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Improving Cytokines and HDL in Diabetic Wistar Rats by Using Combination of Curcumin and Metformin

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Abstract: In type 2 diabetes mellitus, lipid profile is often found in the form of high-density lipoprotein (HDL) decrease. TNFα and IL-6 are proinflammatory cytokines that are elevated in the blood in many patients with diabetes mellitus. Turmeric is one of the natural ingredients that have anti-cholesterol and anti-inflammatory effects through the work of its active substance, curcumin. This study aims to evaluate the effect of turmeric extract combined with metformin on HDL, TNFα, and IL-6 levels in diabetic rats. Twenty-four Wistar rats were injected intraperitoneally with streptozotocin and niacinamide. The diabetic rats were divided into control, metformin, turmeric, and combination groups. The dose of metformin and turmeric were given, respectively, 45mg/KgBW/day and 200 mg/KgBW/day. At the same time, the combo group was given a half dose of each. Blood serum was taken to examine the HDL, IL-6, and TNFα levels. The levels of HDL, IL-6, and TNFαin diabetic rats that received the combination of metformin and turmeric extract ($p<0.05$) were the best among all the groups. It indicated that the combination of metformin and turmeric was better in increasing HDL and reducing IL-6 and TNFα levels better than metformin or turmeric extract alone

Keywords: cytokines; insulin; lipid; turmeric

INTRODUCTION

World Health Organization (WHO) defines diabetes mellitus (DM) as a chronic disorder characterized by metabolic, protein, and fat disorders caused by absolute or relative insulin deficiency.[1] Lack of insulin in the body results in decreased glucose transport through cell membranes. This situation then causes the cells to lack food, thereby increasing fat metabolism in the body. In type 2 diabetes, a lipid profile is often found in the form of increased triglyceride (TG) and decreased high-density lipoprotein (HDL) levels. In contrast, low-density lipoprotein (LDL) levels are not much different from those found in non-diabetic individuals but are dominated by smaller and denser.[2]

In addition, hyperglycemia which occurs in patients with type 2 diabetes, tends to cause oxidative stress at the cellular level. It triggers the auto-oxidation of glucose to form ROS (Reactive Oxygen Species), oxygen radicals that will damage nuclear DNA.[3] Inflammation due to oxidative damage triggered by hyperglycemia plays a role in the progression of DM. TNFα and IL-6 are proinflammatory cytokines widely recognized as markers of vascular inflammation.[4] The American Diabetes Association (ADA), the American Association of Clinical Endocrinologists, and the American College of Endocrinology (AACE) recommend metformin as first-line treatment for patients with type 2 diabetes.[5]

Turmeric (*Curcuma longa*) is one of the natural ingredients that have antioxidant, antitumor, anticancer, anti-cholesterol, and anti-inflammatory properties through the action of its active substance, namely curcumin.[6] There are several research results regarding the effects of curcumin on diabetes which are still contradictory to each other. Gutierres et al. proved that administration of curcumin at a dose of 90 mg/Kg/day could not significantly improve HDL levels[7]. Meanwhile, Hussein et al. proved that giving curcumin 200mg/KgBW/day in hypercholesterolemic rats can increase HDL levels in 2 weeks, 4 weeks, and 6 weeks.[7] Nicol et al. proved an increase in IL-6 levels and TNFα levels that did not change significantly in patients

receiving curcumin. Meanwhile, according to Guo et al., administration of curcumin 300mg/kg for 16 weeks in streptozotocin-induced Sprague-Dawley rats was shown to reduce the expression of TGF-B1, AMPK, and Mitogen-Activated Protein Kinase (MAPK).[8]

Research on antidiabetic agents from natural ingredients has recently become a trend that many scientists carry out. However, until now, research that proves the combination of metformin, as the drug of choice for type 2 DM, and curcumin, a natural ingredient with both antidiabetic and anti-inflammatory potential, has not been widely carried out. In addition, there are results from previous studies that have not been consistent regarding the effect of curcumin on HDL levels. Based on the background stated above, the present study aims to evaluate the effect of turmeric extract combined with metformin on HDL levels, TNFα levels, and IL-6 levels in diabetic Wistar rats.

MATERIAL AND METHOD

This research was conducted in full compliance with ethical standards and approved by the Ethical Committee of the Faculty of Medicine, UNISSULA Semarang, Indonesia (No 356/X/2021/Komisi Bioetik). This study included 24 male 2-month-old Wistar rats (200-250 g). It was conducted in the Food and Nutrition Studies Center, Gadjah Mada University, Yogyakarta. All rats were given standard food and distilled water and acclimatized for 7 days before induction.

Turmeric Extract

Turmeric extract was obtained from PT. Herbal and Pharmaceutical Industry Sido Muncul Tbk with 100% purity turmeric extract in capsule packaging. Each capsule contained 500 mg of powdered extract containing 100 mg of the active substance curcuminoids. Turmeric extract was dissolved in 2 cc of aqua and then given orally to diabetic rats once a day for 14 days. It was started after Streptozotocin-Nicotinamide induction.

Induction of experimental diabetes mellitus and experimental design

Rats fasted for approximately 18 hours, then niacinamide 110 mg was induced intraperitoneally, followed by induction of streptozotocin (STZ) 45 mg, 15 minutes after niacinamide (NA) injection. The niacinamide was provided by Sigma-Aldrich, St. Louis, MO, USA, and the streptozotocin by Nacalai Tesque, Inc., Kyoto, Japan. The behavioral change of rats was observed after diabetes induction. Distilled water and standard food were provided ad libitum. Fasting Blood Sugar was measured on day 3 after induction with *GlucoDr*. The rats were considered diabetic if the fasting blood glucose level was over 250 mg/dL. The diabetic rats were then randomized and divided into 4 groups: one group was the negative control, the second group was given Metformin 45mg/KgBW/day, the third group was given turmeric extract 200mg/KgBW/day, and the fourth group was given Metformin 22.5mg/KgBW/day and 100mg/KgBW/day. The treatment was administered for 28 days. The fasting blood sample was taken from the ophthalmic vein.

Measurements

The fasting blood glucose (FBG) and HDL levels were measured using GOD-PAP enzymatic photometric test. The levels of serum IL-6 and TNFα were examined using ELISA.

Statistical Analysis

The mean levels of HDL, IL-6 and TNFα were analyzed using One Way ANOVA, followed by post-hoc LSD using SPSS ver. 16.0. Data were considered significant if $p<0.05$.

RESULT

Fasting Blood Glucose Level

The fasting blood glucose level in the four rat groups after streptozotocin-niacinamide induction was higher than 250 mg/dL (Figure 1).

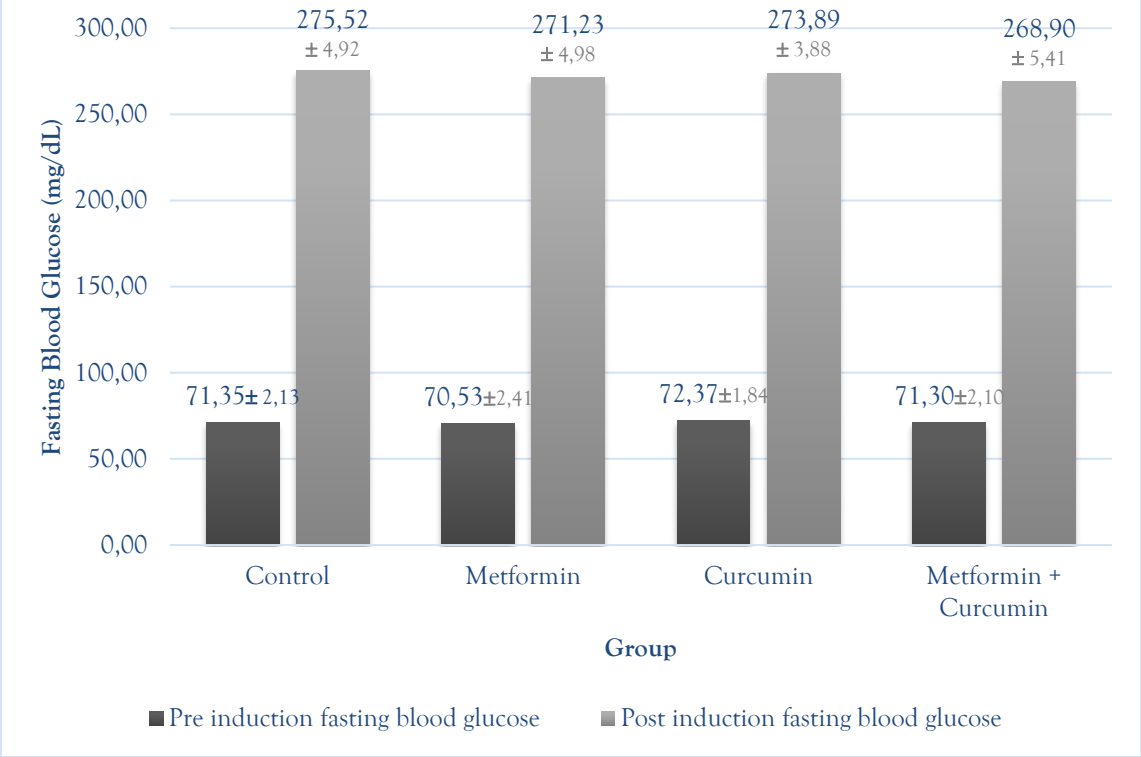

Figure 1. The average ± Deviation standard of Fasting blood glucose level of Wistar rats pre-post diabetes type 2 induction (mg/dL).

Based on the results of descriptive analysis, it was found that the average FBG level in each group was highest in the control group (71.35 ± 2.13) and lowest in the combination group (71.30 ± 2.10). Analysis of the normality of the distribution of the mean FBG levels analyzed using the Shapiro-Wilk test showed that all groups were not normally distributed ($p < 0.05$). The results of the homogeneity analysis using the Levene test revealed that the variance of the data was heterogeneous, with a value of $p = 0.000$ ($p < 0.05$), indicating significant differences between groups. The significance of the difference in the mean levels of FBG between groups was followed by a post hoc test using the Mann-Whitney test. The Mann-Whitney test results are explained in table 1. These results showed that diabetes type-2 induction in Wistar rats was successful.

Table 1. Fasting blood glucose level of Wistar rats pre-post diabetes type 2 induction

Variable	Control	Metformin	Curcumin	Metformin + Curcumin
Control		0.004*	0.004*	0.004*
Metformin	0.004*		0.000*	0.008*
Curcumin	0.004*	0.000*		0.025*
Metformin + Curcumin	0.004*	0.008*	0.025*	

High-Density Lipoprotein Levels

The highest mean HDL level was found in the combination group (72.43 mg/mL ± 3.31). The HDL levels between the metformin group (60.42 mg/dL ± 3.33), curcumin group (67.40 mg/dL ± 2.06), and combination group (72.43 mg/dL± 3.31) were significantly different ($p<0.05$) after test using post-hoc LSD (Figure 2).

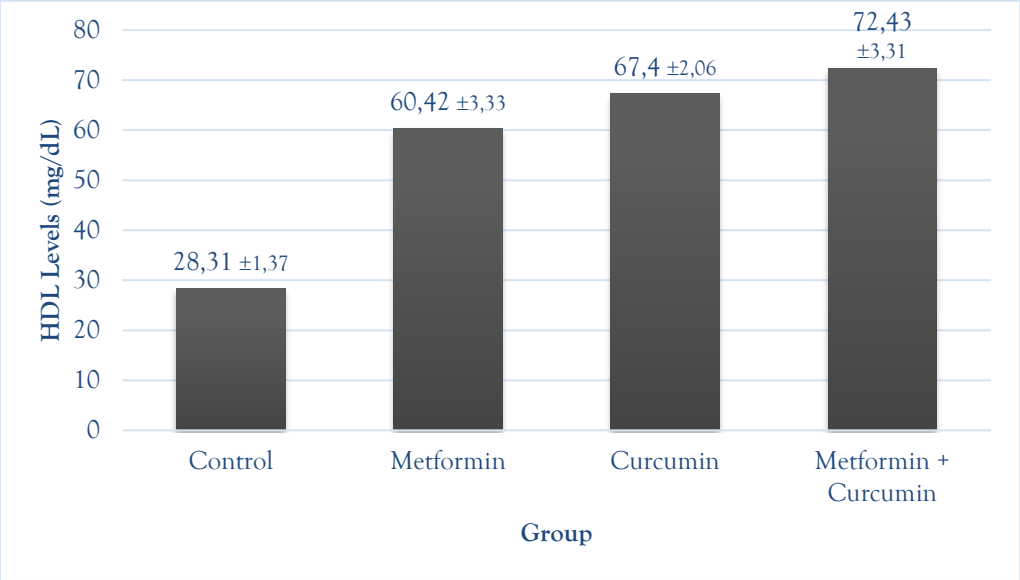

Figure 2. The average ± Deviation standard of HDL level in all groups after oral treatment (mg/dL)

The data were analyzed using the One Way Anova test for each group and obtained a p-value of 0.000, indicating a significant difference in the average HDL level. The LSD post hoc test was conducted to determine the differences between groups. The results of the LSD post hoc test showed that there was a significant difference in mean HDL levels in the control and metformin group (p=0.000); control and curcumin group (p=0.000); and control and combination group (p=0.000). Based on the data above, it revealed that giving turmeric extract at a dose of 200 mg/KgBB/day combined with Metformin 22.5 mg/KgBB/day had the best results in increasing HDL levels, followed by curcumin extract at 200 mg/KgBB/ day.

Interleukin 6 (IL-6) Levels

The metformin group had the highest mean IL-6 level (75.95 ng/L± 3.83) of the three treatment groups. The IL-6 level of the curcumin group (62.81 ng/L ± 2.15) and combination group (58.82 ng/L± 2.15) was measured at the end of treatment with post-hoc LSD and found to be significant (p<0.05) (Figure 3).

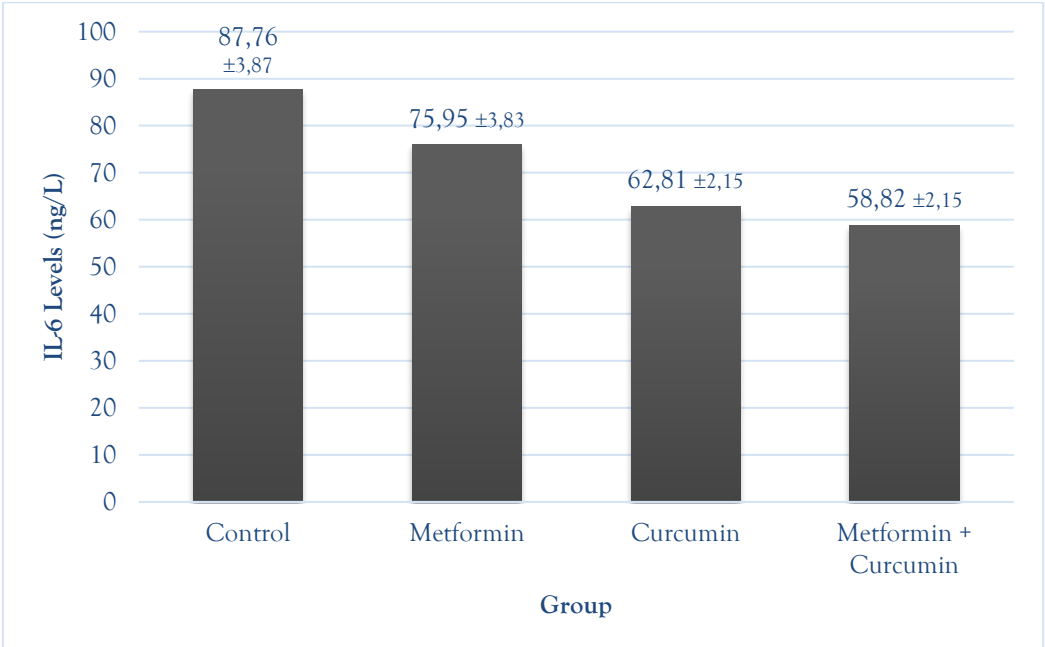

Figure 3. The average ± Deviation standard of IL-6 level in all groups after oral treatment (ng/L)

The data were analyzed using the One Way Anova test for each group and obtained a p-value of 0.000, indicating a significant difference in the average IL-6 level. The LSD post hoc test was conducted to determine the differences between groups. The results of the LSD post hoc test demonstrated that there was a significant difference in the mean IL-6 levels in the control and metformin group (p=0.000); control and curcumin group (p=0.000); and control and combination group (p=0.000). Based on the data above, it revealed that giving turmeric extract at a dose of 200 mg/KgBB/day combined with metformin 22.5 mg/KgBB/day had the best results in reducing IL-6 levels, followed by curcumin extract at 200 mg/KgBB/day.

Tumor Necrosis Factor Alpha (TNFα) levels

The same trend was also found for TNFα. The combination group had the lowest TNFα level (7.56 ng/L± 0.44). TNFα level between metformin group (12.13 ng/L± 0.75) and curcumin group (8.82 ng/L± 0.38) was significantly different (p<0.05) (Figure 4).

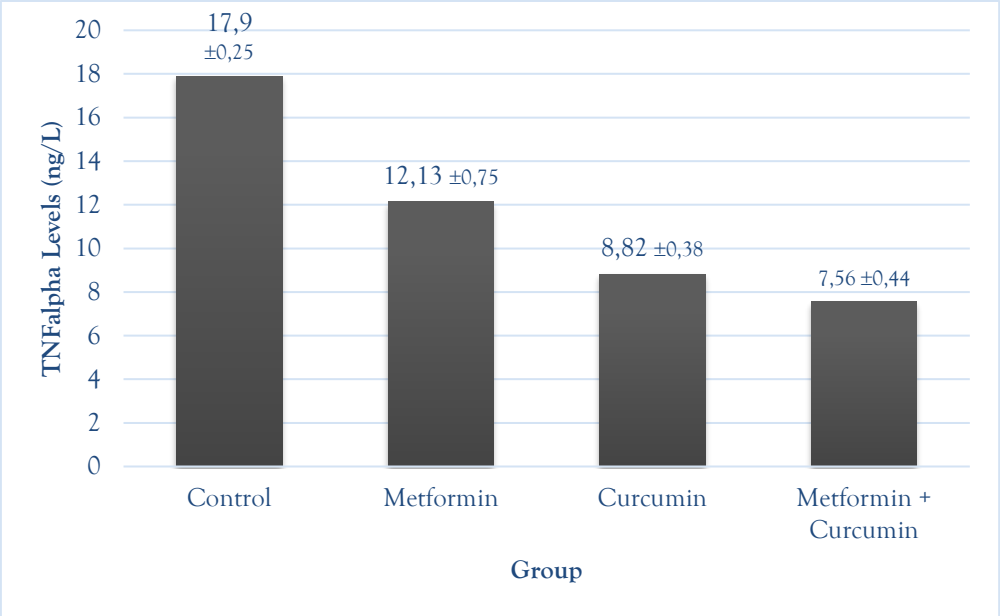

Figure 4. The average ± Deviation standard of TNFα levels in all groups after oral treatment

Data were analyzed using the One Way Anova test for each group and obtained a p-value of 0.000, indicating a significant difference in the average TNFα level. The LSD post hoc test was conducted to determine the differences between groups. The results of the LSD post hoc test showed that there was a significant difference in the mean TNFα levels in the control and metformin group (p=0.000); control and curcumin group (p=0.000); and control and combination group (p=0.000). Based on the data above, it demonstrated that giving turmeric extract at a dose of 200 mg/KgBB/day combined with Metformin 22.5 mg/KgBB/day had the best results in reducing TNFα levels, followed by curcumin extract at 200 mg/KgBB/day.

DISCUSSION

The induction of streptozotocin (STZ) can increase oxidative stress and damage pancreatic cells. Meanwhile, niacinamide functions as an antioxidant that protects cells from the cytotoxic effects of STZ. The combination of the two is suitable as an induction agent in experimental animals that will be used to test the antidiabetic effect of natural ingredients.[9] It aligns with the mean fasting blood sugar level of more than 250 mg/dL in all groups after induction of 45 mg STZ and 110 mg niacinamide.

The combination of metformin and turmeric extract on diabetic rats resulted in the lowest levels of IL-6 and TNFα levels and the highest HDL levels compared to the metformin and control groups. Giving turmeric extract alone resulted in a better decrease in IL-6 and TNFα levels and an increase in HDL levels compared to the group that received metformin alone. In contrast, research conducted by Gutierres et al. proved that the

administration of curcumin at a dose of 90mg/Kg/day could not significantly improve HDL levels. It occurred due to the lack of curcumin doses given to experimental animals[7]. The effect of turmeric extract on type 2 diabetes mellitus can cause an increase in HDL levels and decrease inflammatory factors, such as IL-6 and TNFα. The present study's results align with previous studies revealing that *Temulawak* rhizome extract with the active substance curcumin can improve lipid profile.[10]

Curcumin can activate peroxisome proliferator-activated receptor-γ (PPAR-γ), increasing the production of sterol regulatory element-binding proteins-1 (SREBP-1). Sterol regulatory element-binding proteins-1 is a membrane-bound transcription factor that activates gene coding for enzymes required to synthesize cholesterol and unsaturated fatty acids. SREBP is regulated by various mechanisms at the level of mRNA synthesis, proteolytic activation, and transcriptional activity. The effect of increasing SREBP-1 by curcumin in hepatocytes is to increase HDL levels.[10,11] Peroxisome proliferator-activated receptor-γ (PPAR-γ) is a type of nuclear receptor. PPAR-γ then regulates ATP-binding cassette transporter A1 (ABCA1) in macrophages. ABCA1 transports cholesterol and phospholipids from cells to apoA-1 and other apolipoproteins, providing a pathway for cells to unload excess cholesterol. ABCA1 is required for cholesterol efflux from cells to APOA1 to form nascent HDL. ABCA1 regulation by PPAR-γ contributes to increased HDL levels.[12]

Oxidative stress in type 2 DM can also cause the emergence of reactive oxygen species (ROS) along with increased levels of pro-inflammatory chemokines in cells, inhibiting blood flow into cells and thereby impairing their function. The increase of ROS will ultimately increase the formation of TNFα expression and exacerbate oxidative stress.[12] TNFα can stimulate the production of other cytokines, including IL-6.[13] 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. The blockage of the NF-κB pathway will then suppress cytokine production.[14] In addition to preventing NF-B expression via the MAPK cascade, curcumin also prevented TNFα mediated expression of PKC, JNK, and ERK. Furthermore, inhibition of pro-inflammatory cytokines could potentially improve insulin sensitivity and glucose metabolism in curcumin-supplemented diabetic rats.[15] It aligns with the results of the present study, which proved that the curcumin and combination groups had better levels of IL-6 and TNFα than the group that only received metformin or the control group.

Metformin activates AMP-activated protein kinase (AMPK), which affects lipogenesis, reduces SREBP-1 expression, and suppresses hepatic gluconeogenesis. The effect of reducing SREBP-1 expression is a decrease in fatty acid synthesis.[12] Deregulating the MAPK, AMPK and other cascades is key to developing obesity and diabetes mellitus. Thus, these cascades are now becoming the targets of diabetes mellitus therapy.[16] Previous studies have shown that using 200 mg/kgbb/day of turmeric extract in Wistar rats has been shown to reduce blood sugar levels, reduce body weight, and suppress ROS expression.[17] In preventing complications of type 2 diabetes, curcumin works through several mechanisms. Curcumin inhibits EMMPRIN (a glycoprotein containing two immunoglobulin domains), which is a glycoprotein that increases the change in plaques that are initially stable to become unstable and then eventually rupture.[18]

This study's results align with previous research conducted by Roxo et al., revealing that the combination of metformin and curcumin works synergistically in lowering blood glucose levels, suppressing oxidative stress, and inhibiting complications in type 2 diabetes.[19] Another study conducted by Cao et al. also showed that the combination of metformin and curcumin could inhibit cell apoptosis in mice with nephropathy by suppressing the Caspase-3 pathway and the apoptotic factor Bax and increasing the signaling pathway of the anti-apoptotic factor Bcl-2.[20] Curcumin extract at the molecular level affects the MAPK pathway through various molecular components. In contrast, metformin, the drug of choice for diabetes mellitus at the molecular level, affects the AMPK pathway. Thus, the use of a combination of turmeric extract and metformin can provide the advantage of better efficacy in increasing HDL levels and reducing IL-6 and TNFα levels. The results of the present study showed that the combination group obtained the highest HDL levels and the lowest levels of IL-6 and TNFα compared to the other three groups.

CONCLUSION

The combination of metformin and turmeric extract improved diabetic markers in diabetic rats. This combo could increase HDL levels and reduce IL-6 levels and TNFα levels better than metformin or turmeric extract alone.

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CONFLICT OF INTEREST

No conflict of interest has been declared by the authors.

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