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Polyherbal Formulation of *Apium graveolens*, *Tithonia diversifolia* and *Curcuma longa* acts as an Antidiabetic Agent Through the Regulation of Nrf2 Modulating Biochemical Parameters

GITA SEKAR PRIHANTI*[1], NOVIANA DWI LESTARI[2], ANUNG PUTRI ILLAHIKA[3] & HUSAMAH[4]

[1]Department of Medical Education, Faculty of Medicine, University of Muhammadiyah Malang, Malang 65145, Indonesia; [2]Faculty of Medicine, University of Muhammadiyah Malang, Malang 65145, Indonesia; [3]Department of Anatomy, Faculty of Medicine, University of Muhammadiyah Malang, Malang 65145, Indonesia; [4]Department of Biology Education, Faculty of Teacher and Training Education, University of Muhammadiyah Malang, Malang 65144, Indonesia
*Corresponding author: sekar@umm.ac.id
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ABSTRACT

Nuclear factor erythroid 2-related factor 2 (Nrf2), superoxide dismutase (SOD), malondialdehyde (MDA) and biochemical clinic are molecular components associated with metabolic dysfunctions in diabetes mellitus (DM). Alternative therapeutic approaches for diabetes mellitus may include the use of bioactive compounds derived from natural sources. This study aimed to evaluate the effect of a formulation of celery (A*pium graveolens*), paitan (*Tithonia diversifolia*) and turmeric (*Curcuma longa*), known as SPK, for diabetes treatment. Diabetes was induced in mice through multiple intraperitoneal doses of streptozotocin (50 mg/kg BW for 4 days). The diabetic mice were divided into five groups: healthy mice (N), DM (diabetic mice without treatment), diabetic mice treated with SPK extracts (SPK 1, SPK 2 and SPK 3). The treatments were administered orally once daily for 21 days. On day 22, all mice were euthanised and organ (liver) and blood samples were collected. The liver was analysed using flow cytometry to determine the profiles of Nrf2, SOD and MDA. Blood samples were analysed for biochemical parameter concentrations. All data were statistically analysed using one-way ANOVA ($p \leq 0.05$) with SPSS, followed by the Tukey test. The results showed that SPK administration significantly increased Nrf2 and SOD profiles under diabetic conditions but did not significantly affect the MDA profile. Additionally, in biochemical parameters, SPK administration significantly reduced triglyceride, blood glucose, creatinine, urea/BUN, SGOT and SGPT levels. In conclusion, the SPK formulation has the potential to be used as an alternative treatment for diabetes mellitus.

Keywords: Antidiabetic, *Apium graveolens*, *Curcuma longa*, diabetes mellitus, *Tithonia diversifolia*

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease defined by sustained elevations in blood glucose levels beyond the normal physiological limits (Lestari *et al.*, 2022) resulting from abnormalities in insulin secretion (due to dysfunction of β cells of islet), insulin resistance, or both (Adharini *et al.*, 2020a). This condition arises either from insufficient insulin production by the pancreas or from the body's impaired ability to effectively utilise insulin (WHO, 2016).

According to the International Diabetes Federation (IDF), an estimated 537 million individuals worldwide were living with diabetes in 2021. This figure is projected up rise for 643 million by 2030 and reach up to 783 million by 2045 (IDF, 2021). In Indonesia, the estimated number of adults aged between 20 to 79 years living with diabetes in 2021 was approximately 19,465,100 out of a total adult population of 179,720,500 within the same age range. When calculated based on these numbers, the prevalence of diabetes in the age group of 20 – 79 years is 10.60% (Ministry of Health Republic Indonesia, 2022).

People with diabetes mellitus not only experience cell damage in the pancreatic organs but complications occur to other organs, one of them which is the organ of liver and kidney. This complication occurs as a result of prolonged

hyperglycemia and subsequently induces or increases the production of reactive oxygen species (Stolf *et al.*, 2018). Reactive oxygen species (ROS) are normally detoxified by antioxidants in the body, maintaining a balance between endogenous and exogenous antioxidant systems. However, an imbalance between antioxidant defenses and ROS generation can promote ROS accumulation, leading to oxidative stress in the liver, which serves as the primary organ responsible for the detoxification and metabolism of xenobiotics. Oxidative stress produced in diabetic conditions tends to be involved during the development of pancreatic β cell dysfunction. The increased oxidative stress in the body is presumed to result from a disruption in the nuclear factor erythroid 2-related factor 2 (Nrf2) transcription factor's ability to regulate endogenous antioxidants, leading to low expression of antioxidant enzymes such as superoxide dismutase (SOD) (Jaiswal *et al.*, 2013). Elevated levels of reactive oxygen species (ROS) can result in the formation of aldehydic compounds such as malondialdehyde (MDA), a terminal product of the interaction between ROS and polyunsaturated fatty acids (PUFAs). Consequently, MDA is widely utilised as a biomarker for cellular damage under oxidative stress conditions (Morales & Munné-Bosch, 2019). Furthermore, clinical biochemical parameters such as triglycerides, blood glucose levels, creatinine, urea/BUN (as indicators of kidney function), serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) (as indicators of liver function) are commonly utilised to monitor the effectiveness of therapeutic interventions.

The management of diabetes mellitus largely relies on pharmacological treatments, including with or without insulin; however, these therapies have been associated with a range of side effects (Mostafavinia *et al.*, 2016). However, the high cost of medical treatment for diabetes mellitus is one of the causes of high rates of patient mortality so many people choose to switch the traditional medicine. In the modern era, numerous synthetic drugs are widely used; however, these drugs may induce hypoglycemia if administered at inappropriate doses. Indonesia, as a tropical nation, possesses a wealth of traditional medicinal plants that have been utilised for generations.

Natural therapeutic approaches utilising herbal ingredients have been extensively studied as alternatives or adjuncts to conventional therapies for DM. The combination of celery (*Apium graveolens*), paitan (*Tithonia diversifolia*) and turmeric (*Curcuma longa*), known as SPK, has demonstrated potential as an antidiabetic agent through mechanisms involving antioxidant and anti-inflammatory activities, as well as the enhancement of Nrf2 pathway regulation. Celery contains flavonoids and polyphenols with antioxidant properties, while Mexican sunflower contains bioactive compounds such as sesquiterpene lactones that have been shown to reduce blood glucose levels. Additionally, turmeric contains curcumin, which has been proven to exert hypoglycemic effects and provide protective benefits to pancreatic beta cells. Therefore, this study aimed to evaluate the effect of SPK on Nrf2, SOD, MDA and biochemical (triglycerides, blood glucose, creatinine, urea/BUN, AST/SGOT, ALT/SGPT) levels in diabetic mice.

MATERIALS & METHODS

Experimental Design

Mice were obtained from Kemuning (Number registration: 004/SKKH/X/2024), Central Java, Indonesia in normal and healthy conditions at the age of 7–8 weeks. Mice were divided into five treatment groups consisting of four mice in each group including normal and healthy control (N), diabetes mellitus control (DM), SPK dose 1 (SPK 1), SPK dose 2 (SPK 2) and SPK dose 3 (SPK 3). Mice were then acclimated for seven days before being used as a diabetes mellitus model animal. All experimental procedures were approved under an Ethical Exemption by university of Muhammadiyah Malang (No. E.5.a/011.a/KEPKUMM/1/2025).

Streptozotocin Injection on Mice

The Streptozotocin (STZ) injection was administered on day 15 after the animals had been fed a high-fat diet (HFD) for 14 days. In this study, the administration of a HFD was intended to reproduce the metabolic syndrome or type 2 diabetes. Mice were injected with multiple dosages of STZ intraperitoneally at a dose of 50 mg/kg BW for four consecutive days. Streptozotocin (bioWORLD, bioPLUS™, USA) was dissolved in 0.1 M citrate buffer and pH 4.5.

Blood glucose levels of mice were then measured on the 5th day after the last STZ injection. Mice were confirmed to have DM if blood glucose levels were found to be ≥200 mg/dL.

Preparation of SPK Formulation

The SPK formulation was prepared from a combination of *Apium graveolens, Tithonia diversifolia* and *Curcuma longa*. The plant materials was collected from UPT Laboratorium Herbal Materia Medica Batu, East Java, Indonesia (7.8677°S, 112.5193°E). The bets number each the plant is 240613.SLD.G.BTU. 587.226 (*Apium graveolens*) 240614.PTN.F.M LG.597.228 (*Tithonia diversifolia*) and 2406 14.KNT.L.MLG.596.227 (*Curcuma longa*). The plant materials of *Apium graveolens* and *Tithonia diversifolia* utilised in this study were the leaves, while the rhizomes were used for *Curcuma longa*. The powdered samples of each plant were extracted using sterile distilled water at a ratio of 1:10 (w/v). The extraction was performed using 500 g of plant powder and 5000 ml, boiled in 95 °C, followed by the addition of the plant powder and stirring for approximately 10 minutes. The mixture was then allowed to stand at room temperature for 15 minutes and subsequently filtered. The filtrate was frozen at −80 °C and subjected to freeze-drying to obtain a dry powdered extract (SPK). The resulting extracts were stored at room temperature in airtight containers until further use.

Administration of SPK Formulation

The *A. graveolens* (Assaggaf, 2025) , *T. diversifolia* (Ejelonu et al., 2022) and *Curcuma longa* (Cortez-Navarrete et al., 2023) as known SPK doses were prepared based on preliminary studies that reported effectivenes antidiabetic activities of the plant with modification. Mice confirmed as diabetic were given SPK formulation at three different doses for 21 days. Mice in the SPK 1 group were given a formulation consisting of a combination of *A. graveolens* at a dose of 500 mg/kg, *T. diversifolia* at a dose of 300 mg/kg and *Curcuma longa* at a dose of 200 mg/kg. Mice in the SPK 2 group were given a formulation consisting of a combination of *A. graveolens* at a dose of 200 mg/kg, *T. diversifolia* at a dose of 500 mg/kg and *C. longa* at a dose of 300 mg/kg. Meanwhile, mice in the SPK 3 group were given a formulation consisting of a combination of *A. graveolens* at a dose of 300 mg/kg, *T. diversifolia* at a dose of 200 mg/kg and *C. longa* at a dose of 500 mg/kg.

Organ Isolation and Flow Cytometry Analysis

After the treatment period ended, mice were injected with ketamine (Ketamil®, 100 mg/mL) at a volume of 0.1 ml, administered into the thigh muscle) and continued with surgery to take blood through the heart. The blood that had been put into a serum separator tube used for biochemical analysis. Meanwhile, the liver organ was used for intracellular antibody staining. Cell isolation began with grinding the liver organ in 3 ml of PBS in a petri dish. The sample was put into a propylene tube and centrifuged at 10 °C at 2500 rpm for 5 minutes. The pellet was resuspended with 1 ml of PBS and then transferred as much as 50 µl into a microtube. Furthermore, the sample was added with 50 µl of cytofix and incubated for 20 minutes at 4 °C. Washperm was also added as much as 500 µl to the sample and incubated for 5 minutes at 4 °C. After that, the sample was centrifuged at 10 °C at 2500 rpm for 5 minutes. The pellet obtained was added with 50 µl of intracellular antibody staining consisting of a combination of *rabbit anti-mice-Nrf2 polyclonal antibody, rabbit anti-mice-SOD1 polyclonal antibody* and *rabbit anti-mice-MDA polyclonal antibody* and incubated for 20 minutes at 4 °C. After that, 400 µl of PBS was added to the sample, resuspended and transferred into a cuvette. The sample was ready to run flow cytometry using BD Cellquest Pro™ software (Adharini *et al.*, 2020b).

Serum Biochemical Analyses

On day 22, following the final administration on day 21, all mice were euthanized using ketamine (0.05 ml). Blood samples were then collected directly from the heart, allowed to incubate for 30 minutes to one hour and subsequently centrifuged at 4500 rpm for 15 minutes at 27 °C to separate the serum. The obtained serum was analysed for several biochemical parameters, including triglycerides, blood glucose, creatinine, urea/BUN, AST/SGOT and ALT/SGPT, using a semi-automated analyser (HORIBA ABX SAS, B.P 7290, France).

Data Analysis

Data was analysed statistically through one-way ANOVA using SPSS software and Tukey's post-hoc follow-up test. P value ≤0.05 was regarded as significant.

RESULTS

Expression of Nuclear Factor Erythroid 2-related Factor 2 Profile

Flow cytometry analysis showed a significant decrease in Nrf2 (nuclear factor erythroid 2-related factor 2) expression in the diabetes mellitus (DM) group to 16.71% compared to 34.04% in the normal group (p≤0.05). This indicates the occurrence of oxidative stress due to metabolic disorders. Treatment with SPK formulation at three different doses showed a significant increase in Nrf2 expression with the highest value belonging to the SPK dose 2 group (36.31%), followed by SPK dose 3 (35.41%) and SPK dose 1 (32.18%) (Figure 1a). This finding indicates that the SPK treatment successfully restored Nrf2 expression close to or even beyond the level of the normal group. These results prove the potential of the SPK formulation in maintaining glucose homeostasis, lipid and protein metabolism and antioxidant activity in the body through activation of the Nrf2 pathway which ultimately contributes to the recovery of diabetes mellitus (Hendrawati, 2017). The DM mice were significantly different between healthy mice/normal (N), had the lowest Nrf2 expression. In contrast, the SPK 1, SPK 2, SPK 3 and N groups did not significant differences between the groups. This indicates that the SPK treatment was able to significantly increase the expression of Nrf2 compared to the DM group (Figure 1b).

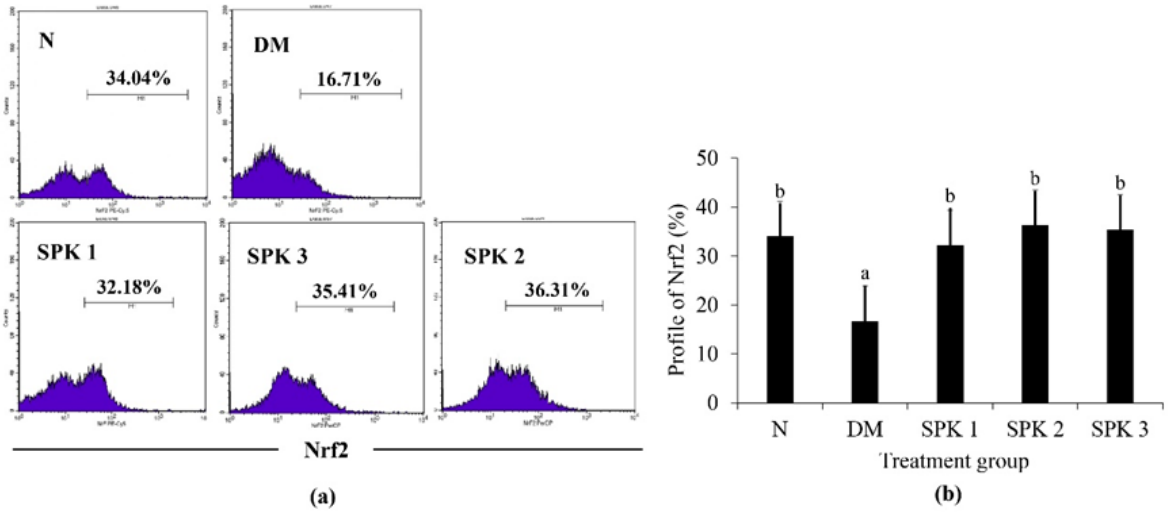

Figure 1. Increased of Nrf2 expression at three different doses of SPK formulation. (a) Flow cytometry analysis of liver cells. (b) Graphic Nrf2 expression difference test using statistical analysis. Bars with different letters (a, b) are significantly different according to Tukey's post hoc test ($p<0.05$), and same letter are not significantly different

Expression of MDA Profile

The results of flow cytometry analysis showed significant differences in MDA levels among the various treatment groups. The normal group (N) had an MDA expression level of 1.94% which indicated a normal condition. In the DM group, there was an increase in MDA expression to 2.04%, indicating an increase in oxidative stress due to ROS activity which causes peroxidation of lipid components in cell membranes (Sunita *et al.*, 2020). After administering the SPK formulation in three different doses, variations in MDA expression were observed. The SPK 1 group showed the highest increase in MDA expression among all groups, at 3.50%, which was significantly higher than the DM and normal groups (p≤0.05). This indicates that the SPK 1 dose triggered a greater oxidative response than the other doses. In contrast, in the SPK 2 group, MDA levels decreased to 1.50%, close to the level of the normal group, suggesting the potential to reduce diabetes-induced oxidative stress. The SPK 3 group showed a further

decrease in MDA levels to 1.24%, even lower than the normal group, indicating that this dose had the strongest antioxidant effect among the other treatment groups (Figure 2a). The results of statistical analysis using one way-ANOVA $p<0.05$ with post-hoc test as the focus of assessment showed that the MDA profile in the SPK 2 and SPK 3 groups was significantly lower than the DM and SPK 1 groups. The same notation "a" on the graph indicates that there is no statistically significant difference between the groups, but the effect of SPK treatment, especially at the SPK 3 dose, is close to normal conditions (Figure 2b) (Shawki *et al.*, 2021).

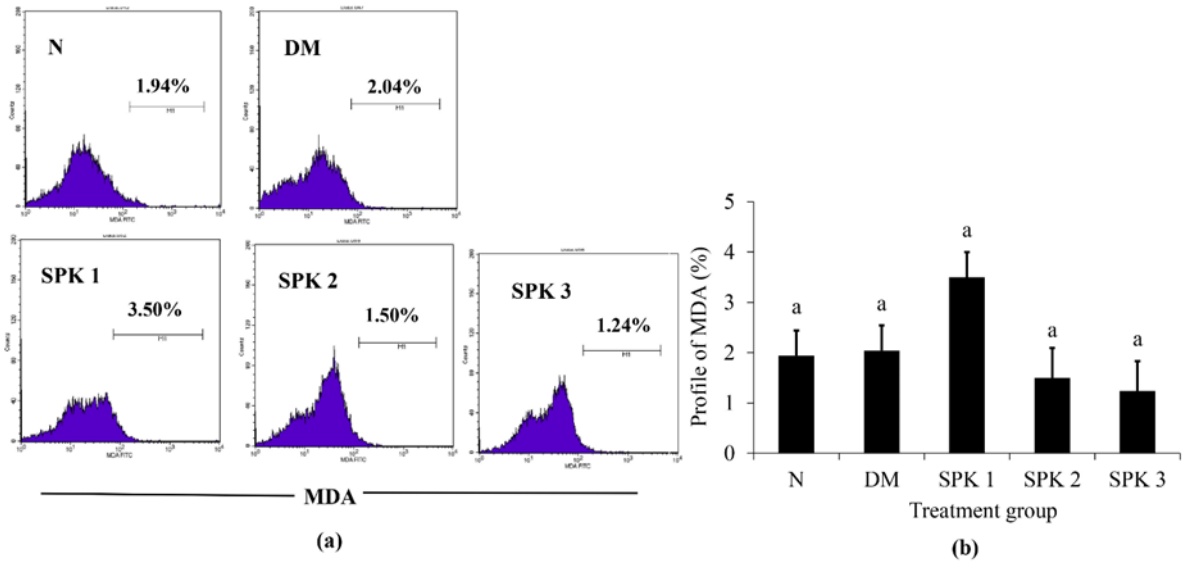

Figure 2. Decrease in MDA expression at three different doses of SPK formulation. (a) Flow cytometry analysis of liver cells stained with rabbit anti-mice-MDA polyclonal antibody. (b) Test for differences in MDA expression using statistical analysis. Bars with different letters (a, b) are significantly different according to Tukey's post hoc test ($p<0.05$), and same letter are not significantly different

Expression of SOD Profile

Flow cytometry analysis showed significant differences in SOD activity between the N and treatment groups. The N group showed an initial SOD activity of 44%, while the DM group had a significant decrease to 33.12% reflecting impaired antioxidant defense due to oxidative stress induced by diabetes. Treatment with the SPK formulation significantly improved SOD activity. The SPK 1 group increased to 51.51% and SPK 2 showed the highest activity of 52.59%, indicating its superior effectiveness in enhancing antioxidant defense. SPK 3 also increased SOD levels to 49.78%, although slightly lower than SPK 1 and SPK 2 (Figure 3a). Increased SOD expression is able to overcome oxidative stress, reduce ROS and increase antioxidant enzymes so as to reduce the pathogenesis of DM (Tiwari *et al.*, 2013). The results of statistical analysis using one way-ANOVA $p<0.05$ with post-hoc test, showed that the DM group were significantly lower SOD values compared to the other groups indicating a decrease in SOD activity due to oxidative stress. Meanwhile, the N group and the SPK treatment group (SPK 1, SPK 2 & SPK 3) showed that the SOD profile in these groups was significantly different compared to the DM group. This confirms the effectiveness of SPK treatment in increasing SOD activity (Figure 3b).

Biochemical Analysis

Each treatment group was analysed biochemically, including triglycerides, blood glucose, creatinine, urea/BUN, AST/SGOT and ALT/SGPT (Table 1). Triglycerides, blood glucose and creatinine showed above the reference value, where there was an increase in the DM group and a decrease in the SPK group at three different doses. Several parameters showed values between the reference values, including urea/BUN and AST/SGOT, where there was also an increase in the DM group and a slow decrease in the SPK group at three different doses. Meanwhile, ALT/SGPT increased in the DM group. Then, there was a decrease in SPK 1 and SPK 2 groups but an increase again in the SPK 3 group. Although the

values were above the reference value, the same subset among the four groups showed no real differences and no significant effects produced by the diabetic model mice and the diabetic model mice treated with the SPK formulation.

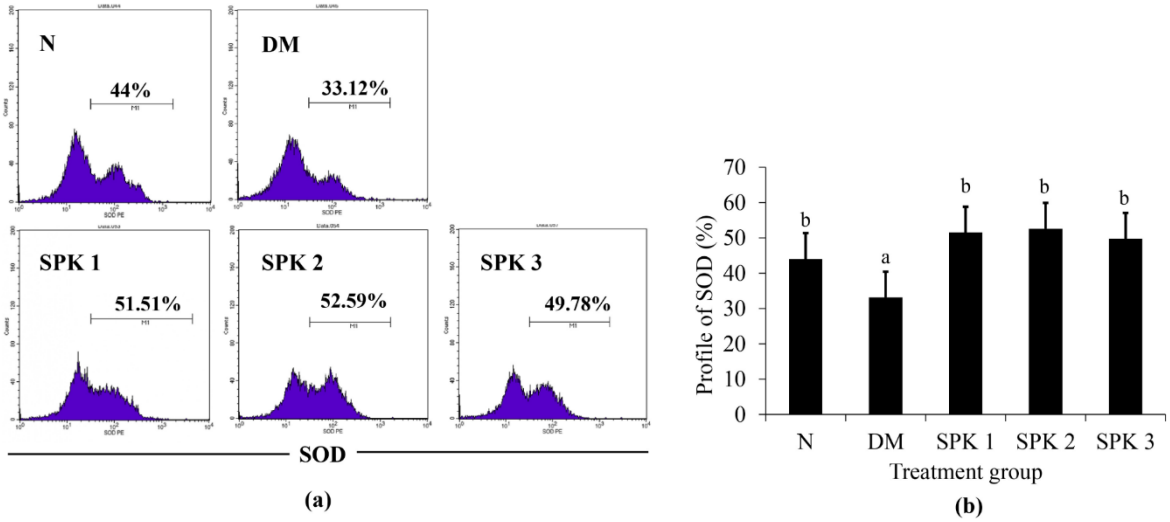

Figure 3. Increased of SOD expression at three different doses of SPK formulation. (a) Flow cytometry analysis of liver cells stained with rabbit anti-mice-SOD1 polyclonal antibody. (b) SOD expression difference test using statistical analysis. Bars with different letters (a, b) are significantly different according to Tukey's post hoc test ($p<0.05$) and same letter are not significantly different

Table 1. Biochemical parameters data by dosage group during the treatment period.

Parameter	Reference Value	Healthy mice	DM mice	SPK 1	SPK 2	SPK 3
Triglycerides (mg/dl)	20 – 114	147.5 ± 42.2^{a}	670 ± 255.2^{b}	222.9 ± 148.2^{a}	309.3 ± 108.4^{a}	324.4 ± 176.2^{a}
Blood glucose (mg/dl)	50 – 160	200.4 ± 62.08^{a}	668.75 ± 49.53^{c}	430.8 ± 146.03^{bc}	366.75 ± 134.09^{ab}	463.2 ± 153.76^{bc}
Creatinine (mg/dl)	0.2 – 0.7	0.55 ± 0.07^{a}	1.03 ± 0.08^{b}	0.69 ± 0.1^{ab}	0.6 ± 0.14^{a}	0.53 ± 0.1^{a}
Urea/BUN (mg/dl)	12.33 – 77.6	14.88 ± 5.88^{a}	29.56 ± 2.85^{b}	8.2 ± 0.73^{a}	8.7 ± 1.74^{a}	8.22 ± 1.48^{a}
AST/SGOT (units/L)	30 – 380	193.6 ± 65.47^{a}	342.27 ± 35.4^{c}	289.87 ± 51.95^{bc}	205.75 ± 11.12^{a}	218.4 ± 66.1^{ab}
ALT/SGPT (units/L)	35 – 80	75 ± 13.3^{a}	101 ± 1^{a}	83.67 ± 18.99^{a}	79.8 ± 32.61^{a}	119.4 ± 79.47^{a}

Values are presented as M + SD; One way ANOVA followed by a Tukey's post hoc test; $P<0.05$ was considered statistically significant; a,b,c Significant p-value, between treatment groups.

DISCUSSION

A multiple intraperitoneal dose of streptozotocin (50 mg/kg body weight, every day for four days) was used to induce the diabetes model in this study. Mice were confirmed diabetic if their blood glucose were ≥200 mg/dL. This study evaluates the effect of SPK administration on several parameters related to diabetes, including nuclear factor erythroid 2–related factor 2 Nrf2, SOD, MDA and biochemical parameters (triglycerides, blood glucose, creatinine, urea/BUN, SGOT and SGPT). High blood glucose levels are not the only cause of DM, which is a complex illness. One of the factors influencing the development of diabetes is oxidative stress. Furthermore, the severity of diabetes may be impacted by high levels of several biochemical parameters. Oxidative stress exerts detrimental effects on physiological functions by promoting inflammatory pathways, inducing cellular damage and contributing to the pathogenesis of various diseases (Steven *et al.*, 2019). The endogenous antioxidant defense system, particularly the superoxide dismutase (SOD) enzyme, is essential for regulating oxidative balance by mitigating excess free radicals and maintaining homeostasis between

pro-oxidants and antioxidants. The use of natural materials is currently widely utilised as an alternative treatment for metabolic diseases such as diabetes. In this study, a combination of celery (*A. graveolens*), paitan (*T.diversifolia*) and turmeric (*C. longa*) was used as an alternative option in treating DM. Based on the research results, CST showed effectiveness in controlling various clinical biochemical parameters related to diabetes mellitus. The active compounds in this combination, such as flavonoids from celery, sesquiterpene lactones and chlorogenic acid from paitan (Tagne *et al.*, 2018) and curcumin from turmeric, possess antioxidant, anti-inflammatory and hypoglycemic properties that contribute to diabetes management.

Previous phytochemical-based studies have also demonstrated the efficacy of plant-derived compounds in managing diabetes and its complications. Silymarin, a complex flavonoid compound, has been shown to improve glycemic control and reduce cardiovascular risk by modulating urotensin II and oxidative stress pathways (Jayaraman *et al.,* 2025). Maslinic acid, a pentacyclic triterpenoid, has been demonstrated to exert antidiabetic effects through activation of the Nrf2 signaling pathway and regulation of oxidative stress and lipid metabolism (Li *et al.,* 2025)). Moreover, β-sitosterol and guggulsterone have been identified as multitarget natural compounds capable of modulating AKT1 and Nrf2 activity, thereby improving insulin sensitivity and mitigating inflammation associated with hyperglycemia (Jayaraman *et al.,* 2025). These findings support the hypothesis that the synergistic combination of *A. graveolens, T. diversifolia* and *C. longa* exerts its therapeutic effects through molecular mechanisms involving antioxidant and anti-inflammatory modulation mediated by Nrf2 activation.

A transcription factor called Nrf2 is crucial for controlling the expression of genes that produce antioxidants and shielding cells from oxidative damage. Based on the analysis results, it shows that under DM conditions, the Nrf2 profile is significantly ($p<0.05$) lower compared to the control and SPK treatment. This is consistent with previous research that indicated a decrease in Nrf2 activity and levels under DM conditions. Chronic hyperglycemia in DM patients leads to an increase in ROS production, which ultimately disrupts the Nrf2 signaling pathway (Hendrawati, 2017).

Numerous causes, such as post-translational changes of NRF2, degradation mediated by the Keap1 protein (Kelch-like ECH-associated protein 1) and severe oxidative damage, can contribute to the decline in Nrf2. Normally, oxidative stress triggers the activation of Nrf2, however diabetes interferes with this process. Antioxidant enzymes like SOD, which are crucial in scavenging ROS, are expressed less frequently as a result. Furthermore, the reduced expression of Nrf2 in diabetes is also a result of insulin resistance and chronic inflammation. Pro-inflammatory cytokines including TNF-α and IL-6, which can prevent Nrf2 activation, can be produced in greater quantities as a result of chronic inflammation. Consequently, increased susceptibility to persistent oxidative damage accelerates tissue injury and promotes the progression of various diabetic complications, including diabetic nephropathy, retinopathy, neuropathy, and cardiovascular disorders (Yi *et al*., 2024).

The decrease in SOD levels in DM conditions in this study is in line with the Nrf2 analysis results, where low Nrf2 levels will result in low SOD levels. This is because Nrf2 is an endogenous antioxidant transcription factor. If the Nrf2 transcription factor is disrupted, it will automatically cause the expression of endogenous antioxidants to be disturbed. SOD is one of the endogenous antioxidants that plays a role in reducing oxidative stress levels (Dodson *et al.*, 2022).

The SOD is the main antioxidant enzyme that plays a role in protecting cells from damage caused by ROS. SOD functions to convert superoxide radicals into hydrogen peroxide (H_2O_2), which is then neutralized by catalase or glutathione peroxidase (GPX). In the condition of diabetes mellitus, the levels and activity of SOD often decrease significantly. The decrease in SOD levels in diabetes is caused by excessive oxidative stress due to chronic hyperglycemia. Excessive ROS production exceeds the capacity of the body's antioxidant system, including SOD, leading to an imbalance between free radical production and antioxidant defense mechanisms (Promyos *et al.*, 2023).

Additionally, non-enzymatic glycosylation caused by high blood glucose levels can also damage the structure and function of SOD, thereby reducing the effectiveness of this enzyme in neutralizing free radicals. Insulin resistance and chronic inflammation in diabetes also exacerbate the decrease in SOD activity. The low levels of SOD contribute to increased cellular damage, endothelial dysfunction and the development of microvascular and macrovascular complications in diabetes. In addition to the profiles of Nrf2 and SOD, MDA was also observed in this study. Based on the analysis results, the MDA profile in all treatments did not show any significant differences. The MDA is the end product of lipid peroxidation that is often used as a marker of oxidative stress. In diabetes patients, increased oxidative stress due to chronic hyperglycemia leads to increased lipid peroxidation, resulting in high levels of MDA in the blood. However, under certain conditions, MDA levels in diabetes patients can be similar to those in healthy individuals. This can be influenced by individual genetic variations as well as compensatory mechanisms from the body's antioxidant system, such as increased activity of certain antioxidant enzymes, which can help control MDA levels to remain within normal limits despite oxidative stress (Yilgor & Demir, 2024).

In addition to parameters related to oxidative stress, the evaluation of clinical biochemical parameters was also observed in this study. Clinical biochemical parameters include triglycerides, blood glucose, creatinine, urea/BUN, AST/SGOT and ALT/SGPT. In the DM condition in this study, these parameters were significantly higher compared to healthy mice. The administration of SPK as a whole was able to lower the levels of these biochemical parameters.

The triglyceride levels in DM conditions in this study were found to be significantly higher compared to healthy mice and all doses of SPK treatment. In line with previous research, triglyceride levels in DM conditions are higher compared to normal conditions. Increased triglyceride levels are often found in diabetes mellitus patients, especially in conditions of insulin resistance. Insulin resistance inhibits the action of this hormone in suppressing lipolysis in adipose tissue, thereby increasing the release of free fatty acids into the bloodstream. Excess free fatty acids will be taken up by the liver and converted into triglycerides (Song *et al.*, 2021). In addition, the activity of lipoprotein lipase (LPL), an enzyme that plays a role in the breakdown of triglycerides in peripheral tissues, is often reduced in diabetes. The decrease in LPL activity leads to an increase in plasma triglycerides (Shang & Rodrigues, 2024).

The blood glucose levels in DM mice are significantly higher compared to healthy mice. In addition, the administration of SPK affects the reduction of blood glucose levels. The condition of hyperglycemia in diabetes mellitus is caused by impaired insulin secretion, insulin resistance, or both. Insulin resistance hampers the body's ability to utilise glucose as an energy source, leading to the accumulation of glucose in the blood. Additionally, damage to the pancreatic beta cells reduces insulin production, further exacerbating the condition of hyperglycemia (Rao *et al.*, 2022).

Subsequently, diabetic mice have elevated levels of SGPT and SGOT, two clinical biochemical measures associated with liver and kidney function, respectively, as well as urea/BUN and creatinine levels. In diabetic patients, elevated BUN and creatinine levels are frequently linked to compromised kidney function. Because of inflammation and oxidative stress, chronic hyperglycemia damages the renal tubules and glomeruli. The kidneys' capacity to filter metabolic waste products, such as urea and creatinine, is diminished by this injury, which raises blood levels of these substances (Jin *et al.*, 2023).

The increase in SGOT and SGPT levels in diabetes patients is often associated with liver damage. Chronic hyperglycemia can cause oxidative stress and fat accumulation in the liver, triggering a condition known as non-alcoholic fatty liver disease (NAFLD). This condition leads to hepatocyte damage, which ultimately increases the release of SGOT and SGPT enzymes into the bloodstream (Boeriu *et al.*, 2023).

Additionally, insulin resistance commonly seen in diabetes patients causes disturbances in fat and glucose metabolism in the liver, which also contributes to the increase in SGOT and SGPT levels. Excessive fat accumulation in the liver (steatosis) leads to chronic inflammation and liver fibrosis, further worsening the

condition and increasing these liver enzyme levels (Boeriu *et al.*, 2023).

The increase in SGOT and SGPT can also be caused by the toxic effects of advanced glycation end products (AGEs) resulting from chronic hyperglycemia. AGEs play a role in damaging the structure and function of liver cells, which subsequently increases the levels of both enzymes in the blood (Mengstie *et al.*, 2022).

This study shows that this combination can lower blood glucose levels by increasing insulin sensitivity and protecting pancreatic beta cells from oxidative damage. Additionally, this combination is also effective in lowering triglyceride levels by inhibiting lipid synthesis in the liver and increasing the activity of the enzyme LPL. The decrease in urea/BUN and creatinine levels indicates protection against kidney damage due to oxidative stress. On the other hand, lower levels of SGOT and SGPT after the administration of this combination indicate protection against liver damage and reduction of hepatocyte inflammation. With complementary mechanisms, the combination of celery, paitan and turmeric provides comprehensive benefits in the management of diabetes mellitus and the prevention of related complications. Within the broader framework of phytochemical research, the present findings reinforce the notion that plant-derived metabolites function as pleiotropic modulators of metabolic homeostasis. Network pharmacology and molecular docking studies have revealed that multiple phytochemicals, including flavonoids and terpenoids, exert their therapeutic effects through convergent pathways involving AKT1, Nrf2 and key inflammatory mediators (Zheng *et al*., 2025). In this context, the observed efficacy of the SPK formulation suggests a coordinated modulation of redox signaling and metabolic regulation, analogous to mechanisms proposed for other well-characterized phytocompounds such as silymarin and maslinic acid. This integrative perspective strengthens the scientific rationale for employing phytochemical combinations as potential adjunctive strategies in diabetes management and the prevention of its complications.

CONCLUSION

In conclusion, the formulation of celery (*A. graveolens*), paitan (*T. diversifolia*) and turmeric (*C. longa*) has demonstrated potential as an antidiabetic agent through its antioxidant and anti-inflammatory mechanisms, as well as the regulation of the Nrf2 pathway. Furthermore, the administration of SPK gives an effect on decrease level of triglyceride, blood glucose, creatinine, urea/BUN, SGOT and SGPT. The findings of this study hold potential for practical applications, including the development of herbal-based supplements or formulation of dietary guidelines aimed at supporting more effective management of diabetes in patients.

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REFERENCES

Adharini, W.I., Nilamsari, R.V., Lestari, N.D., Widodo, N. & Rifa'i, M. (2020a). Immunomodulatory effects of formulation of *Channa micropeltes* and *Moringa oleifera* through anti-inflammatory cytokines regulation in type 1 diabetic mice. *Pharmaceutical Sciences*, 26 (3): 270–278. DOI: 10.34172/PS.2020.43.

Adharini, W.I., Nilamsari, R.V., Lestari, N.D., Widodo, N. & Rifa'i, M. (2020b). The role of *Moringa oleifera*- Ifalmin® formulation in regulation of B220+IgM+ and B220+IgG+ in diabetic mice. *The Journal of Experimental Life Science*, 10 (1): 43–48. DOI: 10.21776/ub.jels. 2019.010.01.08.

Assaggaf, H.M. (2025). Investigating the antidiabetic properties of *Apium graveolens* extract and its inhibition of enzymes associated with hyperglycemia. *International Journal of Biological Macromolecules*, 290: 138873. DOI: 10.1016/j.ijbiomac.2024.138873.

Boeriu, A., Dobru, D. & Fofiu, C. (2023). Non-invasive diagnostic of NAFLD in type 2 diabetes mellitus and risk stratification: Strengths and

limitations. *Life*, 13 (12): 2262. DOI: 10.3390/life13122262.

Cortez-Navarrete, M., Pérez-Rubio, K.G. & Escobedo-Gutiérrez, M.de.J. (2023). Role of *Fenugreek, Cinnamon, Curcuma longa, Berberine* and *Momordica charantia* in type 2 diabetes mellitus treatment: a review. *Pharmaceuticals*, 16 (4): 515. DOI: 10.3390/ph16040515.

Dodson, M., Shakya, A., Anandhan, A., Chen, J., Garcia, J.G.N. & Zhang, D.D. (2022). NRF2 and diabetes: the good, the bad and the complex. *Diabetes*, 71 (12): 2463–2476. DOI: 10.2337/db22-0623.

Ejelonu, O.C., Elekofehinti, O.O., Adanlawo, I.G. & Kundu, R. (2022). TGR5 potentiates GLP-1 secretion and cause pancreatic islet regeneration in response to *Tithonia diversifolia* saponin-rich extract in diabetic model mice. *Phytomedicine Plus*, 2 (1): 100203. DOI: 10.1016/j.phyplu.2021.100203.

Hendrawati, A. (2017). Nuclear factor erythroid 2-related factor 2 (Nrf2) as a therapeutical target in type-2 diabetes mellitus: a review. *The Indonesian Biomedical Journal*, 9 (2): 73–77. DOI: 10.18585/inabj.v9i2.311.

IDF. (2021). *Facts & figures*. https://idf.org/about-diabetes/diabetes-facts-figures/.

Jaiswal, D., Rai, P.K., Mehta, S., Chatterji, S., Shukla, S., Rai, D.K., Sharma, G., Sharma, B., khair, S. & Watal, G. (2013). Role of *Moringa oleifera* in regulation of diabetes-induced oxidative stress. *Asian Pacific Journal of Tropical Medicine*, 6 (6): 426–432. DOI: 10.1016/S1995-7645(13)60068-1.

Jayaraman, S., Prasad, M., Natarajan, S.R., Krishnamoorthy, R., Alshuniaber, M.A., Gatasheh, M.K., Veeraraghavan, V.P., Rajagopal, P. & Palanisamy, C.P. (2025). Molecular mechanisms underlying the effects of beta-sitosterol on TGF-β1/Nrf2/SIRT1/p53-mediated signaling in the kidney of a high-fat diet and sucrose-induced type-2 diabetic rat. *Chemico-Biological Interactions*, 411: 111443. DOI: 10.1016/j.cbi.2025.111443.

Jin, Q., Liu, T., Qiao, Y., Liu, D., Yang, L., Mao, H., Ma, F., Wang, Y., Peng, L. & Zhan, Y. (2023). Oxidative stress and inflammation in diabetic nephropathy: role of polyphenols. *Frontiers in Immunology*, 14: 1185317. DOI: 10.3389/fimmu.2023.1185317.

Li, Q., He, G., Zheng, R., Liu, C., Wang, C., Liu, Z., Li, Z. & Lu, C. (2025). Ferroptosis-related biotargets and network mechanisms of maslinic acid against myocardial ischemia-reperfusion injury: an integrated bioinformatic and experimental approach. *Combinatorial Chemistry & High Throughput Screening*, 28. DOI: 10.2174/0113862073354768241217162514.

Lestari, N.D., Adharini, W.I., Widodo, W., Rahayu, S., Tsuboi, H., Jatmiko, Y.D. & Rifa'i, M. (2022). Anti-inflammatory evaluation of moringa-albumin combination in inhibiting IFN-γ and TNF-α expression in diabetic mouse model. *Research Journal of Pharmacy and Technology*, 15 (2): 628–632. DOI: 10.52711/0974-360X.2022.00103.

Mengstie, M.A., Abebe, E.C., Teklemariam, A.B., Mulu, A.T., Agidew, M.M., Azezew, M.T., Zewde, E.A. & Teshome, A.A. (2022). Endogenous advanced glycation end products in the pathogenesis of chronic diabetic complications. *Frontiers in Molecular Biosciences*, 9. DOI: 10.3389/fmolb.2022.1002710.

Ministry of Health Republic Indonesia. (2022). *Diabetes melitus is our problem*. https://yankes.kemkes.go.id/view_artikel/1131.

Morales, M. & Munné-Bosch, S. (2019). Malondialdehyde: facts and artifacts. *Plant Physiology*, 180 (3): 1246–1250. DOI: 10.1104/pp.19.00405.

Mostafavinia, A., Amini, A., Ghorishi, S.K., Pouriran, R. & Bayat, M. (2016). The effects of dosage and the routes of administrations of streptozotocin and alloxan on induction rate of typel diabetes mellitus and mortality rate in rats. *Laboratory Animal Research*, 32 (3): 160–165. DOI: 10.5625/lar.2016.32.3.160.

Promyos, N., Phienluphon, P.P., Wechjakwen, N., Lainampetch, J., Prangthip, P. & Kwanbunjan, K. (2023). Inverse correlation of superoxide dismutase and catalase with type 2 diabetes among rural Thais. *Nutrients*, 15 (9): 2071. DOI: 10.3390/nu15092071.

Rao, C.H., Liu, L., Gao, J., Du, Z.H. & Gao, C. (2022). Establishment of blood glucose control model in diabetic mice. *International Journal of Ophthalmology*, 15 (12): 1908–1914. DOI: 10.182 40/ijo.2022.12.03.

Shang, R. & Rodrigues, B. (2024). Lipoprotein lipase as a target for obesity/diabetes related cardiovascular disease. *Journal of Pharmacy &*

Pharmaceutical Sciences, 27: 13199. DOI: 10.3389/jpps.2024.13199.

Shawki, H.A., Elzehery, R., Shahin, M., Abo-hashem, E.M. & Youssef, M.M. (2021). Evaluation of some oxidative markers in diabetes and diabetic retinopathy. *Diabetology International*, 12 (1): 108–117. DOI: 10.1007/s13340-020-00450-w.

Song, K., Park, G., Lee, H.S., Choi, Y., Oh, J.S., Choi, H.S., Suh, J., Kwon, A., Kim, H.S. & Chae, H.W. (2021). Prediction of insulin resistance by modified triglyceride glucose indices in youth. *Life*, 11 (4): 286. DOI: 10.3390/life11040286.

Steven, S., Frenis, K., Oelze, M., Kalinovic, S., Kuntic, M., Bayo Jimenez, M.T., Vujacic-Mirski, K., Helmstädter, J., Kröller-Schön, S., Münzel, T., & Daiber, A. (2019). Vascular inflammation and oxidative stress: major triggers for cardiovascular disease. *Oxidative Medicine and Cellular Longevity*, 2019 (1): 7092151. DOI: 10.1155/2019/7092151.

Stolf, A.M., Campos Cardoso, C., Morais, H.d., Alves de Souza, C.E., Lomba, L.A., Brandt, A.P., Agnes, J.P., Collere, F.C., Galindo, C.M., Corso, C.R., Spercoski, K.M., Locatelli Dittrich, R., Zampronio, A.R., Cadena, S.M.S.C. & Acco, A. (2018). Effects of silymarin on angiogenesis and oxidative stress in streptozotocin-induced diabetes in mice. *Biomedicine & Pharmacotherapy*, 108: 232–243. DOI: 10.1016/j. biopha.2018.09.042.

Sunita, R., Sahidan, S. & Hidayat, R. (2020). Evaluation of malondialdehyde in type 2 diabetes mellitus patients as oxidative stress markers in Bengkulu population. *Bioscientia Medicina : Journal of Biomedicine and Translational Research*, 4 (3): 45–54. DOI: 10.32539/bsm.v4i3.146.

Tagne, A.M., Marino, F. & Cosentino, M. (2018). *Tithonia diversifolia* (Hemsl.) A. gray as a medicinal plant: a comprehensive review of its ethnopharmacology, phytochemistry, pharmacoto-xicology and clinical relevance. *Journal of Ethnopharmacology*, 220: 94–116. DOI: 10.1016/j.jep.2018.03.025.

Tiwari, B.K., Pandey, K.B., Abidi, A.B. & Rizvi, S.I. (2013). Markers of oxidative stress during diabetes mellitus. *Journal of Biomarkers*, 2013 (1): 378790. DOI: 10.1155/2013/378790.

WHO. (2016). *Diabetes fact and number*. Geneva: WHO Press.

Yi, M., Cruz Cisneros, L., Cho, E.J., Alexander, M., Kimelman, F.A., Swentek, L., Ferrey, A., Tantisattamo, E. & Ichii, H. (2024). Nrf2 pathway and oxidative stress as a common target for treatment of diabetes and its comorbidities. *International Journal of Molecular Sciences*, 25 (2): 821. DOI: 10.3390/ijms25020821.

Yilgor, A. & Demir, C. (2024). Determination of oxidative stress level and some antioxidant activities in refractory epilepsy patients. *Scientific Reports*, 14 (1): 6688. DOI: 10.1038/s41598-024-57224-6.

Zheng, Z., Gao, J., Ma, Y. & Hou, X. (2025). Cellular and Molecular Mechanisms of Phytochemicals Against Inflammation-Associated Diseases and Viral Infection. *Cell Biology International*, 49 (6): 606-633. DOI: 10.1002/cbin.70011.