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The Comparison Effect between Metformin, Turmeric Extract, and its Combination on Blood Sugar, Mda, and 8-OHdG Levels (Experimental Study in Streptozotocin-Nicotinamide-Induced Type 2 DM Wistar Male Rats)

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Abstract: Patients with type 2 DM experience excessive oxidative stress, resulting in hyperglycemia, and increased lipid and DNA modifications. Interaction of bilayer lipids with reactive oxygen produces lipid peroxidases, forming the end product malondialdehyde (MDA). DNA damage is identified by an increase in 8-hydroxydeoxyguanosine (8-OHdG). Curcumin compounds in turmeric extract can inhibit the increase of Reactive Oxygen Species (ROS). This study aims to prove the different effects of metformin, turmeric extract, and the combination of metformin and turmeric extract on blood sugar levels, MDA, and 8-OHdG in male rats (Wistar strain) induced by Streptozotocin-Nicotinamide. This study is experimental with a post-test-only control group design. The research subjects were 24 Wistar rats and were randomly divided into four groups and induced with Streptozotocin-Nicotinamide. KI is the negative control group, KII was given Metformin 45mg/KgBW/day, KIII group was given a Turmeric extract dose of 200mg/KgBW/day, and KIV was given a combination of turmeric extract dose of 100mg/KgBW/day and Metformin 22.5mg/KgBW/day for 14 days. Blood samples in the ophthalmic vein were taken for blood sugar, MDA, and 8-OHdG examination. The decrease in blood sugar, MDA, and 8-OHdG levels in the KIV group was found to be the lowest, followed by KIII, KII, and KI. One Way Anova test on blood sugar levels, MDA, 8-OHdG showed significant differences between groups with a p-value ≤ 0.000. The administration of a combination of metformin and turmeric extract can reduce blood sugar, MDA, and 8-OHdG levels compared with a single dose of Metformin or Turmeric extract.

KEYWORDS: Turmeric extract, blood sugar, MDA and 8-OHdG

I. INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia that occurs due to abnormalities in insulin secretion, decreased sensitivity of insulin receptors or both1. Type 2 DM starts with the inability of body cells to fully respond to insulin receptors, this situation is called "insulin resistance"2. The causes of type 2 DM are strongly associated with overweight/obesity, increasing age, physical inactivity, certain races, and family history2. Type 2 DM accounts for 90-95% of all diabetes2. According to the basic health research report (RISKESDAS) conducted in 2018, Indonesia experienced an increase in the number of DM patients by 10.9%71. The pathophysiology of type 2 DM is due to insulin resistance in the muscles and liver and damage to pancreatic beta cells. Schawrtz 2016, states that there are 11 important organs that mediate impaired glucose tolerance called the "egregious eleven"3. Excessive Superoxide Anion Radical (O2-) production in the mitochondrial electron transfer chain activates classic metabolic events evidenced in the course of DM, Malondialdehyde (MDA) is a highly toxic byproduct formed in part by lipid oxidation derived from free radicals4. The two most prevalent ROS that can profoundly affect lipids are mainly hydroxyl (HO-) and hydroperoxyl (HO-2) radicals5. Free radicals have the ability to attack DNA and modify, most commonly, the guanine (G) base resulting in the formation of the 8-OHdG modification (known as DNA adduct)6.

Turmeric (Curcuma Longa) is an herbal plant containing curcumin compounds, which are useful as antioxidants, antibacterial, anticancer, antiproliferative, and anti-inflammatory agents7. According to Sun et al 2016, the administration of curcumin 200mg/kg/day for 8 weeks in STZ-induced rats can reduce MDA, ROS, and oxidative stress levels8. Metformin is a first-line

antihyperglycemic drug for diabetes mellitus that works by increasing insulin receptor sensitivity9. Revisiting the use of turmeric as a natural ingredient for affordable antidiabetic treatment10. This study wanted to prove the antidiabetic and antioxidant properties of turmeric by looking at the effect of the combination of metformin and turmeric extract on blood sugar, MDA, and 8-OHdG levels in male rats (Wistar strain) with type 2 diabetes mellitus induced by Streptozotocin-Nicotinamide.

II. MATERIAL AND METHOD

The type of research conducted uses a laboratory experimental method with a post-test-only control group design that uses experimental animals as research subjects. The study began with the selection of Wistar rat samples that fit the inclusion criteria. Wistar rats were acclimatized and then divided into 4 groups. Group K I, K II, K III and K IV were induced with STZ-NA intraperitoneally. After STZ-NA induction, blood sugar levels were checked, declared as diabetic rats if they had blood sugar levels >200mg/dL. The negative control group (K1) was induced with STZ-NA intra-peritoneally without treatment, while the positive control group (K2) was a group of type 2 DM rats that received a dose of metformin 45mg/KgBW/day (K2), Kurkumin dose 200mg/KgBW/day (P1), and a combination of Metformin 22.5mg/KgBW/day and Kurkumin dose 100mg/KgBW/day (P2). The study was ended by drawing blood through the orbital sinus of the eye as much as 1cc and terminated on day 15 for all four groups. MDA levels were measured by the TBARS method, 8-OHdG was measured by the ELISA method and blood sugar levels were measured using a UV spectrophotometer. This research was conducted at the Center for Food and Nutrition Studies at Gajah Mada University, the research time was 15 days starting in January 2022. Data analysis of blood sugar levels, MDA and 8-OHdG using the SPSS 24 program, for Windows normality test with Shapiro Wilk and homogeneity with Levene test. Data results were normally distributed and homogeneous tests were carried out using the One-way Anova test if the results of One-Way Anova $p<0.05$ were followed by the post hoc LSD test.

III. RESULT

Research to prove the different effects of metformin, turmeric extract and a combination of metformin and turmeric extract on blood sugar levels, MDA and 8-OHdG has been conducted on Streptozotocin-Nicotinamide-induced Wistar male rats. The treatment results are shown in Table 1.

Table 1. The mean result of blood sugar, MDA, 8-OHdG levels

Variable	Group					
	KI (n=6) Mean±SD	**KII (n=6) Mean±SD**	**KIII (n=6) Mean±SD**	**KIV (n=6) Mean±SD**	*Levene test*	**p-value ANOVA**
of blood sugar level (mg/dL)	277,6±4,16	100,7±2,11	98,03±3.26	92,61±3,64	0,515	0,000
Saphiro wilk	0,427	0,496	0,796	0,537		
MDA level (nmol/mL)	10,91±0,36	4,92±0,32	3,90±0,22	1,94±0,25	0,520	0,000
Saphiro wilk	0,638	0,415	0,200	0,583		
8-OHdG levels (ng/mL)	6,59±0,57	5,89±0,24	2,68±0,26	1,68±0,58	0,778	0,000
Saphiro wilk	0,604	0,990	0,889	0,124		

Description: KI (DM rats without treatment), KII (Metformin 45mg/KgBW/day), KIII (Turmeric extract 200mg/KgBW/day), and KIV (combination of Metformin 22.5mg/KgBW/day and Turmeric extract 100mg/KgBW/day).

The highest mean blood sugar, MDA, and 8-OHdG levels were found in group I which was not given metformin or turmeric extract, while the lowest blood sugar, MDA, and 8-OHdG levels were found in group IV with a combination of Metformin 22.5mg/KgBW/day and turmeric extract 100mg/KgBW/day.

The results of the normality test and homogeneity test showed that the data of each group were normally distributed and homogeneous ($p>0.05$). Data on blood sugar levels, MDA, and 8-OHdG are normally distributed and homogeneous, so data analysis uses the One-Way Anova parametric test to determine differences between the four groups.

Table 2. LSD post hoc test result on the mean blood sugar levels between two groups

Group	K I	K II	K III	K IV
K I	-	0,000	0,000	0,000
K II	0,000	-	0,186	0,001
K III	0,000	0,186	-	0,012
K IV	0,000	0,001	0,012	-

LSD post hoc test results as shown in Table 2. The post hoc LSD test results as shown in Table 2 show that there is a significant difference in the mean blood sugar levels in groups K I and K II (p=0.000); K I and K III (p=0.000); K I and K IV (p=0.000). In groups KII and KIII as well as KIII and KIV there was no significant difference (p>0.000).

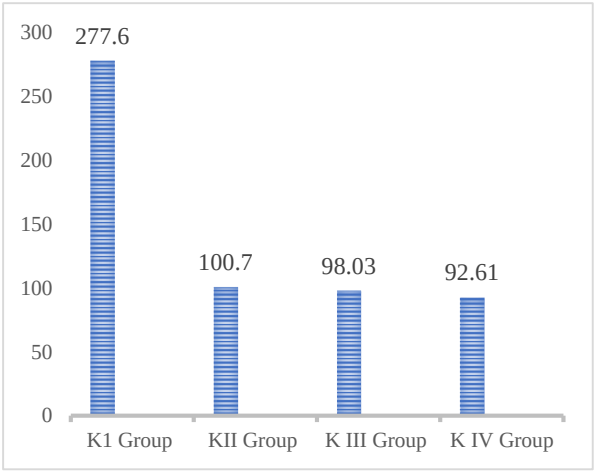

Figure 1. The average levels of blood sugar between groups

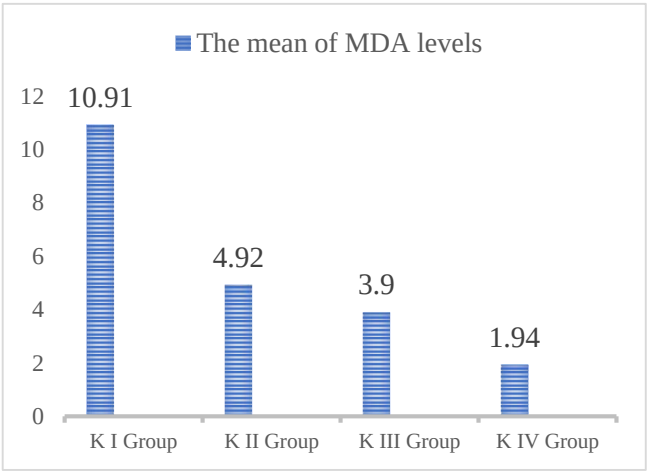

Figure 2. The average levels of MDA between groups

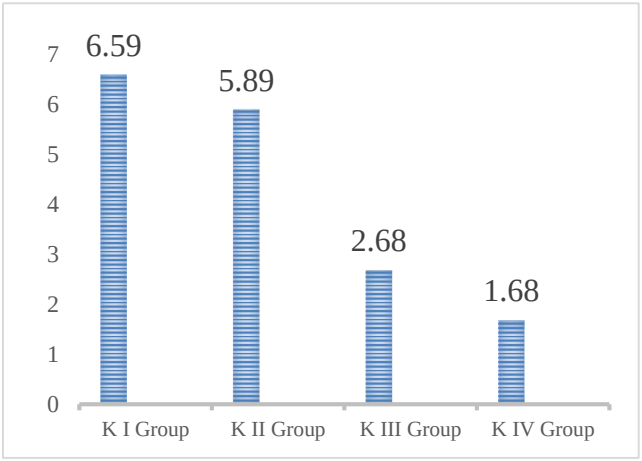

Figure 3. The average levels of 8-OHdG levels between groups

Figures 1,2, and 3 showed that the combination of Metformin 22.5 mg/KgBW/day and turmeric extract 100mg/KgBW/day (K IV) was able to reduce blood sugar, MDA, and 8-OHdG levels the lowest compared to single-dose administration.

Table 3. Test results for differences in mean MDA levels between two groups

Group	K I	K II	K III	K IV
K I	-	0,000	0,000	0,000
K II	0,000	-	0,000	0,000
K III	0,000	0,000	-	0,000
K IV	0,000	0,000	0,000	-

LSD post hoc test results as shown in Table 3. showed that there was a significant difference in the mean MDA levels in groups K I and K II (p=0.000); K I and K III (p=0.000); K I and K IV (p=0.000).

Table 4. Test results for differences in mean 8-OHdG levels between two groups

Group	K I	K II	K III	K IV
K I	-	0,000	0,000	0,000
K II	0,000	-	0,000	0,000
K III	0,000	0,000	-	0,000
K IV	0,000	0,000	0,000	-

LSD post hoc test result as shown in Table 4. showed that there was a significant difference in the mean levels of 8-OHdG in groups K I and K II (p=0.000); K I and K III (p=0.000); K I and K IV (p=0.000).

V. DISCUSSION

The results showed that in KIV, the group that received a combination treatment of metformin 22.5 mg/KgBB/day and turmeric extract 100mg/KgBB/day obtained the lowest blood sugar levels compared to other groups. Curcumin induces Mitogen-Activated Protein kinase (MAP kinase) which can block the activity of NF-κB as a transcription factor that triggers the emergence of cytokines[11]. The MAPK cascade is a signaling pathway that regulates various cellular processes (such as proliferation, differentiation, transformation, apoptosis, and stress response) under both normal and pathological conditions.[12] Blockage of the NF-κB pathway will then suppress cytokine production. Besides preventing the expression of NF-κB through the MAPK cascade, curcumin also prevents the expression of PKC, JNK, and ERK-mediated by the expression of TNF-α[13]. Metformin activates adenosine 5'-monophosphate-activated protein kinase (AMPK), which is referred to as a cellular energy sensor48. Metformin also has the ability to stimulate the release of glucagon-like-peptide-1, thereby increasing insulin secretion and lowering plasma glucose levels[14]. Combination therapy of current antidiabetic agents with natural antioxidants that have beneficial effects on diabetic disorders may be an attractive strategy, this study shows the additive effect of curcumin combined with metformin in reducing hyperglycemia and oxidative stress in diabetic rats, with respect to maintaining the effect of metformin in reducing glycemia and the effect of curcumin in increasing NF-Kb activity[15].

The results of the KIV study, namely the group that received a combination treatment of metformin 22.5 mg/KgBB/day and turmeric extract 100mg/KgBW/day had better values compared to KI, KII, and KIII. There was a decrease in oxidative stress biomarkers, namely MDA and 8-OHdG in the KIV group with the lowest value. These results prove that the combination of metformin and turmeric extract can reduce ROS levels so as to prevent excessive oxidative stress. Oxidative stress plays a major role in the onset and development of various diseases including type 2 DM. The Keap1-Nrf2 pathway is an important antioxidant system that maintains cellular redox balance. Reducing inflammation where trans-localization of Nrf2 as a transcription factor drives various antioxidant responses in cells[16]. Through several other pathways and regulatory proteins, it plays a fundamental role in preventing several diseases and reducing their complications. Regulation of the Nrf2 pathway occurs at the transcriptional and post-transcriptional levels, and this regulation plays an important role in its activity. There is a correlation between the Nrf2 pathway and very important signaling pathways, including PI3 kinase/AKT/mTOR, NF-κB, and HIF-169 factors[16]. Metformin can inhibit oxidative stress through the AMPK/mTOR signaling pathway and oxidative stress. Zhao et al, 2021 research on acute stroke patients and type 2 diabetes showed that metformin can reduce brain tissue inflammation through the AMPK pathway, and reduce the production of inflammatory cytokines, thereby reducing blood-brain barrier damage and promoting neural repair[17]. Metformin can also promote apoptosis of rat hippocampal neurons caused by oxygen-glucose deprivation. After metformin treatment, the expression of AMPK, pAMPK, and Bax decreased significantly, and the expression of mTOR and Bcl-2 increased significantly[17].

The results showed that there was the lowest downward trend in blood sugar, MDA, and 8-OHdG levels in the combination of metformin 22.5 mg/KgBW/day and turmeric extract 100mg/KgBW/day (KIV). Followed by the group with curcumin 200 mg/KgBW/day, the group with metformin 45 mg/KgBW/day (KII), and the negative control group (KI). There were significant differences between groups but the research conducted was preliminary research so further research is needed to assess the use of a combination of turmeric extract and metformin.

VI. CONCLUSION

There are differences in blood sugar, MDA, and 8-OHdG levels between groups, KIV (Metformin dose 22.5 mg/KgBW/day and turmeric extract dose 100 mg/KgBW) has the lowest mean followed by KII (turmeric extract dose 200 mg/KgBW/day) and KII (Metformin dose 45mg/KgBW/day) KI (negative control group) has the highest mean.

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