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Anti-atherogenic Effects of Soybean (*Glycine max*) Seed and Ginger (*Zingiber officinale*) Rhizome Extracts on Type 2 Diabetic Rat Model

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ABSTRACT

Diabetes mellitus (DM) is linked to an increase in dyslipidemia, which is associated with atherosclerosis, one of the leading causes of cardiovascular disease. Soybean (*Glycine max*) and ginger (*Zingiber officinale*) are functional foods, although the potency of herbs to suppress atherogenic DM has not been adequately demonstrated. This study was therefore conducted to examine the anti-atherogenic effects of *G. max*, *Z. officinale*, and their combination in a type 2 diabetic rat model. Extracts were prepared from *G. max* seeds and *Z. officinale* rhizome. Sprague Dawley rats were obtained and given a combination of high fructose high lipid diet (HFHLD) and a single dose of streptozotocin (25 mg/kg BW) intraperitoneally to induce diabetes. The rats were administered orally with *G. max* (5000 mg/kg BW), *Z. officinale* (500 mg/kg BW), and their combination for four weeks. Blood samples were collected from the heart. The lipid profile was calculated to determine the amounts of non-HDL-c, CRI-1 (TC/HDL-c), CRI-2 (LDLc/HDLc), and AIP [log 10 (TG/HDL-c)]. The results revealed that the oral administration of *G. max* (5000 mg/kg BW), *Z. officinale* (500 mg/kg BW), and their combination significantly ($p<0.05$) lowered non-HDL cholesterol levels by 20, 40, and 50%, respectively, compared to the diabetic group, while AIP levels were reduced significantly ($p<0.05$) by 20, 30, and 30%, respectively. CRI-2 was significantly ($p<0.05$) lowered by 30% in all test groups, but only *Z. officinale* reduced CRI-1. The findings of this study show that *Z. officinale* is more effective at inhibiting atherogenesis than *G. max* and their combination.

Keywords: Antiatherogenic, Diabetes mellitus, *Glycine max*, Soybean, *Zingiber officinale*.

Introduction

Dyslipidemia is linked to an increased risk of cardiovascular disease in people with diabetes mellitus (DM). An increase or decrease in the lipid fraction in plasma characterizes lipid metabolic disorders, also known as dyslipidemia.[1] According to the Centres for Disease Control and Prevention, dyslipidemia affects 70-97% of diabetic patients and is a major cause of cardiovascular complications.[2,3] This condition is the leading cause of death in diabetic patients. Insulin secretion problems, insulin resistance, or both can cause dyslipidemia in people with diabetes.[4] The insulin hormones play a role in lipid metabolism, and disrupting their secretion increases lipid mobilization by activating lipase.[5] This condition enhances adipose tissue lipolysis and the generation of free fatty acids. This would stimulate cholesterol synthesis, as well as triglyceride and low-density lipoprotein (LDL)-cholesterol production. As a result, it contributes to hyperlipidemia, which is linked to cardiovascular complications.[6] Reduced insulin secretion lowers HDL-cholesterol levels through lowering lecithin-cholesterol acyltransferase (LCAT) and apolipoprotein A1 (ApoA1) synthesis.[7] It causes dyslipidemia, which is linked to the development of atherosclerosis as a DM cardiovascular consequence. The level of non-high-density lipoprotein (non-HDL) cholesterol and the Atherogenic Index of Plasma (AIP) are strong predictors of atherosclerosis.

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Coronary heart disease (CHD), stroke, and aneurism are cardiovascular complications of DM due to atherosclerosis[3,6]. Most of the deaths in DM cases are caused by cardiovascular complications.[7]
Glycine max and *Zingiber officinale* are two functional foods that have been used to treat a variety of ailments. According to some animal studies, *G. max* seed possesses hypoglycemic action and can repair the lipid profile in diabetic rats [8,9] In *G. max*, isoflavone compounds such as daidzin and genistein were predicted to be lead substances.[10,11] *Z. officinale* rhizome active compounds gingerol and shogaol, on the other hand, have antioxidant and anti-hyperlipidemia properties.[12,13] Traditional healers typically utilize a combination of herbs to treat diseases, but most of the studies still use single herbs to evaluate their bioactivity. The effects of *G. max* seed and *Z. officinale* rhizome on DM atherogenesis have yet to be completely investigated.
The present study was aimed at investigating the anti-atherogenic effects of *G. max* seed extract, *Z. officinale* rhizome extract and their combination on diabetic rats.

Materials and Methods

Sources of plant materials
Glycine max seeds and *Zingiber officinale* rhizomes were obtained from Malang, East Java, Indonesia, in June 2017. They were identified at Balai Materia Medika, Batu, Malang with certificate specimen numbers: 074.241/102.7/2017 and 074/211/201.7/2017, respectively. The *Z. officinale* rhizomes were dried and ground to powder form.

Preparation of Glycine max and Zingiber officinale extracts
The *Z. officinale* rhizome powder (50 g) was extracted using an aqueous solvent utilizing the infusion process (250 ml). Meanwhile, the *G. max* seeds (80 g) were cooked in 100 mL of water and blended to reduce particle size. Filtration was used to separate the extract from the waste. A rotary evaporator was used to evaporate both extracts until they were concentrated.

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Sources of experimental animals and maintenance
Male Sprague-Dawley (SD) rats (2 months old with a body weight of 180-200 g) were obtained from Gajah Mada University, Yogyakarta, Indonesia. The animals were maintained individually in an automated animal room at 25 ± 1ºC with a 12:12-hour light-dark cycle. They were fed standard feed, water *ad libitum*, and fasted overnight before the experiments.

Ethical approval
The animals were handled following the ethical principles authorized by the Commission of Ethical Research at Brawijaya University in Malang, Indonesia, with certificate number 823-KEP-UB.

Animal grouping and treatment
The normal diet (ND) and high-fructose high-lipid diet (HFHLD) food were freshly mixed and given to the animals every two days. Diabetic rats were induced by HFHLD and a single dose of streptozotocin (25 mg/kg BW) intraperitoneal. A fasting blood glucose level of more than 126 mg/dL was used to confirm diabetes in rats.[14] The rats in the experiments were divided into five groups of five rats each. The control group received ND for eight weeks, while the diabetic and treatment groups received HFHLD. The treatment groups were separated into three groups: the first received 5000 mg/kg BW of *G. max* extract, the second received 500 mg/kg BW of *Z. officinale* extract, and the third received their combinations (5000: 500 mg/kg BW) for four weeks. Body weight and food intake were monitored weekly. After an overnight fast, blood samples were taken from the heart. Blood samples were promptly centrifuged at 4500 rpm, and the serum was separated and stored at -20ºC.

Determination of lipid profiles
The plasma concentrations of total cholesterol (TC), triglycerides (TG), LDLc, and HDLc were estimated using the Chod-Pap method.[15]

Estimation of non-HDL cholesterol level
The non-HDL cholesterol level was estimated with the mathematical formula below:
Non-HDLc = TC – HDLc.

Determination of the Castellis Risk Index (CRI)
Castellis Risk Index (CRI) was based on three key lipid profile values: TC, LDLc, and HDLc, and was divided into two categories, CRI-1 and CRI-2.[16] They were determined as follow:
CRI-1 = TC/HDLc ratio
CRI-2 = LDLc/HDLc ratio

Atherogenic Index Plasma (AIP)
Atherogenic Index Plasma (AIP) was proposed by Dobiasova and Frohlich in 2001. AIP is a logarithmically transformed ratio of TG to HDLc.[17]
AIP = Log (TG/HDLc) ratio.

Statistical analysis
The data were presented as mean ± SD. One-way ANOVA was used for statistical analysis. For mean comparisons, the least significant difference (LSD) test was performed, and $p < 0.05$ was considered statistically significant.

Results and Discussion

Effects of Glycine max seed extract and Zingiber officinale extract on body weight, food consumption and blood glucose level.
Table 1 indicates a decrease of body weight on test group compared to diabetic group at post-treatment. On the other hand, the body weight tends to increase compared to at pre-treatment except in the normal group. Food consumption on test group and normal group increased compared to diabetic group. The administration of *G. max* seed extract and combination decreased fasting blood glucose (FBG) level compared to diabetic group ($p<0.05$). *G. max* extract increased the secretion of insulin *β*-cells of the pancreas or secretagogue, which is controlled by isoflavones compound.[9] Therefore it produces a reduce fasting blood glucose level. However, *Z. officinale* rhizome extract cannot decrease FBG level, the level is not different compared to diabetic group ($p>0.05$). It is caused by antioxidant activity of herbs which protect pancreatic cells from damage and also insulin sensitizer, as a result their hypoglycemia activity is lower than *G. max* extract.[12,13]

Effects of Glycine max seed extract and Zingiber officinale extract on lipid profiles
Table 2 shows that the administration of *G. max* seed extract, *Z. officinale* rhizome extract, and their combination significantly ($p<0.05$) decreased TC, TG, and LDLc levels compared to the diabetic group, while HDLc levels were increased. In the diabetic group, HDLc levels were reduced significantly ($p<0.05$) compared to the normal group, while TC, TG, and LDLc levels were increased. In a diabetic rat model, oral administration of *G. max* and *Z. officinale* extracts, as well as their combination, lowered blood TC, TG, and LDLc levels. This effect is linked to the anti-hyperlipidemia, insulin secretagogue, and antioxidant properties of the active compounds.[9,10,12,13] Isoflavone in *G. max* can reduce cholesterol levels by inhibiting HMG CoA reductase, thereby reducing hepatic cholesterol synthesis.[10,18]
G. max extract also increased the secretion of insulin β-cells of the pancreas, which is controlled by isoflavones.[9,19] Stigmasterol and lanosterol in *G. max* inhibit DPP-4, thereby allowing an increase in hormone and insulin secretion to be retained.[20,21] In a previous study, stigmasterol was discovered in *Urena lobata* leaf extract to inhibit DPP-4 activity.[22] Very low-density lipoprotein (VLDL) and chylomicrons are catabolized by insulin-stimulated lipoprotein lipase (LPL). Therefore, the rise in triglycerides, LDLc, and total cholesterol levels could be prevented.[5] *Z. officinale* rhizome extract enhances the synthesis of 7-hydroxylase and causes the conversion of hepatic cholesterol to bile salts.[23] This leads to a decrease in hepatic cholesterol synthesis. Administration of *G. max* seed extract, *Z. officinale* rhizome extract, and their combination increased serum HDLc levels in diabetic rats. The active ingredient in *Z. officinale*, which functions as an antioxidant and insulin sensitizer, is responsible for this action.[24,25] *Z. officinale* contains phenolic compounds, including gingerol and shogaol, which protect pancreatic cells from injury and hence preserve insulin production.[26,27] Free fatty acid levels in the blood are lowered by insulin hormone, which enhances the activity of the enzyme Lecithin Cholesterol Acyl Transferase (LCAT), which aids in HDLc maturation.[28]

Effects of Glycine max seed extract and Zingiber officinale rhizome extract on non-HDLc
The oral administration of *G. max* seed extract (5000 mg/kg bw), *Z. officinale* rhizome extract (500 mg/kg bw), and their combination were able to significantly ($p < 0.05$) reduce non-HDLc levels by approximately 20, 40, and 50%, respectively, when compared to the diabetic groups (Figure 1). Meanwhile, non-HDLc levels were significantly ($p < 0.05$) increased by more than 6-fold in the diabetic groups. *G. max* seed extract, *Z. officinale* rhizome extract, and their combination decreased non-HDLc. *G. max* contains the active compound isoflavone, which reduces cholesterol levels through inhibition of HMG CoA reductase. Furthermore, it decreases hepatic synthesis of cholesterol.[10,23,25] *G. max* also inhibits lipolysis through decreasing hormone-sensitive lipase (HSL) activation of adipose tissue, which is regulated by the active compound saponin.[10,29]
It prevents FFA levels from rising, lowering cholesterol hepatic production and non-HDLc levels in the process. They aid in the prevention of atherosclerosis or anti-atherogenesis.[18]

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Table 1: Body weight, food consumption and blood glucose level of diabetic rats

Group	Normal	Diabetic	*G. max*	*Z. officinale*	Combination
Body weight pre treatment (g)	352.7 ± 15.8	300.5 ± 31.0	244.8 ± 17.7	225.0 ± 36.0	297.3 ± 30.5
Body weight post treatment (g)	336.6 ± 29.7	335.3 ± 38.7	253.2 ± 33.1	282.5 ± 45.3	317.5 ± 31.8
Food consumption (%)	74.7 ± 8.0	68.3 ± 13.0	89.4 ± 8.0	87.0 ± 11.0	72.0 ± 27.0
FBG pre-treatment (mg/dL)	105.4 ± 7.5	201.3 ± 35.0	182.6± 43.1	168.5 ± 35.8	163.5 ± 11.5
FBG post-treatment (mg/dL)	105.4 ± 7.5[a]	139.0 ± 14.9[b]	109.0 ± 13.2[c]	132.3 ± 17.9[b]	124.0 ± 12.5[d]

Data are expressed as mean ± SD; n = 5; Means with different letter are significantly different; $p < 0.05$, LSD test.

Table 2: Lipid serum profiles of study groups

Group	TC (mg/dL)	TG (mg/dL)	LDLc (mg/dL)	HDLc (mg/dL)
Normal	87.2 ± 3.6[a]	65.2 ± 7.1[a]	4.6 ± 0.8[a]	78.4 ± 3.1[a]
Diabetic	107.4 ± 3.6[b]	214.2 ± 50.2[b]	12.6 ± 48.1[b]	55.0 ± 6.0[b]
G. max	95.2 ± 6.1[c]	128.6 ± 17.2[c]	1.2 ± 15.9[c]	51.5 ± 2.5[c]
Z. officinale	90.2 ± 8.1[d]	106.4 ± 16.7[d]	2.5 ± 19.1[c]	61.2 ± 2.3[d]
Combination	85.6 ± 7.9[e]	96.2 ± 12.1[e]	1.4 ± 16.5[c]	47.5 ± 6.1[e]

Data are expressed as mean ± SD; n = 5; Means with different letter are significantly different; $p < 0.05$, LSD test.

Polyphenol substances in *Z. officinale* may increase the enzyme 7-hydroxylase and stimulate the hepatic conversion of cholesterol to bile acid. Furthermore, the mechanism will inhibit the synthesis of hepatic cholesterol.[23,27] Herb administration raises HDLc levels, therefore decreasing non-HDLc involved in the atherogenesis process.

Effects of Glycine max seed extract and Zingiber officinale rhizome extract on CRI-1 level

Figure 2 demonstrates that giving *Z. officinale* rhizome extract (500 mg/kg BW) to diabetic rats significantly ($p>0.05$) reduced CRI-1 levels (TC/HDLc ratio) by about 20%, but administration of *G. max* seed extract and its combination to diabetic rats reduced CRI-1, but not considerably. The CRI-1 level was more than 2-fold higher in the diabetic groups than in the normal groups. *Z. officinale* stimulates the 7-hydroxylase enzyme and causes hepatic cholesterol conversion to bile acid, which is regulated by polyphenol chemicals. As a result, hepatic cholesterol synthesis will be reduced.[23]

This results in a decrease in the CRI-1 level, which is linked to atherogenesis. *Z. officinale* rhizome-derived phenolic compounds, such as gingerol and shogaol, have an antioxidant action by scavenging superoxide anion.[26] These chemicals prevent oxidative damage in the cell pancreas, allowing insulin hormone release to continue.[27] Insulin hormone reduces free fatty acid levels in the blood while increasing LCAT activity. The HDLc maturation process is aided by this enzyme.[28] The decrease of free fatty acids will inhibit lipase-sensitive hormones, hence, the production of HDLc in the body will be sufficient.[28,29]

It also controls LDLc levels by lowering VLDL.[18] By inhibiting HMG CoA reductase synthesis, phytosterol in *G. max* serves as an anti-hyperlipidemic, preventing cholesterol formation and the conversion of VLDL to LDL.[29,31] *G. max* isoflavone promotes LPL to convert VLDL to LDLc and enhances insulin production.[5] This lowers LDLc levels in the blood, lowering the CRI-2 level. *Z. officinale* reduced hepatic cholesterol synthesis by increasing the 7-hydroxylase enzyme and hepatic cholesterol conversion to bile acid.[23] This also results in a decrease of LDLc levels which is involved in the atherogenesis process.[28,30] The phenolic component in *Z. officinale* can boost insulin secretion while also lowering chylomicron and VLDL levels.[20,32]

Effects of Glycine max seed extract and Zingiber officinale rhizome extract on AIP

Figure 4 reveals that oral administration of *G. max* seed extract (5000 mg/kg BW), *Z. officinale* rhizome extract (500 mg/kg BW), and their combination significantly ($p<0.05$) reduced AIP by 20, 30, and 30%, respectively. AIP was raised 6-fold in diabetic rats compared to normal controls. Isoflavone from *G. max* binds triglycerides from the diet, reducing triglycerides absorption in the intestine.[10] Increased GLUT-4 expression, which promotes glucose absorption into cells and reduces lipolysis, may help to lower insulin resistance.[10,25] The antioxidant impact of *Z. officinale* could explain the regulation of insulin secretion.[26] Insulin can lower plasma levels of free fatty acids and triglyceride synthesis.[27,28] The activation of the LCAT enzyme is increased by lowering free fatty acid levels in plasma, which aids HDLc maturation.[28] The mechanism reduces triglyceride levels while boosting HDLc, resulting in a drop in AIP.[18] The AIP value is determined by HDLc levels; raising HDLc levels results in a low atherogenic index, thereby lowering the risk of atherosclerosis.

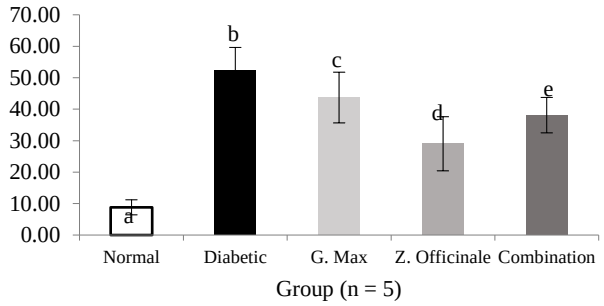

Figure 1: Non-HDL-c levels in diabetic rats treated with *Glycine max* seed extract, *Zingiber officinale* rhizome extract, and their combination. Different letters represent different effects ($p < 0.05$).

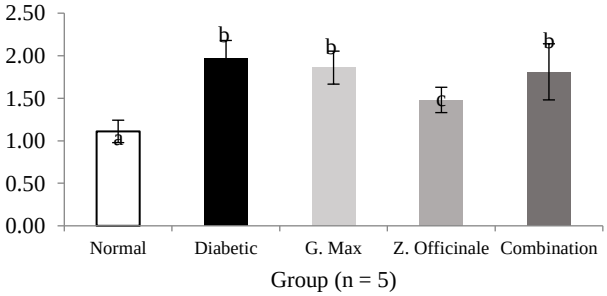

Figure 2: CRI-1 (TC/HDLc ratio) levels in diabetic rats treated with *Glycine max* seed extract, *Zingiber officinale* rhizome extract, and their combination. Different letters represent differents effect ($p < 0.05$).

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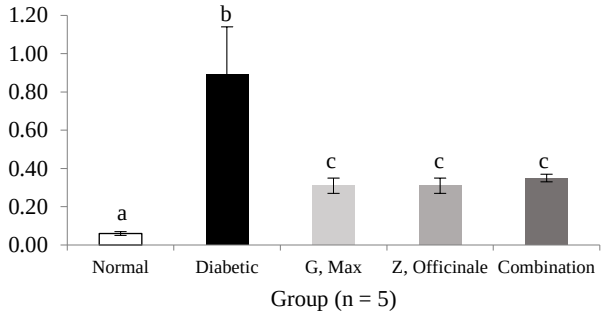

Figure 3: CRI-2 (LDLc/HDLc ratio) levels in diabetic rats treated with *Glycine max* seed extract, *Zingiber officinale* rhizome extract, and their combination. Different letters represent different effects ($p < 0.05$).

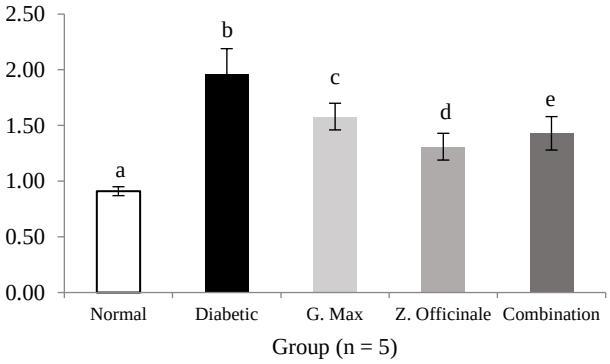

Figure 4: IAP in diabetic rats treated with *Glycine max* seed extract, *Zingiber officinale* rhizome extract, and their combination. Different letters represent different effects ($p < 0.05$).

Also, it aids in the repair of lipid metabolism by lowering cholesterol synthesis.[27] They help to lower the CRI-1 level, which is important in the atherogenesis process. This supports Al Amin's findings that *Z. officinale* rhizome (500 mg/kg bw) lowered overall cholesterol levels in diabetic rats.[30]

Effects of Glycine max seed extract and Zingiber officinale rhizome extract on CRI-2 level

When compared to the diabetic group, oral administration of *G. max* seed extract (5000 mg/kg BW), *Z. officinale* rhizome extract (500 mg/kg BW), and their combination significantly ($p<0.05$) reduced CRI-2 levels (LDLc/HDLc ratio) by 30% each (Figure 3). The CRI-2 level was raised more than 9-fold in the diabetic groups compared to normal controls. The combination of *G. max* seed extract and *Z. officinale* rhizome extract reduced the LDLc/HDLc ratio. Isoflavone was able to activate Peroxisome Proliferator-Activated Receptor (PPAR), a lipid metabolism regulator.

Conclusion

The findings of this study show that *Z. officinale* rhizome extract is more effective as an anti-atherogenic agent on diabetic rats than *G. max* seed extract and their combination, lowering non-HDLc, CR-1, and AIP levels.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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