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Effect of Sorghum (*Sorghum Bicolor L.)* Ethanol Extract in Preventing Significant Increased Blood Glucose Levels, Interleukin-6 (IL-6) and Malondialdehyde (MDA) in White Rats (*Rattus Norvegicus*) Wistar Male Strain with Nicotinamid-Streptozotocin Induced Diabetes Mellitus

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ABSTRACT

Introduction: The prevalence of type 2 diabetes mellitus in recent years continues to increase worldwide due to various factors such as lifestyle, lack of physical activity, and obesity. Sorghum (Sorghum bicolor L.) has a role as an antioxidant in preventing diseases caused by inflammation and oxidative stress. This study aimed to analyze the effect of ethanol extract of sorghum (Sorghum bicolor L.) in preventing increased blood glucose levels, inflammation, and oxidative stress in male Wistar strain white rats with diabetes mellitus induced by nicotinamide-streptozotocin. ***Methods:*** This study was an experimental post-test-only control group design using male rats as subjects. The sample was divided into 4 groups: the placebo group (P0), the negative control group which received placebo (P1), treatment group 1 (P2) which received ethanol sorghum extract 600mg/kg body weight, treatment group 2 (P3) which received ethanol sorghum extract 800mg/kg body weight. Each group was induced to NA-STZ to make the male rats in hyperglycaemic states. Then the blood samples were taken and examined for IL-6 levels, MDA levels, and glucose levels. ***Results:*** The average blood glucose level in the P3 group was not significantly lower than that in P2 ($p=0.805$). The mean IL-6 level in the P3 group showed significantly lower results compared to the P0 ($p<0.001$) and P2 groups ($p<0.001$). The mean MDA level in the P3 group showed significantly lower results compared to the P0 ($p=0.001$) and P2 groups ($p=0.001$). ***Conclusion:*** The ethanol extract of sorghum (Sorghum bicolor L.) can prevent the increase in blood glucose, IL-6, and MDA levels in white rats (Rattus norvegicus) male Wistar strain diabetes mellitus induced by nicotinamide-streptozotocin.

Keywords: sorghum ethanol; blood sugar; IL-6; MDA

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease caused by metabolic disorders. The prevalence of DM in Indonesia in 2019 was 10.7 million aged 20-79 years. [1] The prevalence of diabetes mellitus, especially type 2, in recent years, has continued to increase worldwide due to various factors, especially lifestyle, lack of physical activity, and obesity.[2] Imbalance in glucose homeostasis due to type 2 DM slowly affects and causes the degeneration of various organs which eventually leads to aging.[3]

Free radicals, especially reactive oxygen species, are formed in excess in DM through glucose oxidation and non-enzymatic protein glycation.[4] ROS at high concentrations can cause oxidative damage to biomacromolecules such as lipids, proteins, and DNA. Endogenous antioxidant systems are needed to fight damage caused by ROS. Lipid oxidation produces many secondary products including malondialdehyde (MDA), a toxic molecule that is widely used as a biomarker of oxidative stress.

Uncontrolled type 2 DM is associated with increased levels of various proinflammatory cytokines, including interleukin-6 (IL-6) levels which are associated with DM and its complications such as obesity, cardiovascular disease, and chronic kidney disease. [5]

Sorghum (Sorghum bicolor L.) is the fifth staple food after wheat, corn, rice, and barley. Pigmented sorghum seeds such as red sorghum contain high antioxidants such as polyphenols, especially tannins which have various roles as antioxidants in preventing diseases caused by inflammation and oxidative stress.[6]

The purpose of this study was to analyze the effect of the administration of ethanol extract of sorghum (*Sorghum bicolor L.)* in preventing increased blood glucose levels, inflammation, and oxidative stress in white rats (*Rattus norvegicus*) male Wistar strain diabetes mellitus induced by nicotinamide-streptozotocin (NA-STZ).

METHODS

This study was an experimental post-test-only control group design using male rats, aged 2-3 months, with a body weight of 200-250 grams. The material used in this study was NA-STZ. The sample was divided into 4 groups: the placebo group (P0), the negative control group which received placebo (P1), treatment group 1 (P2) which received ethanol sorghum extract 600mg/kg body weight, treatment group 2 (P3) which received ethanol sorghum extract 800mg/kg body weight. Each group was induced to NA-STZ to make the male rats in hyperglycaemic states. Then the blood samples were taken and examined for IL-6 levels, MDA levels, and glucose levels. Data is recorded and analyzed.

RESULTS

The subjects used in this study were 30 male Wistar rats, aged 2-3 months, with a body weight of approximately 200-250 grams. The variables observed in this study were IL-6, MDA, and blood glucose levels. The normality of IL-6, MDA, and blood glucose levels was tested using the Shapiro-Wilk test. The data was normally distributed with a p-value> 0.05. The result in each group were shown in Table 1.

TABLE 1: Descriptive Analysis of Variable Blood Glucose, IL-6, and MDA levels.

Variables	Groups	n	Mean	SD	Min	Max
Blood glucose	P0	7	92,71	13,07	79,00	116,00
	P1	7	470,00	106,69	335,00	600,00
	P2	7	327,86	125,16	167,00	480,00
	P3	7	300,71	127,74	119,00	426,00
IL-6	P0	7	4,05	0,30	3,74	4,55
	P1	7	5,83	0,29	5,50	6,29
	P2	7	3,99	0,22	3,72	4,45
	P3	7	2,54	0,21	2,33	2,93
MDA	P0	7	0,57	0,04	0,49	0,61
	P1	7	0,95	0,02	0,91	0,98
	P2	7	0,52	0,04	0,49	0,59
	P3	7	0,20	0,01	0,19	0,20

Analysis of the effect of treatment between groups after administration of sorghum ethanol extract on blood sugar, IL-6, and MDA levels was assessed by the Kruskal-Wallis test.

The results of the significance analysis of the Kruskal-Wallis test obtained a p-value <0.001. To find out the comparison of mean blood glucose levels that differ significantly between groups, it is necessary to carry out a Mann-Whitney test.

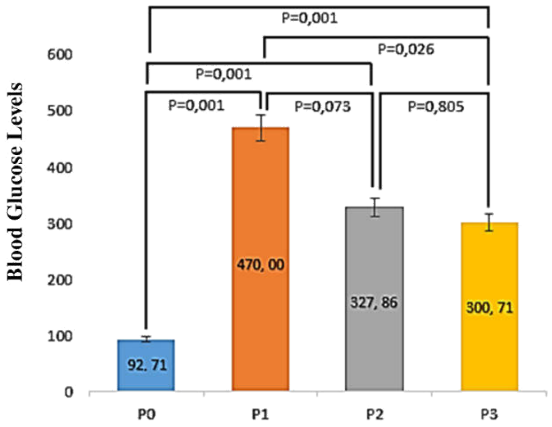

FIGURE 1: Comparison of Mean Blood Glucose Levels between Groups.

The results of the study are presented in Figure 1. It shows that the mean blood glucose level to P0 was significantly lower than P1 (p=0.001), P2 (p=0.001), and P3 (p=0.001). The average blood glucose levels in the P2 and P3 groups compared to P1 showed that the mean results for P2 were not significantly lower (p=0.073), while the average for P3 was significantly lower (p=0.026). The average blood glucose level in the P3 group was not significantly lower than that in P2 (p=0.805).

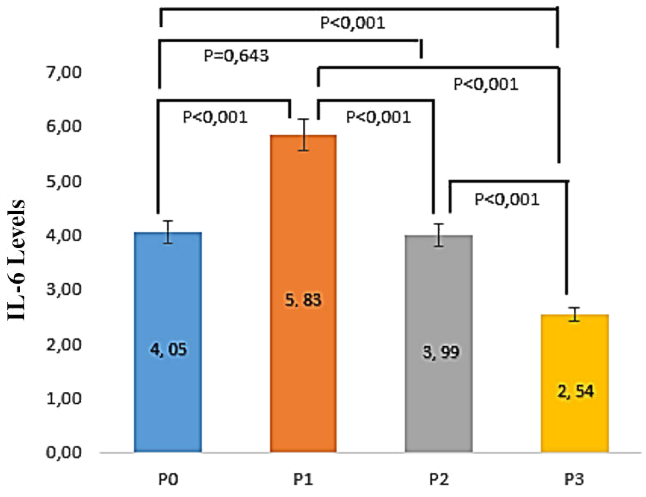

FIGURE 2: Comparison of Mean IL-6 Levels between Groups.

The results of the study are presented in Figure 2 showing that the mean IL-6 level in the P1 group was significantly higher than that in the P0 (p<0.001), P2 (p<0.001), and P3 (p<0.001) groups.

A comparison of the mean IL-6 levels in the P0 and P2 groups did not show a significant difference (p=0.643). The mean IL-6 level in the P3 group showed significantly lower results compared to the P0 (p<0.001) and P2 groups (p<0.001).

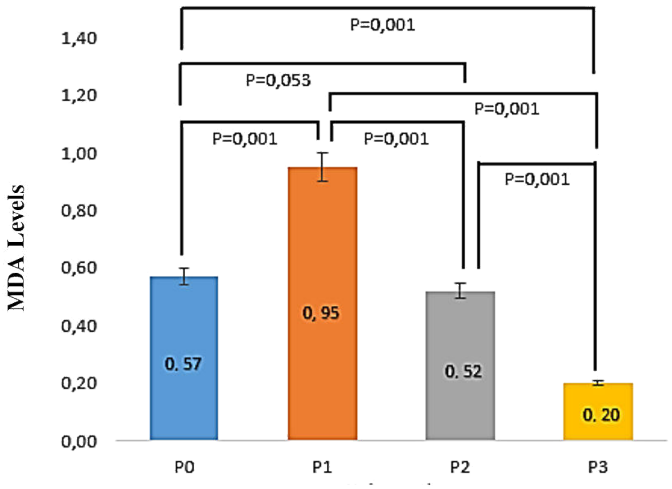

FIGURE 3: Comparison of Mean MDA Levels between Groups.

The results of the study are presented in Figure 5.3 showing that the mean MDA level in the P1 group was significantly higher than that in the P0 (p=0.001), P2 (p=0.001), and P3 (p=0.001) groups. The mean MDA level of the P0 group compared to the P2 did not show a significant difference (p=0.053). The mean MDA level in the P3 group showed significantly lower results compared to the P0 (p=0.001) and P2 groups (p=0.001).

DISCUSSION

Administration of the NA-STZ combination causes partial damage to pancreatic β cells such as type 2 diabetes mellitus. Damage to pancreatic β cells causes a disturbed balance of insulin and glucagon where insulin production decreases, and excess absolute or relative glucagon levels cause higher hepatic glucose production resulting in hyperglycemia.[7]

Administration of sorghum ethanol extract to the study groups P2 and P3 showed a lower average blood glucose level than the P1 group with the mean at T3 showing significant results, but P2 not showing significant results. This shows that the administration of sorghum ethanol extract can prevent an increase in the average blood glucose level in the P3 group given a dose of 800 mg/kg BW. Similar results of administration of sorghum extract were shown in another study where increased plasma glucose and triacylglycerol levels in DM rats showed significantly lower results in DM rats given 250 mg/kg BW of sorghum extract.[8] Research by giving sorghum flour 5 grams/day lowered fasting blood glucose levels in DM rats.[9]

Kim & Park's research, DM rats given sorghum extract with 80% fermented ethanol found lower blood glucose levels

in DM rats given a dose of 0.6 g/kg BW sorghum extract compared to DM rats who were not treated, but the results were not significant at a dose of 0.4 g/kg BW.[10] The difference in the effectiveness of sorghum extract at a similar dose of 600 mg/kg BW from this study compared to Kim & Park's research could be due to differences in extract solvents where Kim & Park used 80% fermented ethanol. Fermentation techniques can effectively increase the amount of nutrients from a food ingredient.[11]

Sorghum is rich in bioactive compounds such as polyphenols including phenolic acids, flavonoids, 3-deoxyanthocyanidins, tannins, and phytic acids.[12] Another ingredient in sorghum is phenolic acid.[13] Sorghum phenolic works to inhibit liver gluconeogenesis enzymes thereby increasing endogenous insulin sensitivity.[14] The sorghum ethanol extract in this study had a phenol content of 6351.26 mg/100 g, this level was higher than avocado leaf 6.42 mg/100 g, kratom stem extract 2359 mg/100 g, and senggani flower extract 4397 mg/100 g.[15]

From this study, the P2 and P3 groups that received sorghum ethanol extract showed significantly lower mean IL-6 levels compared to the P1 group that did not receive treatment. The mean IL-6 level in the P2 group compared to P0, which is the normal group, did not show a significant difference, while the mean in the P3 group was significantly lower than that in P0. The results showed that the administration of sorghum ethanol extract prevented an increase in the mean IL-6 levels in the P2 and P3 groups. Comparison of the results of the P0 and P2 groups did not differ significantly, so it can be concluded that the administration of sorghum ethanol extract not only prevented an increase in IL-6 levels in P2 but could reduce it to reach IL-6 levels in the P0 group, even in the P3 group which had a significantly lower average than P0 and P2. This shows the potential of sorghum ethanol extract in DM in reducing IL-6 with greater potency at higher extract doses.

This is consistent with research conducted by Rhodes & Kresovich regarding the effect of sorghum ethanol extract at doses of 0, 15, 30, and 60 μg/mL on mouse macrophages stimulated with lipopolysaccharide on cell viability levels, TNF-α and IL-6 which showed results significantly decreased levels of TNF-α and IL-6.[16] The results of a study conducted by Hong et al stated that there was the suppression of inflammation from the activity of sorghum extracts where there was a decrease in NO, IL-6, and ROS production. The anti-inflammatory activity is more correlated with the total tannins than the phenolic extract of sorghum. The tannin-rich sorghum extract has inhibitory activity against the hyaluronidase enzyme which is associated with inflammation.[17]

The mean MDA level in the P2 group compared to P0 did not show a significant difference, in the P3 group, it was significantly lower than P0 and P2. The results of the study showed that the administration of sorghum ethanol extract prevented the increase and could reduce the average MDA level. The P3 group showed a decrease in the average MDA level compared to the P0 group. This shows that the dose of the extract affects the magnitude of the decrease in MDA levels.

Research with 60% ethanol extract of sorghum was able to have the effect of inhibiting MDA production.[18] The decrease in MDA levels in this study was due to the antioxidant properties of sorghum extract which is rich in bioactive compounds such as polyphenols including phenolic acids and flavonoids, 3-deoxy anthocyanidin, tannins, and phytic acid.[12]

The tannin content that can be found in sorghum acts as an antioxidant scavenger to fight free radicals, contributing to the prevention of chronic diseases such as cardiovascular disease and cancer. Tannins work to chelate hydrogen ions and inhibit lipid peroxidation by inhibiting cyclooxygenase activity.[19] Hydroperoxides have toxic effects on cells either directly or through degradation into hydroxyl radicals which are highly toxic and can react with transition metals such as iron or copper to form stable aldehydes such as MDA which damage cell membranes. Flavonoids can work to donate hydrogen ions to bind to lipid peroxyl radical species to become non-radical products to neutralize free radicals. [18]

CONCLUSION

The ethanol extract of sorghum (Sorghum bicolor L.) can prevent the increase in blood glucose, IL-6, and MDA levels in white rats (Rattus norvegicus) male Wistar strain diabetes mellitus induced by nicotinamide-streptozotocin.

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