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by Siska Siska

Submission date: 28-Dec-2020 12:05PM (UTC+0700)
Submission ID: 1481599907
File name: jahe.pdf (352.14K)
Word count: 3468
Character count: 19338

COMBINATION OF GINGER EXTRACT (*Zingiber officinale* Roscoe) AND ZINC AS METABOLIC SYNDROME IN HIGH CHOLESTEROL DIET DIABETIC MICE

Hardi Sunaryo[1)], Siska[1)], Dwitiyanti[1)], Rizky Arcinthya Rachmania[1)]
[1)]Faculty of Pharmacy and Science, University of Muhammadiyah Prof. DR. HAMKA, Indonesia

Abstract: Metabolic syndrome including the presence of obesity, insulin resistance and dyslipidemia that predisposes type 2 diabetes. The purpose of this study to prove the effectiveness of the combination of the ethanol extract of ginger (*Zingiber officinale* Roscoe) with Zinc (Zn) as a hypolipidemia, and hypoglycemia through the measurement of oxidized LDL, total cholesterol, triglycerides in diabetic mice fed high-cholesterol diet. This study used 54 male mice were divided into 9 groups: normal control group, negative control, positive control, ginger extract (ZO) group dose of 37.5 mg/kg, Zn 20 mg/kg and five groups with a test dose variation that ginger dose 1 (37.5 mg/kg) + Zn 20 mg/kg, ginger dose 2 (75 mg/kg) + Zn 20 mg/kg, ginger dose 3 (150 mg/kg) + Zn 20 mg/kg of each test group given the extract for 14 days. Animal models used were mice induced by streptozotocin (STZ) and feed hypercholesterolemia. The results obtained from this study, combined with ginger extract 75 mg/kg and zinc 20 mg/kg can lower blood glucose levels by 39.87 %, comparable to metformin ($p \geq 0.05$). The combination of ginger extract 75 mg/kg with zinc 20 mg/kg can lower total cholesterol 65.88%, LDL cholesterol 74.62% and triglyceride levels 55.41%, comparable to atorvastatin ($p \geq 0.05$). The addition of Zn to ginger (ZO) extract can increase hypolipidemia activity and hypoglycemic activity of ginger extract.

Keywords: hypoglycemia, hypolipidemia, *Zingiber officinale* Roscoe and Zinc, ginger extract, metabolic syndrome, diabetic mice

Introduction

Metabolic syndrome including the presence of obesity, insulin resistance and dyslipidemia that predisposes type 2 diabetes is becoming more prevalent in recent years [Alberti *et al* 2006, Bergman *et al* 2006]. Per recent estimates, approximately 215 million people worldwide suffer from diabetes and 80–90% of them from type 2 diabetes [Procopiou and Philippe *2005*]. The modern lifestyle of increased intake of high-calorie cafeteria fast food associated with decreased energy expenditure also contributes to the current rising prevalence of obesity and type 2 diabetes [Aude *et al* 2004]. Recent epidemiological studies also revealed that 90% of all patients with type 2 diabetes are or have been overweight, and indicated that obesity is a strong risk factor and cause of type 2 diabetes and associated metabolic disturbances [Bray and Bellanger 2006, Kahn *et al* 2006]. The events of hyperglycemia and hyperlipidemia, and their association present major risk factors for the development of diabetic and cardiovascular complications [Lender and Sysko 2006]. To reduce these serious complications and negative outcome of the metabolic syndrome, the control not only of blood glucose but also of lipids is necessary [Moller 2001]. Therefore, new medicinal agents with dual properties on controlling both blood glucose and lipids are in great demand. The currently available therapeutic options such as dietary modification or a combination of synthetic antidiabetic, hypolipidemic drugs have their own limitations and undesirable side-effects [Lender and Sysko 2006]. Hence, there is an increased demand to search and evaluate traditional approaches for the treatment of metabolic disorders, particularly the use of herbal medicines [Srinivas *et al* 2009].

Various studies have shown that hyperglycemia, hypercholesterolemia, and oxidation of LDL cholesterol is a major cause of atherosclerosis and coronary heart disease (CHD). Thus, to reduce mortality due to CHD can be done by lowering blood sugar, cholesterol, and prevent the oxidation of LDL cholesterol. During this time, for the treatment of hyperglycemia and hyperlipidemia used diabetes drug combinations and cholesterol-lowering drugs, it is may not necessarily able to prevent atherosclerosis, because both of diabetes and hypercholesterolemia will increase the oxidative stress that can stimulate the formation of oxidized LDL (ox-LDL). Therefore, the treatment of hyperlipidemia Diabetes Mellitus (DM) need additional antioxidants.

Ginger is known to contain several potentially bioactive substances, mainly gingerols and their related dehydration products, the shogaols, as well as volatile oils including sesquiterpenes, such as b-bisabolene and (-)-zingiberene, and monoterpenes, mainly geranial and neural. Gingerols have been shown to inhibit both prostaglandin and leukotriene biosynthesis and angiogenesis.

In addition, several ginger components exhibit serotonin receptor-blocking activity [Zainab *et al* 2006].

Previous studies reported that ginger extract (*Zingiber officinale* Roscoe) can lower cholesterol levels, blood sugar, lipid peroxidation, and inhibit the development of atherosclerosis after given for 6 weeks in mice induced by alloxan [Elshater et al 2009]. Zn (Zn-carbonate form) is added to the diet (1 g / kg BW) for 8 weeks can reduce atherosclerosis in rabbits a diet high in cholesterol [Ren et al 2006]. Clinical trials of zinc supplementation as an antioxidant for 7 years did not cause metabolic syndromes [Czernichow *et al* 2009].
Due to the above reasons, research must be done to prove whether the combination of the two substances are antioxidants and can prevent hypocholesterolemia synergistically and more secure in DM mice a diet high in cholesterol. In addition, the expected combination with Zn will reduce the dose of ginger extract thus applicable in clinical.

METHODOLOGY

Extract preparations and phytochemical screening

Dried ginger rhizome was made into powder to obtain a diameter of 40 mesh, the powder obtained macerated with 70% ethanol for 3 days, then the residue was filtered and remacerated with 70% ethanol until there were no active substance in powder. The filtrate was concentrated by vacuum filtration rotary evaporator at a temperature of not more than 60°C to obtain a thick extract. Then, thick extract was dried in an oven temperature of 50°C. Further conducted phytochemical screening.

Hypercholesterolemia feed preparation

Hypercholesterolemia feed made from standard feed containing 12% egg yolk contains cholesterol, equivalent to 0.5% [Priyanto *et al* 2012].

Treatment of animal

The study begins with acclimatization then hypercholesterolemia feeding for 30 days. After the mice were induced hyperglycemia by administering streptozotocin (STZ) dose of 50 mg / kg BW given intraperitoneally. Sugar levels will increase after 7 days of administration of STZ. After an increase in blood glucose levels, test animals given the drug comparison and test material for 14 days, with the division of the group as follows: Group 1 (normal): Mice with a normal diet, the group who were given standard food and drink. Group 2 (negative control): diabetic mice + dietary hypercholesterolemia as a negative control. Group 3 (positive control 1): diabetic mice + dietary hypercholesterolemia + metformin (65 mg/kg) as a positive control. Group 4 (positive control 2): diabetic mice + dietary hypercholesterolemia + atorvastatin (5.7 mg/kg) as a positive control. Group 5 (a single dose of 1 extract ZO): diabetic mice + dietary hypercholesterolemia + ginger extract dose of 37.5 mg/kg. Group 6 (zinc): diabetic mice + dietary hypercholesterolemia + Zinc 20 mg/kg. Group 7 (doses 1 extract ZO + zinc): diabetic mice + dietary hypercholesterolemia + ginger extract dose of 37.5 mg/kg and Zinc 20 mg/kg. Group 8 (doses 2 of extract ZO + zinc): diabetic mice + dietary hypercholesterolemia + ginger extract dose of 75 mg/kg and Zinc 20 mg/kg. Group 9 (doses 3 of extract ZO + zinc): diabetic mice + dietary hypercholesterolemia + ginger extract dose of 150 mg/kg and Zinc 20 mg/kg.
At the end of 8 weeks after treatment mice were anesthetized and killed. After that was done the examination of blood glucose levels, total cholesterol, LDL (Low Density Lipoprotein), and triglycerides.

Measurement of blood glucose levels

Measurement of blood glucose levels was done by the end of week 4 after treatment. Mice blood collection through the orbital sinus. Measurement of blood glucose levels of mice performed using clinical spectrophotometer.

Measurement of total cholesterol

10 mL serum was mixed with enzyme reagent (kit) 1000 mL, and then mixed with a vortex and incubated for 10 minutes at a temperature of 20-25°C or 5 minutes at a temperature of 37°C, and then read with a spectrophotometer clinical.

Measurement of LDL

100 µL serum was mixed with 1000 µL precipitation reagent. The mixture was shaken and incubated for 5 minutes at a temperature of 37°C, then centrifuged. Supernatant was taken as 100 mL inserted into the microtube then was mixed with 1000 mL of the enzyme. LDL cholesterol levels were measured with a spectrophotometer clinical. Levels of LDL cholesterol was calculated as the difference of total cholesterol and cholesterol in supernatant.

Measurement of triglyceride levels

Serum triglyceride concentrations were measured by enzymatic colorimetric test by using *GPO-PAP method* with *Lipid Clearing Factor (LCF)* (Human, Germany). Serum triglyceride concentrations were measured by pipetting 10 mL of standard triglyceride solution (200 mg/dL) and serum samples and then mixed with reagent of the triglyceride kit (triglyceride kit reagent, Human, Germany), and incubated for 10 minutes at room temperature. The absorbance of the standard and serum samples were read by spectrophotometer at 546 nm

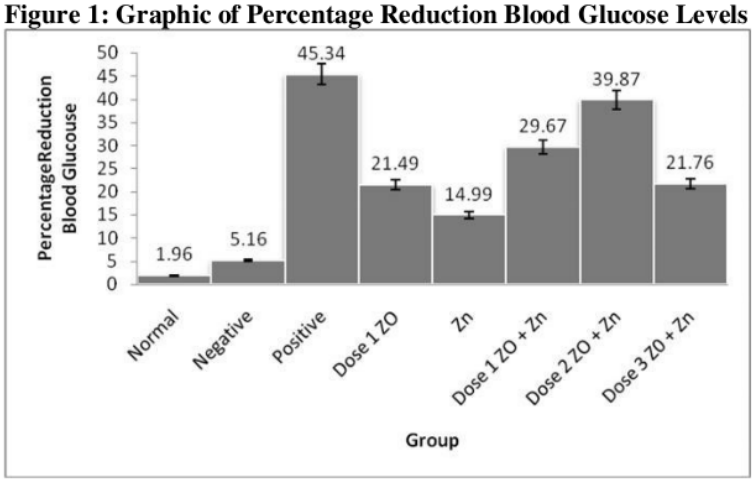
wave length. Triglyceride concentrations of the samples were calculated by diving the sample's absorbance with standard's absorbance and multiplied 200 mg/dL.

Statistical analysis

The data obtained were analyzed by one-way ANOVA. In the analysis of this data determined in advance of data homogeneity and normality of the data from each data and followed by one-way ANOVA test with a significance level of 95%. Then see the differences between the treatment groups, if there was a difference between the treatment groups followed by Tukey's test.

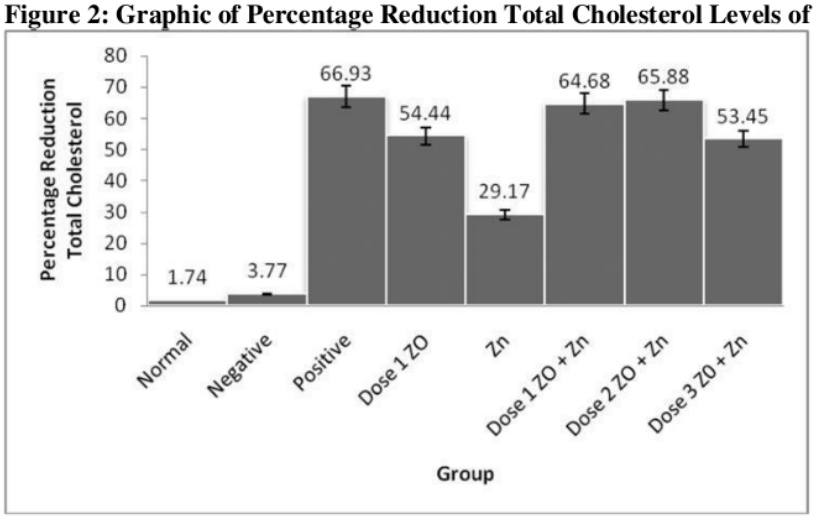
RESULTS AND DISCUSSION

Figure 1: Graphic of Percentage Reduction Blood Glucose Levels of Mice



Ginger extract used were obtained from the Research Institute for Spices and Medicinal Plants. Ginger extract who has been powdered extracted with 70% ethanol. Reasons for the selection of 70% ethanol solvent because it is non-toxic solvent, neutral, molds and bacteria is difficult to grow, good absorption, the heat that required for concentration less.

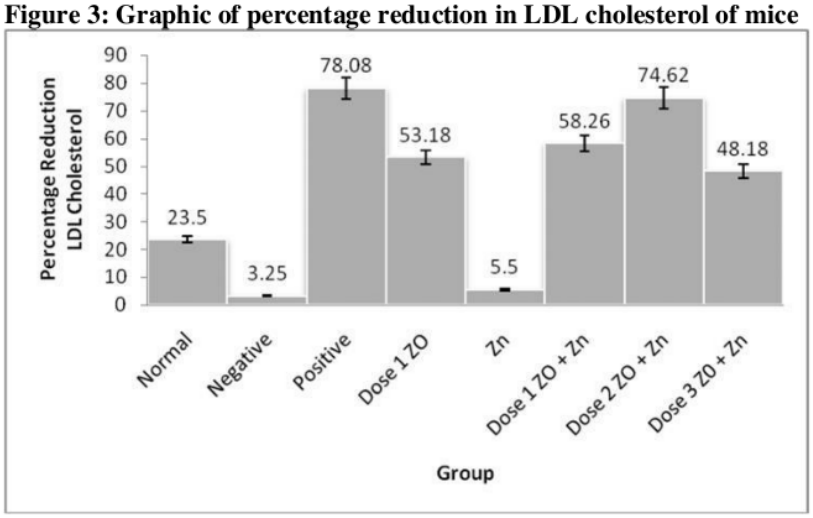
Figure 2: Graphic of Percentage Reduction Total Cholesterol Levels of Mice



Extraction results obtained ginger rhizome yield 6.918%. Based on known phytochemical screening of chemical compounds contained in extracts of ginger are alkaloids, flavonoids, tannins, and triterpenoids. This study used 56 mice were divided into 9 groups. In this study, using three groups: control group of normal control, negative control and positive control. Variations test dose was divided into five groups of different dose variations that ginger extract 37.5 mg/kg, a dose of zinc; 20 mg/kg, a dose 1 ginger extract 37.5 mg/kg +

Zn 20 mg/kg, a dose 2 ginger extract 75 mg/kg + Zn 20 mg/kg, and doses 3 ginger extract 150 mg/kg + Zn 20 mg/kg of each group was given ginger extract for 14 days.

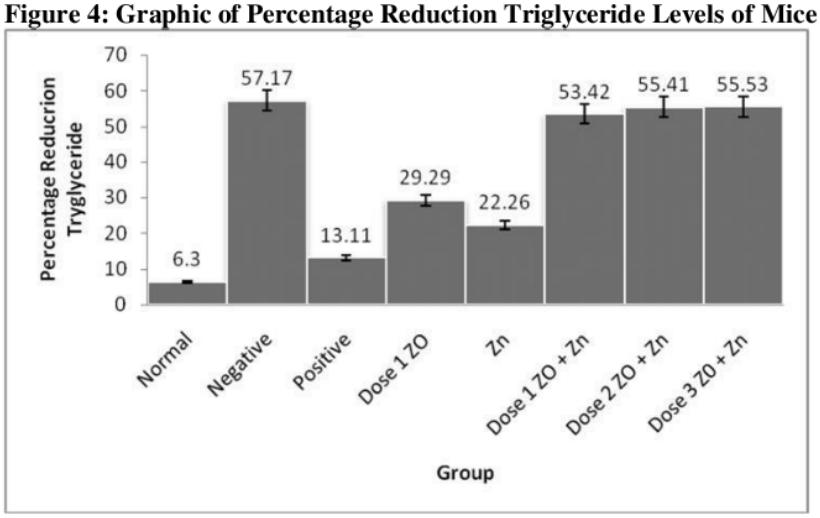
Figure 3: Graphic of percentage reduction in LDL cholesterol of mice



Induction of hyperglycemia in STZ can increase blood glucose levels an average of 97.93 mg/dl to 202.18 mg/dl. Giving combination ginger extract 75 mg/kg and Zn 20 mg/kg shown to lower blood glucose levels mice by 39.87%, with a decrease comparable to the positive control metformin 1.3 mg/20 g BW mice **(Figure 1)**.

The combination of ginger extract 75 mg/kg with zinc 20 mg/kg can lower total cholesterol 65.88% **(Figure 2)** , LDL cholesterol 74.62% **(Figure 3)** and triglyceride levels 55.41% **(Figure 4)**, comparable to atorvastatin 0,114 mg/20g BW mice ($p \geq 0.05$).

Figure 4: Graphic of Percentage Reduction Triglyceride Levels of Mice



This is possible due the extract ginger + zinc can lower blood glucose levels by stimulating the beta cells of the pancreas to produce more insulin or by working directly on the liver (hepatic) and decrease liver glucose production. Decreased glucose levels can also be caused by the effect of ginger extract and zinc which act as antioxidants. Comparison of glucose levels can be seen in Figure 1.

The major pungent component of ginger is gingerol, a mixture of homologues with 10, 12 and 14 carbons in the side chain designated (6)- (8)- and (10)-gingerols. Gingerols can be converted to shogaols and zingerone by dehydration and retro-aldol reaction, respectively. Zingerone and shogaol are found in small amounts in

fresh ginger. These ginger components have been shown to have a variety of pharmacological effects, including anti-inflammatory, anti-emetic, cardiotonic and gastroprotective properties [Zainab *et al* 2006]. Several reports have detailed serotonin receptor-blocking activity of ginger and its components. Since 5-hydroxytryptamine has been reported to induce hyperglycemia in rats, investigated the effect of ginger juice on serotonin-induced hyperglycemia and hyperinsulinemia in normoglycemic rats and reported that ginger juice inhibited this inductive effect. In addition, reported that ginger juice partially alleviated the hyperinsulinemia observed in STZ induced diabetic rats [Zainab *et al* 2006].

This hypoglycemic action of ginger may be due to effects involving serotonin receptors, an increase in pancreatic secretion of insulin from b cells or release of bound insulin. Further work is needed to investigate how ginger lowers glucose levels in diabetic rats. In addition, the results also demonstrate that raw ginger can potentially reverse the nephropathy in STZ-induced diabetic rats. Since urinary albumin levels are a selective marker of glomerular injury and elevated levels of urinary albumin are a harbinger of progressive nephropathy, future studies should focus on the effects of ginger administration on urinary albumin levels. It should be noted that we have previously reported that the doses of ginger used in the present study are not toxic in rats. Therefore, it can be concluded from these studies that raw ginger has significant potential in the treatment of diabetes. Further study is underway to investigate the active component(s) of ginger responsible for the observed beneficial effects in the diabetic condition [Zainab *et al* 2006].

Mode of action of Zingiber officinale on glycemic control: (1) Ginger inhibits enzymes in carbohydrate metabolism. The key enzymes controlling carbohydrate metabolism associated with hyperglycemia and type 2 diabetes are α-amylase and α-glucosidase. (2) Ginger increases insulin release and sensitivity. Ginger promotes glucose clearances in insulin responsive peripheral tissues, which is crucial in maintaining blood glucose homeostasis [Li Yiming *et al* 2012].

Measurement of total cholesterol and LDL cholesterol clinical spectrophotometer. Decrease in total cholesterol and LDL positive control group comparable with ginger extract dose 75 mg/kg + Zn 20 mg/kg. This is possible due the mechanism of cholesterol reduction associated with the ability of the combination of ginger extract and zinc to the inhibition of cholesterol synthesis in the liver enzyme that cholesterol will come out into the intestine, another possibility is because the mechanism of cholesterol reduction associated with the ability of the combination of ginger and zinc binding bile acids and stimulate the secretion of bile so that cholesterol will come out with the bile into the intestine for further discarded and delaying gastric emptying and stimulates insulin secretion and its effect, improve the metabolism of carbohydrates and fats. Ginger also has a protective effect against diabetes complications in the liver, kidneys, eyes and nerves. Increased excretion of bile acids may prevent reabsorption (cholesterol synthesis of bile acids) that occurs reverse blocking synthesis (inhibits the enzyme of hydroxy methyl glutaril synthetase). The state will reduce cholesterol in the blood. Comparison of percent reduction in total and LDL cholesterol levels can be seen in Figure 2 and 3.

Ginger improves lipid profiles with impaired insulin-stimulated glucose metabolism is a common feature in obese and diabetic subjects. It is well established that insulin resistance in peripheral tissues is tightly associated with elevated circulating lipids and tissue lipid accumulation [Li Yiming *et al* 2012].

The measurement results of triglyceride levels obtained ginger dose 1 + Zinc, ginger dose 2 + Zinc and ginger dose 3 + Zinc groups comparable to the positive control group. Comparison of the levels of triglycerides can be seen in Figure 4.

Results from various studies using experimental animals indicate if the free radical damage has occurred, the antioxidants in the body cannot repair it, but only can prevent further damage by preventing oxidation. Therefore, there must be efforts to enhance antioxidant status in the body that can be done by eating foods that contain antioxidant nutrients and non-nutrients such as antioxidants in ginger.

The [6]-gingerol is an effective anti-diabetic agent via its ability to enhance insulin sensitivity and to decrease hyperlipidemia in type 2 diabetic animals. It seems likely [6]-gingerol is beneficial against oxidative stress, thereby being helpful in delaying or preventing complications of diabetes and aging [Amar *et al* 2009]

As an antioxidant, phenolic compounds of ginger capable off chain reactions by means of reacting with lipid radicals, and turn it into a stable product. Zinc can maintain the immune system and stimulate the antioxidant enzymes. Based on the literature [Hadi Sunaryo *et al* 2015] ginger extract plus zinc can lower levels of MDA and increase the activity of SOD and catalase in the liver and blood of mice.

Conclusion

The combination of ginger extract 75 mg/kg and zinc 20 mg/kg can lower blood glucose levels by 39.87 % comparable to metformin not significant. The combination of ginger extract 75 mg/kg and zinc 20 mg/kg can lower total cholesterol 65.88%, LDL cholesterol 74.62%, and triglycerides levels 55.41% comparable to

atorvastatin not significant. The addition of Zn to ginger extract can increase hypolipidemia activity and hypoglycemic activity of ginger extract (*Zingiber officinale* Roscoe).

Acknowledgement

Our thanks to Kopertis Region III Directorate General of Higher Education, Ministry of Education and Culture that has funded this research through competitive grants program year 2013-2014.

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