Hypolipidemic Effects of *Rosa Damascena* Mill. Extract in Streptozotocin-induced Diabetic Rats

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Abstract

Background: Diabetes mellitus is a chronic hyperglycemic condition with a lot of complication which can lead to death. Vascular complication in diabetes commonly caused by dyslipidemia which characterized by decreased HDL, elevated LDL, Cholesterol and Triglyceride. *Rosa damascena*- an ornamental plant-thoughts to have anti-hyperglycemia and antioxidant effects because of its large amount of polyphenolic components . This study analyzed hypolipidemic activity properties of an ethanol extract of *Rosa damascena* by measuring the lipid profile using various doses. **Methods**: This research is experimental study with randomized post-test only control group design. Twenty male Wistar rats divided into 5 groups; The groups were Healthy Control Group (HCG), Diabetes Group (DG), and Extract Group: P1 (250 mg/kgBW), P2 (500 mg/kgBW), P3 (1000 mg/kgBW). All treatment groups were injected by single-dose streptozotocin 50 mg/kgBW to induce diabetes, and given the *Rosa damascena* ethanolic extract oral treatment for 2 weeks. Statistical results showed that *Rosa damascena* significantly decrease cholesterol (58.2±15.19), LDL (11±2.44) optimally in dose 250 mg/KgBW and triglyceride (96.7±44.2) optimally in dose 500 mg/kgBW after 14 days. **Conclusion**: *Rosa damascena* extract seems to be great candidate for anti-hyperlipidemic drugs.

Keywords: *Lipid profile, Rosa damascena, Streptozotocin*

Introduction

According to WHO database in 2016, 8.5% diabetes mellitus patients are adults within range 18 years to 65 years old. Diabetes mellitus is a chronic hyperglycemic condition that caused by disruption of insulin secretion, insulin works or even both[1,2].

This condition escalate morbidity and mortality of diabetes patients which caused by its vascular complication itself [3]. Vascular complication in diabetes divided into microangiopathy and macroangiopathy. These angiopathy could get worse due to atherosclerosis lesion which formed faster than non diabetes patients [2]. Microvascular complications in diabetes are neuropathy, nephropathy and retinopathy; while macrovascular complications are coronary artery disease, peripheral artery disease, artery renalis sclerosis and even stroke [4].

Dyslipidemia is one of the main factor that contribute forming atherosclerosis lesion marked by poor high density lipoprotein (HDL), elevating low density lipoprotein (LDL) and rising Triglyceride (TG) [2,5]

One of the plant that claimed has potency to decrease blood glucose and control lipid profile was red rose (*Rosa damascena* Mill.) [6]. Red rose contain a large amount of polyphenolic components; some of them are kaemfrol, *cyanidine 3,5* , *D-glucoside*, *quarcetin*, and gallat acid [7]. The highest antioxidant activity of anthocyanin in *Rosa damascena* was *cyanidine 3,5, D-glycoseides* or known as *cyanidine* [8]. The *cyanidin* found to have anti-hyperglicemia and antioxidant effects [7,9].

Roses are easy to grow in tropical country like Indonesia, they sprout under upland and lowland between 1500 height above mean sea level [10]. Indonesian Central

Bureau of Statistic mentioned that roses is one of the ornamental plants which has the second largest harvest area after *Chrysanthemum* [11].

The present study aimed at investigating lipid lowering agents of ethanol extract of *Rosa damascena* with various doses in diabetic rats.

Material and Methods

Experimental criteria and animals care

The animals which used in this research were 20 male white rats Wistar Strain, age ± 12 weeks with body weight between 150-200 grams, all animals were healthy with no handicap which characterized by shining and clean fur, pink mucous membrane around the eyes and agile. The criteria for the exclusion were sick and disabled. If the experimental animals were dead along experiment they went to dropout criteria.

All animal samples were obtained from Department Pharmacology, Faculty of Medicine, Universitas Airlangga. They were kept under laboratory standard condition with temperature 12 hours light/dark cycle. There were 5 cages with size 500 x 300 x 150 mm (length x width x height). Each cage consists of 4 rats. They were fed on standard pellets with water *ad libitum*.

Animal labs were injected with low dose streptozotocin 50 mg/KgBW protocol. Fasting blood glucose levels were measured 5 days after the injection, if their glucose level are above 200 mg/dl then it will be considered diabetic. Easy touch glucometers and glucostrip were used to measure fasting glucose levels [12].

Animals were divided into 5 groups with random sampling. The groups were Healthy Control Group (HCG), Diabetes Group (DG), *Rosa damascena* Group: P1 (250 mg/KgBW), P2 (750 mg/KgBW), P3 (1000 mg/KgBW). All experiment procedures in this study were approved by the Ethics Committee Faculty of Dental Medicine Universitas Airlangga number 456/HRECC.FODM/X/2020, Surabaya, Indonesia, and were performed in an ethical manner with strict adherence to the animal research guide and purpose.

Rosa damascena Extract

100 grams of dried *Rosa Damascena* was soaked in 96% ethanol solution for 3 x 24 hours before it was evaporated at temperature of 50˚C to get viscous extract. This extract was added with CMC-Na 0,1% to increase viscosity and prevent particle from clotting [13].

The extract will be given in three different doses which are P1 as much as 250 mg/kgBW, P2 as much as 500 mg/kgBW, and P3 as much as 1000 mg/kgBW. Ethanol extract dissolved with destilled water before giving to rats orally.

Biochemical Analysis

After 14 days treatment, blood plasma was examined to measure the lipid profile each unit sample. Procedure was required anesthesia and termination since it was using cardiac puncture technique. Blood was collected into vacutainer plastic serum tube using 5 ml syringe with 23G needle. All blood samples were sent to Surabaya Health Center Laboratory to be analyzed using automatic analyzer.

Statistical Analysis

Lipid profile from each group were expressed as mean±SD. The data were statically analyzed using *Anova* (normal distribution) and *Kruskall Wallis* (abnormal distribution) continue with post hoc test with multiple comparison every each group. Values of $p<0.05$ were considered significant[14].

Results

a. Cholesterol

Table 1. Effect of ethanol extract of *Rosa damascena on* cholesterol (mg/dl) in streptozotocin-induced diabetic rats.

Group	N	X ± SD	Median (Min-Max)
HCG (Health Control Group)	4	64.5±3.10a	65.5 (60-67)
DG (Diabetes Group)	4	138.5±38.72b	147 (87-173)
P1 (Extract RD 250 mg/kg BW)	4	58.2±15.69a	59.5 (41-73)
P2 (Extract RD 500 mg/kg BW)	4	65±7.74a	67 (54-72)
P3 (Extract RD 1000 mg/kg BW)	4	61.7±13.2a	57.5 (51-81)

Noted: superscript showed significance difference between groups with α=0.05

The lowest cholesterol level was found in group P1 (60.4±14.5) followed with group P3 (62±11.4) and P2 (65.8±6.9). All groups cholesterol mean was analyzed with *Anova* followed with *Brown-Forsythe* and post hoc *Games-Howell*. Since *Games-Howell* test showed there was no differences, *independent sample t* test was carried out. The result was there are significant differences between diabetes group, health control group and treatment group.

b. High Density Lipoprotein (HDL)

Table 2. Effect of ethanol extract of *Rosa damascena* on HDL (mg/dl) in streptozotocin-induced diabetic rats

Group	N	X ± SD	Median (Min-Maks)
HCG (Health Control Group)	4	30.8±0.81	30 (29-31)
DG (Diabetes Group)	4	31.2±3.40	30.5 (28-36)
P1 (Extract RD 250 mg/kg BW)	4	28.2±5.12	28 (23-34)
P2 (Extract RD 500 mg/kg BW)	4	31.5±5.97	32 (24-38)
P3 (Extract RD 1000 mg/kg BW)	4	29.5±3.87	29.5 (25-34)

HDL level shows the data was normally distributed so it was analyzed with *Anova*. It was found that there was no significant differences between groups, the highest HDL level was in P2 group (31.5±5.97) while the lowest was in P1 group with (28.2±5.12) as shown as in table 2.

c. Low density Lipoprotein (LDL)

Table 3. Effect of ethanol extract of *Rosa damascena on* LDL (mg/dl) in streptozotocin-induced diabetic rats.

Group	N	X ± SD	Median (Min-Max)	P
HCG (Health Control Group)	4	10.7±1.25a	11 (9-12)	0,031*
DG (Diabetes Group)	4	29.2±11.44b	32 (14-39)	
P1 (Extract RD 250 mg/kg BW)	4	11±2.44ac	10.5 (9-14)	
P2 (Extract RD 500 mg/kg BW)	4	13.2±1.25cd	13 (12-15)	
P3 (Extract RD 1000 mg/kg BW)	4	11±2.70ad	10 (9-15)	

Noted: superscript showed significance difference between groups with α=0.05

Low Density Lipoprotein data was abnormally distributed, therefore *Kruskall- Wallis* test was carried out and followed with *Mann whitney U* test to find differences between groups. Table 3 showed that there was significant differences between LDL levels in all groups.

d. Triglyceride

Table 4. Effect of ethanol extract of *Rosa damascena* on Triglyceride (mg/dl) in streptozotocin-induced diabetic rats

Group	N	X ± SD	Median (Min-Maks)
HCG (Health Control Group)	4	122.2±13.74a	120 (108-141)
DG (Diabetes Group)	4	1179.2±362.77b	1145 (772-1654)
P1 (Extract RD 250 mg/kg BW)	4	175±114.4a	183.5 (49-284)
P2 (Extract RD 500 mg/kg BW)	4	96.7±44.2a	100 (43-144)
P3 (Extract RD 1000 mg/kg BW)	4	207.2±81.9a	177 (149-326)

Noted: superscript showed significance difference between groups with α=0.05

The lowest triglyceride level was found in group P2 (96.7±44.2) followed with group P1 (175±114.4) and P3 (207.2±81.9). All groups triglyceride value was analyzed with *Anova* followed with post hoc *Tukey* HSD. From this test, it was found that there were significant differences between HCG, diabetes group and extract group.

Discussion

Streptozotocin is a chemical compound which often used to induce diabetes in animal labs. It enters beta pancreas cells through Glucose Transporter 2 (GLUT2) and cause DNA alkylation. This lead to poly ADP-rybosilation and make DNA damage which set off beta

cell necrotizing [15]. Furthermore, mononuclear cells that infiltrate Langerhans islets make the beta cell damage much worse and conduct hyperglycemia [15,16] .

While T cells and macrophages are infiltrating, adipose cells activating pro inflammation cytokine which makes insulin resistance in adipose tissue. Hormon sensitive lipase are activated and cause triglyceride breakdowns into free fatty acids and glycerol. Free fatty acids went to bloodstream to get to the liver and synthesized into triglyceride again [17].

In this study we found that *Rosa damascena* could lowering cholesterol, LDL and triglyceride significantly but not increasing HDL. 250 mg/KgBW of *Rosa damascena* extract have better effect in lowering cholesterol (58.2±15.19) and LDL (11±2.44) than other doses. On the other hand 500 mg/KgBW of *Rosa damascena* extract optimally decrease triglyceride (96.7±44.2) much better than other doses.

Several in vitro studies on cell culture have shown pancreatic *α-glucosidase* and *α amylase* inhibition activates by anthocyanins [6,18]. Antocyanin were mentioned could increase insulin sensitivity and glucose uptake in vital organs such as muscle and adipose tissue and suppress lipogenic factors [19]. Other study revealed that administration of *cyanidine-3-O-glucosidase* able to reduce glycerol and free fatty acids released by mouse embryonic cells during hyperglycemia phase [20]. Anthocyanin can also reduce body weight by decreasing levels of SREBP-1 mRNA (Sterol Regulatory Element-Binding Protein) and inhibiting enzyme that synthesize fatty acids and triglycerol [21].

There are several possibly mechanism of action of *Rosa damascena* extract in reducing triglyceride and cholesterol in this study. Gholamhusein in 2010 found that *Rosa damascena* as pancreatic *lipase* inhibitor. *Rosa damascena* has been shown to have strong anti porcine pancreatic *lipase* activity, measured by turbidimetric assay[22]. Another mechanism was *Rosa damascena* induce the activation of *Peroxisome Proliferator Receptor γ (PPAR γ)* in adipose tissue. This condition will deactivate hormone sensitive lipase, breakdown triglyceride and reduce free fatty acids [23]. *Rosa damascena* also has *3-hydroxy-r-methyl-glutaryl-CoA reductase* activity. Another study showed that 0.15mg mL^{-1} of rose extract has 60% inhibitory effect on activity of *HMG-CoA reductase* [24].

Although the real mechanism of *Rosa damascena* as pancreatic *lipase* inhibitor and *HMG-CoA reductase* inhibitor are not known yet, *Rosa damascena* extract is able to control dyslipidemia. All three doses of *Rosa Damascena* extract showed better effect in lowering cholesterol, LDL and triglyceride.

Conclusion

The result of this study showed that *Rosa damascena* ethanol extract can decrease cholesterol, LDL and Triglyceride but not rising HDL in diabetic rats. This anti-hyperlipidemic effect was probably exerted at least by three mechanism including inhibits pancreatic *lipase*, increase *PPAR γ* and inhibits *HMG-CoA* reductase. Therefore, *Rosa damascena* extract seems to be great candidate for anti-hyperlipidemic drugs.

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References

1. World Health Organization, Global Report on Diabetes, *WHO* (2016) 2016: 11-12.
2. Decroli Eva. Diabetes Mellitus type 2. *Central Publishing of Internal Medicine Department, Faculty of Medicine, Andalas University* (2019): 1-2
3. Agrawal RP, Ola V, Bishnoi P, Gothwal S, sirohi P, Agrawal R. Prevalence of Micro and Macrovascular Complications and their Risk Factors in Type-2 Diabetes Mellitus. *Journal of the association of physicians of india*; (2014) 62: pp. 504-508.
4. Lotfy M, Adeghate J, Kalasz H, Singh J, Adeghate E. A Chronic Complications of Diabetes Mellitus: A Mini Review. *Current Diabetes Reviews* (2017) 13: 3-10.
5. Ozder Aclan. Lipid profile abnormalities seen in T2DM patients in primary healthcare in Turkey: a cross-sectional study. *Lipids in Health and Disease*

(2014) vol. 13:183

6. Gholamhoseinian A, H.Fallah H, Sharififar F. Inhibitory effect of methanol extract of Rosa damascena mill. flowers on α-glucosidase activity and postprandial hyperglycemia in normal and diabetic rats. *Phytomedicine* (2009)16: 935–941
7. Nayebi N, Khalili N, Kamalinejad M, Emtiazy M. A systematic review of the efficacy and safety of Rosa damascena Mill. with an overview on its phytopharmacological properties. *Complementary Therapies in Medicine* (2017) 34: 129-140.
8. Priska M, Peni N, Carvallo L, Dala Ngapa Y. Review: Antocyanins and Their Uses. *Chemical Cakra* (2018) 6: 79-97.
9. Boskabady MH, Shafei MN, Saberi Z, Amini S. Pharmacological effects of rosa damascena. *Iranian journal of basic medical sciences*, (2011) 14: 295–307.
10. Julianto TS. *Indonesian flower Essential Oil* (2016) : 115-116.
11. BPS. Indonesian ornamental plants statistic. *Indonesian Central Bureau of Statistic* (2017): 11-18.
12. B Purwanto, P Liben. Animal models in diabetes research. *Experimental Animal Research Protocol Series*, (2014) : 27-30.
13. Zahedi-Amiri Z, Taravati A, Hejazian L B. Protective Effect of Rosa damascena Against Aluminum Chloride-Induced Oxidative Stress. *Biological Trace Element Research* (2019) : 120-127.
14. Duncan RC, Knapp RG, Miller, MC. Test of hypothesis in population Means. In: *Introductory Biostatistics for the health sciences* (1977) John Wiley and Sons Inc: 71-96.
15. Gheibi S, Kashfi K, Ghasemi A. A practical guide for induction of type-2 diabetes in rat: Incorporating a high-fat diet and streptozotocin. *Biomedicine & Pharmacotherapy;* (2017) 95:605–613.
16. Goyal SN, Reddy NM, Patil KR, Nakhate KT, Ojha S, Patil CR, et al. Challenges and issues with streptozotocin-induced diabetes. A clinically relevant animal model to understand the diabetes pathogenesis and evaluate therapeutics. *Chem Biol Interact* (2016) 244: 49–63.
17. Jocken JWE. Adipose Triglyceride Lipase and Hormone-Sensitive Lipase Protein Expression Is Decreased in the Obese Insulin-Resistant State. *The Journal of Clinical Endocrinology & Metabolism,* (2007) 92 :2292–2299.
18. Akkarachiyasit S, Charoenlertkul P, Yibchok-anun S, Adisakwattana S. Inhibitory activities of cyaniding and its glycosides and synergistic effect with acarbose against intestinal a-glucosidase and pancreatic a-amylase. *Int. J. Mol. Sci* (2010) 11: 3387–3396.
19. Beatrice Scazzocchio B, Rosaria Varì R, Carmelina Filesi C, Massimo D'Archivio M, Carmela Santangelo C et al, Cyanidin-3-*O*-β-Glucoside and Protocatechuic Acid Exert Insulin-Like Effects by Upregulating PPARγ Activity in Human Omental Adipocytes. *Diabetes* (2011) 60:2234-2244.
20. Guo H, Guo J, Jiang X, et al. Cyanidin-3-O-b-glucoside, a typical anthocyanin, exhibits antilipolytic effects in 3T3-L1 adipocytes during hyperglycemia: Involvement of FoxO1-mediated transcription of adipose triglyceride lipase. *Food & Chemical Toxicology* (2012) 9: 3040-3047.
21. Belwal, Tarun, Seyed F, Seyed M, Solomon H. Dietary Anthocyanin and Insulin Resistance: When Food Becomes a Medicine. *Nutrients* (2017) 9: 1111.
22. Gholamhoseinian A, Shahouzehi B, Sharififar F. Inhibitory effect of some plant extracts on pancreatic lipase. *Int J Pharmacol* (2010) 6: 705-711.
23. Mohammadi A, Fallah H, Gholamhosseinian A. Antihyperglycemic Effect of Rosa Damascena is Mediated by PPAR.γ Gene Expression in Animal Model of Insulin Resistance. *Iranian Journal of Pharmaceutical Research* (2017) 16: 1080-1088.
24. Gholamhoseinian A, Sharifi-Far F, Shahouzehi B. Inhibitory activity of some plant methanol extracts on 3-Hydroxy-3-Methylglutaryl coenzyme a reductase. *Int J Pharmacol* (2010) 6: 705-711.