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The Effect of Cinnamon Bark Extract (*Cinnamomum burmannii*) on Blood *Aspartate aminotransferase* (AST) and *Alanine aminotransferase* (ALT) Levels in Hyperglycemic Rats

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ABSTRACT

Diabetes mellitus is a metabolic disease with hyperglycemia characteristics due to abnormalities in insulin secretion. Hyperglycemia conditions in diabetics can cause increased production of Reactive Oxygen Species or excessive free radicals and will trigger oxidative stress. One organ that has a risk due to hyperglycemia, for example, is liver damage. Cinnamon bark plant (Cinnamomum burmannii) is one of the plants that contain antidiabetics and antioxidants. The purpose of this study was to determine the effect of cinnamon bark extract on AST and ALT levels in hyperglycemic rats. This study is an experimental study with the design of The Post Test – Only Control Group using 30 rats divided into five groups, namely negative control group (K-), positive control group (K+), treatment group 1 (Dose 100 mg/kgBW), treatment 2 (Dose 200 mg/kg BW) and treatment 3 (Dose 300 mg/kgBW) then continued administration of cinnamon bark extract (Cinnamomum burmannii) for 30 days. Examination of AST and ALT levels is measured using an enzymatic kinetic method spectrophotometer. Data were analyzed with One-Way ANOVA and Post-Hoc Bonferroni. The results showed that cinnamon extract can reduce AST, ALT, and blood glucose levels at low doses of 100 mg/kgBW as evidenced by a P value of < 0.05 meaning that cinnamon extract (Cinnamomum burmannii) can reduce AST and ALT levels in hyperglycemic rats.

Keywords: Hyperglycemia, Cinnamon Bark Extract, AST, ALT.

INTRODUCTION

Diabetes mellitus (DM) is one of the biggest global health diseases of the 21st Century. The International Diabetes Federation predicts that there are 463 million people experiencing Diabetes mellitus in the world, namely the age range of 20-79 years in 2015 there were 415 million, and it is expected to increase again in 2014 to 642 million. Prevalence of Diabetes mellitus in the world in 2015, one in eleven adults have diabetes mellitus, judging from the aspect of gender, men still have a higher prevalence rate than women, and seen from the environmental aspect people living in urban areas have a higher prevalence than people living in rural areas [(1).]

Diabetes mellitus is a metabolic disease with characteristics of hyperglycemia that occurs due to abnormalities in insulin secretion. Glucose is formed in the liver

from food consumed normally and then circulates in a certain amount in the blood. Insulin is a hormone produced in the pancreas that controls glucose levels in the blood by regulating its production and storage [(2)]. Hyperglycemia in diabetics occurs due to the inability to secrete insulin, insulin action, or both. Increased blood glucose can cause excessive production of Reactive Oxygen Species (ROS) or free radicals and will trigger oxidative stress. Oxidative stress will cause damage to pancreatic beta cells which can lead to diabetes [(3)].

Diabetes mellitus is a metabolic disease with characteristics of hyperglycemia that occurs due to abnormalities in insulin secretion. Glucose is formed in the liver from food consumed normally and then circulates in a certain amount in the blood. Insulin is a hormone is one of the experimental animals used for experimental research where alloxan can induce diabetes in experimental animals, the administration of alloxan is one of the fastest ways Normally it circulates in a certain amount in the blood.

Insulin is a hormone that is one of the experimental animals used for experimental research where alloxan can induce diabetes in experimental animals, the administration of alloxan is one of the fastest ways to produce experimental diabetic conditions (hyperglycemia) in experimental animals. aloxan effectively damages the beta cells of Langerhans islets characterized by a reduction in the diameter of Langerhans islet cells and impaired beta cell function so that it is no longer able to increase insulin secretion leading to an increase in glucose levels in the blood [(4)].

Uncontrolled hyperglycemia can cause damage to systems in the body, which will lead to complications. The treatment of Diabetes mellitus that has been used using synthetic drugs and insulin injections can cause long-term complications and abnormalities in several organs [(3)]. In the United States, diabetes mellitus is one of the causes of liver disease, diabetes is also an indication for liver transplantation. Diabetes mellitus is a risk for liver disorders such as cirrhosis of the liver, 30% of people with the disease have a clinical abnormality of Diabetes mellitus. Diabetic patients are at risk of organ complications such as acute liver disease, chronic liver disease, and hepatocellular carcinoma [(5)].

In hyperglycemia, liver damage is caused by hepatic cell death induced by oxidative stress, which can trigger an increase in oxidative stress and cause injury to the liver [(6)]. The liver can not detect the presence of glucose in the blood in hyperglycemia, this makes the liver produce glucose continuously. To meet the energy needs in the formation of glucose, fatty acids will be broken down to form energy. This will increase the oxidation of free fatty acids and cause fat accumulation in the liver. An increase in free radicals will cause DNA mutations and trigger the production of *Reactive Oxygen Species* [(7)].

Increased levels of Reactive Oxygen Species can induce inflammation and necrosis in liver tissue. Liver tissue that is inflamed and necrosis will stimulate liver cells to produce collagen for the process of forming liver fibrosis. Severe liver fibrosis will progress to the stage of liver cirrhosis and will then lead to the occurrence of hepatocellular carcinoma, liver damage can be detected through liver function tests [(7)]. Liver function tests are needed to help in the diagnosis of patients, especially in patients with Diabetes mellitus with impaired liver function. The necessary liver function test examination includes a specific examination for inflammation of the liver parenchyma, namely, *Serum Glutamic Oxaloacetic Transaminase* (SGOT) or by other names *Aspartate aminotransferase* (AST) and *Serum Glutamic Pyruvic Transaminase* (SGPT) or other names *Alanine aminotransferase* (ALT) aims to determine the inflammation that occurs in the body and is usually an indication of a disorder (inflammation) in the liver [(8)].

The enzymes *Aspartate aminotransferase* and *Alanine aminotransferase* are related to the parenchyma of liver cells, the difference, *Aspartate aminotransferase* is found in the liver, heart (heart muscle), skeletal muscle, kidneys, and brain, and erythrocytes,

whereas *Alanine aminotransferase* is found more abundantly in the liver, (clinically the amount of low concentrations is ignored and found in the kidneys, heart, and skeletal muscles) therefore *Alanine aminotransferase* is a more specific indicator of liver inflammation than *Aspartate aminotransferase*. *Aspartate aminotransferase* can be elevated in diseases that can affect other organs, such as myocardial infarction, acute pancreatitis, and anemia [(8)].

To avoid the side effects of anti-diabetic drugs and improve liver function, traditional medicine can be given as one of the alternative therapies that can work as hypoglycemic and hypolipidemic. In addition to being cheap, traditional medicine also has minimal side effects. One of the traditional ingredients that can lower blood glucose levels and lipid profile levels is *Cinnamomum burmannii* or cinnamon. Cinnamon has a bioactive component of the polyphenol group that has activities. To avoid the side effects of anti-diabetic drugs and improve liver function, traditional medicine can be given as one of the alternative therapies that can work as hypoglycemic and hypolipidemic. In addition to being cheap, traditional medicine also has minimal side effects. One of the traditional ingredients that can lower blood glucose levels and lipid profile levels is *Cinnamomum burmannii* or Cinnamon. Cinnamon has a bioactive component of the polyphenol group that has activities similar to insulin (*mimetic insulin*). This bioactive component is *doublylinked procyanidin type-A polymerase* which is part of *catechins* or *epicatechins* which are hereinafter referred to as *methylhydroxychalcone polymers* (MHCP). Research conducted on bark extracts sweet (*Cinnamomum sp*.) with a dose of 200 mg/kgBW within 30 days to have a significant effect on lowering blood glucose levels [(9)].

In addition, cinnamon bark extract with *trans-cinnamaldehyde* levels is a source of antioxidant compounds with its ability to capture free radicals or *radical scavengers* to improve liver function. Cinnamon is a spice plant that contains many compounds that are useful for humans. In cinnamon, many phytochemical compounds from the *phenylpropanoids* class are found in the form of *cinnamic acid*. This compound functions as an antioxidant that can prevent the formation of free radicals, remove radicals before damage appears, repair oxidative damage, and eliminate damaged molecules inside the cell. Antioxidant compounds can be used to inhibit or slow down the oxidation process. One of the oxidation processes in the body is due to frequent consumption of drugs. Drugs are one of the indirect inducers of the formation of Reactive Oxygen Species which are then causing mitochondrial dysfunction [(10)].

MATERIALS & METHODS

Materials

Alloxan; 96% ethanol; Sterilized water injection; Aquades (Aquabidest); 70% alcohol; Handschoen; blood; SGOT Reagent; SGPT Reagent; Cinnamon bark ethanol extract; Rat (Rattus norvegicus)

Method

This research is an experimental laboratory research using the Post Test-Only Control Group design which uses experimental animals, namely male Wistar rats as research objects. The research sample was 40 rats which were then divided into 5 experimental groups, namely, negative control group (K-), positive control group (K+) alloxan induction, treatment group (P1) dose of 100 mg/KgBW, treatment group (P2) dose 200 mg/KgBW, treatment group (P3) dose 300 mg/KgBW.

1) Alloxan Induction

The induction process for experimental animals (male rats) will begin by restricting feed for 30 hours before being injected. The rats were not given food but, only given water to drink. After the fasting period, the rats will be manually detained and receive an intraperitoneal injection of 100 mg/kg BW alloxan in the lower right abdomen of the rats correctly. Next, the rats are placed back in their cages with water and *ad libitium* commercial feed [(11)]. Next, the mice

were placed back in their cages and given standard food and water. After 7 days, the mice that were induced with alloxan had their blood glucose levels checked to determine the hyperglycemic effect before being given treatment.

1) Making cinnamon bark extract

Cinnamon bark extract is made using the maceration method with a mixture of 96% ethanol. The cinnamon bark is first dried and ground, then 1,000 gr of cinnamon bark powder is soaked in 96% ethanol with a volume of 1,000 ml for 72 hours at room temperature until it settles. After three days the soaking results were put into an evaporation flask. The evaporation flask is installed on the evaporator and fill the water bath with water until it is full. All series of equipment are installed including the rotary evaporator, water bath heater (set to a temperature of 60 °C), connected to electricity. Then wait until the ethanol solution separates from the active substance that is already in the evaporation flask, then leave it until the ethanol flow stops dripping into the collection flask (± 1.5 to 2 hours for one flask). The extraction results are put into a glass bottle and stored in the refrigerator or freezer [(12)].

2) Examination of blood AST levels

Prepare all the necessary tools and materials, pipette into the test tube mixed reagent (R1+R2), Blank 100 ul and sample or control 100 ul, then put 1000 ul of AST mono reagent into the test tube, and add 100 ul of sample serum, then put it into a test tube that has filled the reagent. Homogenized and incubated for 5 minutes after which it was measured using a spectrophotometer with wavelength 340 nm, readings are taken at minutes 1,2,3, and after that the AST level results from the serum samples examined.

3) Examination of blood ALT levels

Prepare all the tools and materials that will be needed, pipette into the test tube mixed reagent (R1+R2), 100 ul blank and 100 ul sample or control, then put 1000 ul of ALT mono reagent into the test tube, and add 100 ul of sample serum, then put it into a test tube that has filled the reagent. Homogenize and incubated for 5 minutes after which measured using a spectrophotometer with wavelength 340 nm, the reading is taken at minute 1,2,3, and after that record ALT levels from the serum samples are examined.

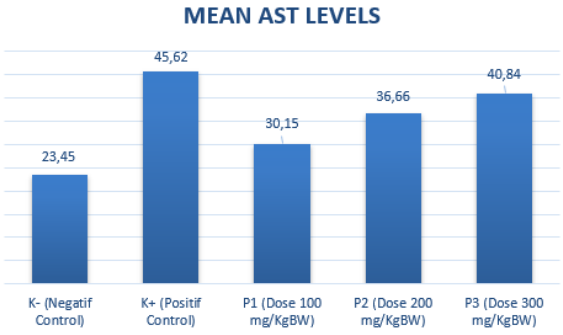
STATISTICAL ANALYSIS

Data were analyzed with the SPPS-16.0 program. For data that is numerical and categorical, the Shapiro-Wilks test will be used to determine the normality of the data as a condition for using One-Way ANOVA.

RESULT

Effect of Cinnamon Bark Extract (*Cinnamomum burmannii*) on Blood *Aspartate aminotransferase* (AST) Levels

The results of measuring AST levels in hyperglycemic rats given cinnamon bark extract (*Cinnamomum burmannii*) can be seen in the following bar diagram.



Based on the diagram above, it can be seen that the average blood AST level given cinnamon bark extract was the lowest in treatment group 1 (dose 100 mg/KgBW), namely 30,15 compared to treatment group 2 (dose 200 mg /KgBW) and treatment 3 (Dose 300 mg/KgBW).

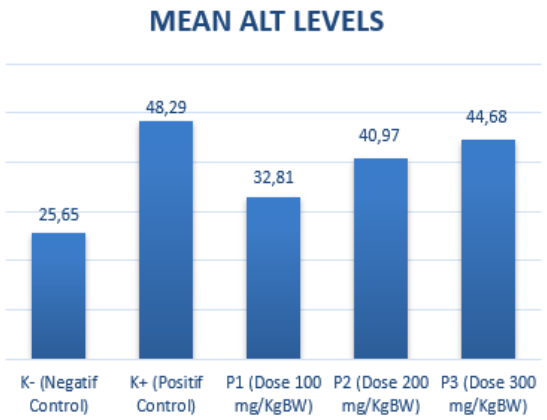
Next, a one-way ANOVA test was carried out on blood AST levels in rats.

Group	Mean	Std. Error	P *Value*
Negatif Control (-)	23,46	2,399	
Positif Control (+)	45,62	2,238	
P1 (Dose 100 mg/KgBW)	30,16	1,954	,000
P2 (Dose 200 mg/KgBW)	36,67	2,493	
P3 (Dose 300 mg/KgBW)	40,84	2,775	

Based on the results of statistical analysis, cinnamon bark extract had a significant effect on the blood of Aspartate aminotransferase (AST) levels in hyperglycemic rats ($p < 0.05$). Based on the results of the analysis, it can be concluded that cinnamon bark extract affects blood AST levels in hyperglycemic rats.

Effect of Cinnamon Bark Extract (*Cinnamomum burmannii*) on Blood *Alanine aminotransferase* (ALT) Levels

The results of measuring ALT levels in hyperglycemic rats given cinnamon bark extract (*Cinnamomum burmannii*) can be seen in the following bar diagram.



Based on the diagram above, it can be seen that the average blood ALT level given cinnamon bark extract was the lowest in treatment group 1 (dose 100 mg/KgBW), namely 32,81 compared to treatment group 2 (dose 200 mg /KgBW) and treatment 3 (Dose 300 mg/KgBW). Based on the above results, it can give an idea that the administration of cinnamon bark extract that can reduce ALT levels in mice with hyperglycemia is the most effective in low doses of 100 mg/KgBW.

Next, a one-way ANOVA test was carried out on blood ALT levels in rats.

Group	Mean	Std. Error	P *Value*
Negatif Control (-)	25,65	2,040	
Positif Control (+)	48,29	2,555	
P1 (Dose 100 mg/KgBW)	32,81	1,988	,000
P2 (Dose 200 mg/KgBW)	40,98	2,696	
P3 (Dose 300 mg/KgBW)	44,69	2,049	

The results of the statistical analysis of One Way ANOVA in all five groups of mice were ($P < 0.05$). These results showed significant differences between the groups, and it can be assumed that cinnamon bark extract can affect blood ALT levels in mice.

DISCUSSION

The results of measuring the effect of cinnamon bark extract on AST and ALT levels in hyperglycemic rats given cinnamon bark extract, in the treatment groups 1 and 2 rats experienced a significant decrease compared to the positive control group, in the rat group treatment 3 The average value of AST and ALT levels has increased again, this show that the lower the concentration of the dose of cinnamon bark extract given, it will have a good effect to maintain AST levels at normal values. The decrease in AST and ALT levels at low doses of 100 and 200 mg/KgBW, shows that the content of flavonoids and polyphenols in cinnamon bark extract (*Cinnamomum burmannii*), has a protective effect on the liver against free radicals sourced from loans, on the other hand, in high doses of extract, namely 300 mg/KgBW, there is an increase in AST and ALT levels, this shows that cinnamon bark extract (*Cinnamomum burmannii*) has a hepatotoxic effect on the liver.

The liver is susceptible to damage caused by various toxic compounds. The causes of liver damage vary. Most of the causes of the damage are due to viruses that spread fecal-oral, parenteral, perinatal, sexual, etc. Causes other than viruses are toxic compounds such as drugs, toxins, fungi, and alcohol. The use of drugs, one of which is the effect of administering herbal medicines

in high doses continuously for a long period and with high doses can trigger liver damage which results in changes in the histological of the liver organ such as fat degeneration and cell necrosis resulting in permanent damage and cell death [13].

Cinnamon is generally well tolerated however, in large doses, it can cause hepatotoxicity due to the coumarin content, This is by the opinion [14], stating that the bark extract of cinnamon bark extract in high doses shows potential for nephrotoxicity and hepatotoxicity because it contains coumarin compounds in high intensity. Administration of cinnamon bark extract at low doses showed a better effect than high doses. Various substances have an optimal range of doses and the potential for toxic effects to occur at high doses.

One of the phenolic compounds in cinnamon is coumarin. Kumarin is a class of compounds that represent the 2H-1-*benzopyr*-2-*on derivative*. These compounds are widely found in several plants, including vegetables, herbs, fruits, and medicinal plants [15]. Excess coumarin compound content can cause hepatic damage.

Combined is metabolized through the main pathway 3,4-epoxide as well as the 7-*hydroxylation* pathway leading to detoxification. The 7-*7-hydroxylation pathway* in humans is generally metabolized by CYP2A6. These metabolisms produce *7-hydroxylamine* and o-HPAA causing hepatotoxicity [16]. Generally, these compounds are at levels that are safe to consume at certain levels [17].

Health risks may also occur in the consumption of large amounts of cinnamon with high coumarin content over a relatively long period. This is because the absorption of coumarin in cinnamon tea has almost the same absorption value as isolated coumarin [16].

The administration of aloxan is known to cause damage to liver function. Studies show that aloxan can cause morphological damage to the liver, especially to liver tissue and other organs [17]. Administration of alloxan to aloxane-induced diabetic rats can also increase blood glucose levels and cause changes in liver tissue [18]. In addition, aloxan at certain doses can cause damage to the liver organ [19].

Health disorders in the liver organ occur due to toxic compounds or free radicals, so various enzymes in the cytosol will enter the blood circulation and cause a difference in permeability in the cell membrane so that the level of transaminase enzymes in the blood will increase. The increase in the levels of AST and ALT enzymes in serum is caused by the destruction of cells containing the enzyme transaminase so that the enzyme transaminase enters the blood circulation. The levels of ALT and AST enzymes can reach 20-100 times the normal limit [20].

Administration of cinnamon bark extract at doses of 100 and 200 mg/KgBW can affect ALT levels and can have a beneficial effect by stabilizing membranes In addition, administration of extracts at high doses of 300 mg/KgBW can cause a state of necrosis, ALT enzymes can increase due to rupture of liver cells.

Cinnamon can inhibit liver damage through *peroxisome proliferator-activated receptor-gamma* (PPARY). Inactivation of PPAR in hepatocytes is associated with increased body weight and liver, as well as decreased serum AST enzyme levels In addition, PPARY activation is associated with other beneficial effects such as decreased regulation of proinflammatory cytokine expression, such as *Tumor Necrosis Factor-Alpha* (TNF-α), interleukin-6 (IL-6), dan C-reactive. Protein (CRP), is involved in its pathogenesis *Non- Alcoholic Fatty Liver Disease* (NAFLD) [21].

Oxidative stress and immune system disorders play a role in contributing to liver dysfunctions such as *Non-Alcoholic Fatty Liver Disease* (NAFLD). In cinnamon, it can increase oxidative stress and prevent NAFLD by decreasing the production of reactive substances of oxygen species, liver protein expression from oxidative stress, pro-inflammatory cytokines, and chemokines. NAFLD also binds to liver insulin resistance and adipose tissue as well as decreased insulin sensitivity throughout the body. Cinnamon can increase insulin resistance by increasing the regulation of glucose transporter 4 in peripheral tissues,

stimulating the release of insulin from pancreatic beta cells, and acting on adenosine monophosphate-activated protein kinase in peripheral tissues and liver, therefore in cinnamon content this mechanism can increase liver enzymes [21]. cinnamon is safe for human consumption, with limits a maximum of 5 mg/Kg [22], the content components contained in cinnamon bark *of Cinnamomum burmannii* are transcinamaldehid (60.17 %), eugenol (17.62 %) and coumarin (13.39 %) [15]. In addition, an increase in blood sugar levels at high doses indicates that cinnamon extract contains a high concentration of antioxidants, antioxidants at high concentrations have hepatotoxic properties and can damage the liver causing toxicity [23]. However, it should be noted that excessive consumption of cinnamon can cause some side effects as follows: increased heart rate and danger of bleeding, skin irritation, and interactions with antibiotics.

CONCLUSION

Based on research that has been carried out, cinnamon bark extract (*Cinnamomum burmannii*) has effect on reducing on blood *Aspartate aminotransferase* (AST) and *Alanine aminotransferase* (ALT). The best reduction in AST and ALT levels is at a low dose, namely 100 mg/KgBW.

Declaration by Authors

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