kg) and a treatment group (alloxan and rosella 2 ml/200 g/day). The measured of levels including glucose, HDL, and triglycerides was carried out after 14 days. The data was analyzed by T-test. The results showed that there was a decrease in fasting blood glucose levels and glucose levels 2 hours post prandial which were statistically significant in the treatment group at the beginning and end of the study (p=0.0001; p=0.0001). There was a statistically significant increase in HDL levels and a decrease in triglycerides before and after treatment in the treatment group (p=0.01; p=0.001). The conclusion of this study shows that the red roselle flowers steeping have the capacity of hypoglycemic and hypolipidemic of red rosella flower steeping on diabetic rats.

Keywords: *Blood Glucose, HDL, Rosella, Triglycerides, Diabetic*

1. INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia that occurs due to abnormal insulin secretion, insulin action, or both. Diabetes is one of the main threats to human health in the 21st century. The number of diabetics in Indonesia ranks 4th in the world [1,2].

Insulin disorders in diabetes mellitus affect lipid metabolism and cause lipidemia. Dyslipidemia is a metabolic disorder characterized by an increase in one or more of total cholesterol, LDL, triglycerides, and a decrease in HDL concentrations in blood circulation. Dyslipidemia characterized by hypertriglyceridemia and hypercholesterolemia can cause atherosclerosis, which is the state where there is an accumulation of plaque in the intima layer of the arterial wall. Further development of atherosclerosis causes cardiovascular diseases. Cardiovascular disease is the leading cause of mortality in many developed and developing countries [3,4,5].

The magnitude of the incidence, prevalence, and mortality of diabetes and cardiovascular disease in Indonesia illustrates the importance of prevention and early management of these diseases. The more patented drugs for diabetes and cardiovascular disease sufferers, the more expensive and affordable the treatment costs will be, especially for patients in developing countries like Indonesia. This situation causes Indonesian people to prefer herbal plants as alternative medicines that are more affordable [6]. One of the plants that are thought to have antidiabetic and hypolipidemic properties is the Red Rosella Flower. Research on the potential for hypoglycemic and hypolipidemic red rosella flowers steeping needs to be done. Red Rosella *(Hibiscus sabdariffa* L. *)* is one of the medicinal plants considered to have a therapeutic effect. Therefore, the effect of steeping of Red Rosella *(Hibiscus sabdariffa* L.*)* towards the levels of blood glucose and blood triglycerides in diabetes mellitus need to be investigated. This study aims to identify the capacity of the red rosella flowers steeping on blood glucose, HDL, and triglyceride levels in diabetic rat.

2. METHODS

This research is experimental laboratory research. It used pre and post-test control design to determine the effectiveness of the administration of red rosella steeping on the levels of fasting blood glucose, 2-hour postprandial glucose, HDL, and triglycerides in *Rattus norvegicus* induced by alloxan. The research was conducted by the Pusat Antar Universitas (PAU) Laboratory of Gadjah Mada University Yogyakarta.

The samples tested were 14 *Rattus norvegicus* randomly divided into two groups. Each group consisted of 7 rats. Group 1 was as negative control group given alloxan 130 mg/kg BW and group 2 was as a treatment group given alloxan 130 mg/kg BW and steeping red roselle tea 2 ml/200 gram BW / day for 14 days.

The inclusion criteria in this study were such as female *Rattus norvegicus Sprague Dawley*, which was three months old, weighing 150 -186 grams. *Rattus norvegicus* experiencing weight loss or illness was excluded from the study sample. The independent variable in this study was the tea steeping of red roselle (*Hibiscus sabdariffa* L.), while the dependent variable was the level of fasting blood glucose and 2-hour postprandial blood glucose, HDL, and triglyceride blood on each subject.

Materials used were calyx of roselle red *(Hibiscus sabdariffa* L.), Alloxan, hot water (~ 80 ˚C), and reagent GPO. Meanwhile, the tools used were a rat cage, scales, a *sonde* oral rat instrument, a spectrophotometer, a syringe, microcapillary pipettes, incubator, vertex, and centrifuge tubes. The process of red roselle tea steeping required several tools such as pipettes, filters, water heaters, thermometers, spoons, measuring cups, and scales.

The research preparation begins with the preparation of tested animals by selecting female healthy *Rattus norvegicus* randomly divided into groups. *Rattus norvegicus* was acclimatized for seven days before treatment. During the acclimatization, *Rattus norvegicus* were given only water and pellets. Upon the process of acclimatization, *Rattus norvegicus was* weighed and then was given no food and drink. After that, fasting blood glucose levels were measured. Two hours after that, the 2-hour postprandial glucose was measured. On day 8, *Rattus norvegicus* in all groups were induced by using alloxan. To identify the reaction after the administration of alloxan, *Rattus norvegicus* was only given water and pellets for 3 days. The examination on the level of fasting blood glucose, 2-hour postprandial blood glucose, HDL, and triglyceride blood was performed upon the alloxan induction on the 11th day.

The provision of red roselle tea was conducted with the following steps: red rosella calyx was weighed 0.094 g /kg mm, and then steeped with a water temperature of ~ 80 ˚C 2 ml for 3 minutes. Each *Rattus norvegicus* was given 2 ml /200 gram mm of steeping water of red rosella. The research was conducted on day 12 by adding the steeping water of red rosella one time a day with appropriate doses based on the weight of each rat by using a *sonde* instrument in group 2. The treatment was conducted for 14 days. On day 26, upon the treatment of red rosella, the blood of all tested rats was taken to examine the fasting blood glucose levels, 2-hour postprandial blood glucose, HDL, and blood triglycerides. The measurement of blood glucose levels used the GOD-PAP enzymatic method, while the measurement of HDL levels used the chylomicron deposition method. The measurement of triglyceride levels used the GPO enzymatic method. The levels of fasting blood glucose, 2-hour postprandial glucose, HDL, and triglyceride blood in this study used a numerical scale that it was analyzed by using paired t-test and independent t-test.

3. RESULTS

Before the research was conducted, *Rattus norvegicus* was acclimatized for 7 days. The weight of the *Rattus norvegicus* in each group was measured at the end of the acclimatization period. The weight measurement was used as a reference for the calculation of the dose of alloxan, which certainly included in the inclusion criteria. The weight had to range between 150 - 186 grams. The alloxan dose used in this study was 130 mg/kg BW. The results of *Rattus norvegicus* weight measurements are shown in Table 1.

Table 1. The average of *Rattus norvegicus* weight

Group	Min (gr)	Max (gr)	Mean±SD (gr)
Negative Control	164	179	169.14 ± 5.24
DM + Rosella	150	186	169.42 ± 14,83

Table 1 shows the weight average of *Rattus norvegicus* in all groups, which is included in the inclusion criteria ranging from 150 - 186 grams. Based on the normality test, the weight average of *Rattus norvegicus* in all groups showed normal distribution (p>0.05) with significance level p = 0.206 in group 1 and p = 0.134 in group 2.

This experimental research used a sample of alloxan-induced *Rattus norvegicus*. Before the research was conducted, *Rattus norvegicus* was acclimatized for 7 days. At the end of the acclimatization period, the rats were fasted or were not given any meal consumption. On day 8, the level of fasting blood glucose and 2-hour postprandial glucose was measured. Furthermore, on day 11, the level of fasting blood glucose, 2-hour postprandial glucose, HDL, and triglycerides were measured before the treatment was conducted.

Table 2. The Average of Fasting Blood Glucose and 2-Hours Postprandial Glucose Levels Before and After the Alloxan Induction

Variables	Groups	Before (mg/dl)	After (mg/dl)	p
Fasting Blood Glucose	Control	79.01 ± (1.46)	213.59 ± (2.81)	0.0001
	DM + Rosella	78.08 ± (2.22)	212.39 ± (1.36)	0.0001
2-Hour Postprandial glucose	Control	81,06 ± (1,66)	216,18 ± (3,03)	0.0001
	DM + Rosella	79,99 ± (1,88)	213,66 ± (1,87)	

Table 2 shows a significant increase in the average level of fasting blood glucose upon the alloxan induction both in the control group (p =0, 0001) and the DM+Rosella group (p=0.0001). The blood glucose level before the alloxan induction becomes a reference to a normal level, which is 78.55 mg/dl. There was a significant increase in the average level of 2-hour postprandial blood glucose upon the alloxan induction both in the control group (p = 0,000 1) and the DM + Rosella group (p=0.0001). The level of 2-hour postprandial glucose before the alloxan induction becomes a reference to a normal level, which is 80.53 mg/dl.

The result of the measurement of HDL and blood triglycerides after the alloxan induction showed a higher level than the normal level. The normal level of HDL in rats

showed 80 - 83 mg/dl, while the normal level of the blood triglyceride in rats showed 71 -74 mg/dl [8,9]. On day 12, the treatment of the steeping of red rosella tea was carried out to group 2 (*Hibiscus sabdariffa* L.) once a day with 2 ml/200 grams BW/day for 14 days. On day 26, the fasting blood glucose level, 2- hour postprandial glucose, HDL and blood triglycerides were measured for the second time.

Table 3. The Average of Fasting Blood Glucose and 2-Hours Postprandial Glucose Levels Before and After Threatment

Variables	Groups	Before (mg/dl)	After (mg/dl)	p
Fasting Blood Glucose	Control	214.02 ± (2.81)	214.57 ± (2.95)	0.391
	DM + Rosella	212.39 ± (1.36)	93.13 ± (1.17)	0.0001
2-Hour Postprandial glucose	Control			0.313
	DM + Rosella	216.18 ± (3.03)	217.9 ± (3.13)	0.0001

The level of fasting blood glucose of the intra-group was analyzed using the Paired t-test. The result in Table 3 shows a slightly significant increase in the average level of fasting blood glucose in the control group (p = 0.39 1). In contrast, the DM+Rosella group experienced a significant decrease (p=0. 0001). The deviation of the average level in fasting blood glucose in the two groups was analyzed using the Independent t-test and showed statistically significant differences (p=0.0001). Based on the average level, the DM+Rosella group experienced a substantial decrease in a fasting blood glucose level. The average level of 2-hour postprandial blood glucose in the control group experienced a slightly insignificant increase (p=0.313). Meanwhile, the DM+Rosella group experienced a significant decrease (p = 0, 0001). The deviation of the average level of 2-hour postprandial blood glucose in the two groups indicated a significant difference (p=0.0001). Based on the average level of the DM+Rosella group, there was a substantial decrease in the level of 2-hour postprandial blood glucose.

Table 4. The Average level of HDL Before and After the Treatment

Group	Before (mg/dl)	After (mg/dl)	p
Control	44.12 ± (2.56)	43.09 ± (2.54)	0.069
DM + Rosella	44.81 ± (1.40)	68.68 ± (2.78)	0.01

The HDL level of the intra-group was analyzed using the Paired t-test. The result in Table 4 shows that the average level of HDL in the control group experienced a slightly insignificant decrease (p=0.069). Meanwhile, the DM+Rosella group experienced a significant increase (p=0.0001). The deviation in the average level of HDL between the two groups was analyzed using the Independent t-test and showed a statistically significant difference (p=0.01). Based on the average level of the DM+Rosella group, there was a substantial increase in the HDL level.

Table 5. The Average Level of Triglycerides Before and After the Treatment

Group	Before (mg/dl)	After (mg/dl)	p
Control	149.73 ± (7.21)	149.89 ± (7.11)	0.078
DM + Rosella	146;03 ± (2.43)	92.98 ± (2.17)	0.001

Table 5 shows that the average level of blood triglyceride in the control group experienced a slightly insignificant increase (p=.,078), while the DM+Rosella group experienced a significant decrease (p=0.0001). The deviation in the average level of blood triglyceride between the two groups showed a significant difference (p=0.0001); thus, only the DM+Rosella group experienced a significant decrease in the triglyceride level.

4. DISCUSSION

Before the research was conducted, *Rattus norvegicus* was acclimatized for 7 days. Each rat was placed in a cage with the same environmental condition (temperature and humidity) to minimize external factors that can interfere with the result of the study. At the end of the acclimatization, the weight of *Rattus norvegicus* was measured to be used as a reference for the alloxan dose administration. The administration aimed to damage the pancreatic beta cells; thus, the diabetes mellitus occurred. There were 3 reaction phases in alloxan-induced subjects. Firstly, there was hyperglycemia, which lasted 1-4 hours after the induction. Secondly, it was followed by hypoglycemia lasting for 6 to 12 hours. Finally, the hyperglycemia occurred permanently in 12 to 24 hours after the induction was carried out [10].

At the end of the acclimatization, the rats also underwent a fasting period. On the 8th day, the fasting blood glucose level and 2-hour postprandial glucose were measured using enzymatic methods with GOD-PAP reagent. The result of the measurement of the average level of fasting blood glucose and 2-hour postprandial glucose before the alloxan induction showed the average normal blood glucose level in the rats. Besides that, all rats underwent the alloxan induction on day 8.

Three days after the alloxan induction (day 11), the level of fasting blood glucose, 2-hour postprandial glucose, HDL, and triglycerides were measured before the treatment was conducted. The result of the measurement of fasting blood glucose level and 2-hour postprandial glucose showed a significant increase in all groups. A considerable increase occurred as alloxan was a diabetogenic agent and cytotoxic analog glucose. The alloxan was accumulated in the pancreatic β cells through the GLUT2 glucose transporter. The reduction of alloxan produced dilaurate acid along with the radical oxygen, which would then be transformed into hydrogen peroxide (H2O2), and then radical hydroxyl eventually arose. If there were metal ions, such as Fe, Cu, and Zn, they

would damage the pancreatic beta cells. Thus, insulin was unable to be produced, and then the hyperglycemia occurred [11].

The result of the measurement of HDL and triglyceride levels showed a significant decrease in HDL level and an increase in triglyceride levels. It was caused by diabetes due to the alloxan induction that tended to increase the blood glucose level and dyslipidemia. Dyslipidemia was characterized by an increase in small dense LDL levels, a decrease in HDL levels, and an increase in plasma triglyceride level [12]. In a state of diabetes, insulin resistance occurred, which can increase the activation of sensitive lipase hormone in adipose tissue. This increase resulted in excessive free fatty acids in the blood. Fatty acids were carried out to the liver to be converted into triglycerides and became part of VLDL in the blood circulation. VLDL in a state of insulin resistance contained a considerable amount of triglycerides (large VLDL) that the triglyceride level in the blood tended to increase. Triglycerides in VLDL would be exchanged with LDL cholesterol. Triglycerides contained in LDL would be hydrolyzed by the hepatic lipase enzyme (usually increase in insulin resistance) to produce a small dense LDL that is easily oxidized. Triglycerides in large VLDL would also be exchanged with ester cholesterol from HDL that would then produce HDL with a massive amount of triglycerides. This kind of HDL cholesterol was highly efficiently catabolized by the kidney and that the serum HDL levels decreased [13,14,15].

On day 12, the treatment was conducted in group 2 in the form of the administration of the steeping of the red roselle tea (*Hibiscus sabdariffa* L.) once a day with 2 ml/200 grams BW/day for 14 days. After 14 days of the treatment (Day 26), the measurement of fasting blood glucose level, 2-hour postprandial glucose, HDL, and triglyceride level were conducted for the second time. The result showed a significant decrease in fasting blood glucose level and 2-hour postprandial glucose in group 2 due to the anthocyanins contained in the red rosella tea, which can inhibit the alpha-glucosidase enzyme located in the small intestinal wall (brush border membrane). Furthermore, it also can hinder the alpha-amylase pancreatic enzyme, which worked to hydrolyze polysaccharides in the intestinal lumen. The inhibition of both enzymes resulted in the disruption of digestion and absorption of carbohydrates and that the glucose cannot be absorbed by the intestine. Finally, the blood glucose level tended to decrease [15,16,17].

The result also showed a significant increase in HDL level and a decrease in triglyceride levels in group 2 due to the content of rosella anthocyanins, which had an inhibitory effect of CETP (cholesteryl ester transfer protein). CETP was a hydrophobic glycoprotein that bonded to HDL and played a role in the redistribution of cholesteryl esters from HDL to VLDL (very low-density lipoproteins) and LDL [18,19,20]. The CETP caused lipoproteins with a massive amount of triglycerides, such as VLDL and LDL, that increasingly became more atherogenic. In addition, triglycerides from VLDL and LDL were transferred into HDL so that HDL would change into a small dense of HDL. The decrease in blood triglyceride levels due to the anthocyanins in rosella could also increase the activity of lipoprotein lipase enzymes in fatty tissue and liver. The activity resulted in the transformation of the hydrolysis of plasma triglycerides into fatty acids and glycerol to get increased. However, this situation caused the blood triglyceride level to decrease [21,22].

This research proved that the red rosella flowers steeping could reduce the level of blood glucose and triglycerides, and increase the HDL level.

5. CONCLUSION

The red rosella flowers steeping have the capacity of hypoglycemic and hypolipidemic on diabetic rats.

AUTHORS' CONTRIBUTIONS

All authors have contributions on the research.

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REFERENCES

[1] Fauci, A.S., Kasper, D.L., Braunwald, E., Hauser, S.L., Longo, D.L., Jameson, J.L., *et al*. (2018). Harrison's Principles of Internal Medicine (20th ed.). USA: McGraw-Hills.

[2] Animaw, W & Seyoum, Y. (2017). Increasing prevalence of diabetes mellitus in a developing country and its related factors. *PlosOne*. 10, 1371.

[3] Kahn, C.R., & Shechter, Y. (2006). Insulin, Oral Hypoglycemic Agents and The Pharmacology of The Endocrine Pancreas. In Goodman, L.S. *The Pharmacological Basis of Therapeutics* (8th ed.). USA: Mc Millan Publishing Company, pp. 1463-1490.

[4] Pooja, O.C. & Mello, D.P. (2009). Antioxidant and antihyperlipidemic activity of Hibiscus sabdariffa Linn. leaves and calyces extracts in rats. *Indian Journal of Experimental Biology, 47*, 276-282.

[5] Guyton, A.C., & Hall J.E. (2016). *Buku Ajar Fisiologi Kedokteran.* Jakarta: EGC, pp. 1074, 1089-90.

[6] Subroto, A. (2006). *Ramuan Herbal untuk Diabetes Melitus*. Jakarta: Penebar Swadaya.

[7] Hirunpanich, V., Utaipat A, Noppawan, P. M., Nuntavan, B., Hitoshi, S., Angkana, H., *et al*. (2005). Hypocholesterolemic and antioxidant effects of aqueous extracts from the dried calyx of *Hibiscus sabdariffa* L. in hypercholesterolemic rats. *Journal of Ethnopharmacology, 103*, 252-60.

[8] Rajagopal, K. & Sasikala, K. (2008). Antihyperglycaemic and antihyperlipidaemic effects of *Nymphaea stellata* in alloxan-induced diabetic rats. *Singapore Med J*, 49(2), 137-141.

[10] Minakshi. & Pundir, C.S. 2005. Preparation of reusable enzyme strip for determination of serum triglyceride. *Indian Journal of Chemical Technology, 12*, 539-42.

[11] Walde, S.S., Dohle, C., Schott-Ohly, P. & Gleichmann, H. (2002). Molecular target structures in alloxan-induced diabetes in mice [Abstrak]. *Life Sciences*, *71*, 1681–169.
[12] Szkudelski, T. (2001). The Mechanism of Alloxan and Streptozotocin action in β cells of rat pancreas. *Physiol. Res*, *50*, 536-46.
[13] Goldberg, I.J. (2001). Diabetic Dyslipidemia: Causes and Consequences. *The Journal of Clinical Endocrinology & Metabolism,86, 965-97.*
[14] Pejic, R.N. & Lee, D.T. (2006). Hypertriglyceridemia. *J.Am.Board.Fam.Med, 19*, 310-6.
[15] Hansawasdi, C., Kawabata, J., & Kasai, T. (2000). ὰ-Amylase inhibitors from Roselle (Hibiscus sabdariffa Linn.) tea. *Bioscience. Biotechnology. Biochemistry*, *64*(5), 1041-43.
[16] Qin, Y., Xia, M., Ma, J., Hao, Y.T., Liu, J., Mou, H.Y., *et al.* (2009). Anthocyanin supplementation improves serum LDL- cholesterol and HDL-cholesterol concentrations associated with the inhibition of cholesteryl ester transfer protein in dyslipidemic subjects. *The American Journal of Clinical Nutrition, 90* (3), 485 – 492.
[17] Wu Y. Ding Y. Tanaka Y. Zhang W. Risk Factors Contributing to Type 2 Diabetes and Recent Advances in the Treatment and Prevention. Int J Med Sci. 2014; 11(11): 1185–1200.
[18**]** Indriawati R, The hepatoprotective capacity of steeping *kersen* leaves (*Muntingia calabura* L.) on diabetic rat. Electronic Journal of General Medicine, 2020, 17(5): em225.
[19] Schröders JW. Hakimi S. Dewi M. Weinehall FS. Nichter L et al. How is Indonesia coping with its epidemic of chronic noncommunicable diseases? A systematic review with meta-analysis PLoS One. 2017; 12(6): e0179186.
[20] Murray R. Granner D. Mayes P. Rodwell, V. Harper's Biochemistry, 25th edn. Stanford: Appleton and Lange. 2018.
[21] Chauhan AS. Plants Having Potential Antidiabetic Activity: A Review. Der Pharm Lett. 2010;2(3):369-387.
[22] Indriawati, R. (2010). the effect of giving lidah buaya (aloe vera) juice to decrease amount of blood glucose and triglycerides in rattus norvegicus induced by alloxan. *Majalah Ilmu Faal Indonesia*. 9(2).
[23] Carjavall-zarrabal, O., Waliszewski, S.M., Barradas-dermitz, D.M., Orta-flores, Z., Hayward-jones, P.M., Nolasco-hipolito, C., *et al.* (2005). The consumption of hibiscus sabdariffa dried calyx ethanolic extract reduced lipid profile in rats. *Plant Foods for Human Nutrition*, *60*, 153-159.