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Effect of Bekatewak Flour on Fasting Blood Glucose Levels (Study on Type 2 Diabetes Mellitus Rats Fed a High-Fat Diet)

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Abstract: High-fat diet (HFD) causes oxidative stress, which is a trigger for insulin resistance in type 2 diabetes mellitus. The provision of foods rich in antioxidants, such as rice bran, tempeh, and Javanese ginger, is needed to reduce oxidative stress. This study aimed to determine the effect of giving Bekatewak (a combination of rice bran, tempeh, and Javanese ginger) flour on fasting blood glucose levels of type 2 diabetes mellitus rats induced by HFD. This study was true experimental research using a pre- and post-test randomized controlled group design with a total of 18 rats as samples. There were three groups: the negative control group (K-), the positive control group (K+), and the treatment group (P). The study results showed a significant difference in fasting blood glucose levels before and after the administration of Bekatewak flour at a dose of 540 mg/200 g/bodyweight ($p<0.05$). There were significant differences in fasting blood glucose levels after intervention between K- and K+, K- and P, and K+ and P groups ($p<0.001$). Bekatewak flour has the potential to be developed as a functional food based on natural antioxidants to help control blood glucose levels and prevent insulin resistance caused by a high-fat diet.

Keywords: bekatewak, hyperglycemia, high-fat diet

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INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by hyperglycemia, an abnormal physiological condition marked by continuously elevated blood glucose levels due to impaired insulin secretion, insulin action, or both . The diagnosis of diabetes mellitus is established when fasting blood glucose (FBG) levels are ≥ 126 mg/dL (Soelistijo et al., 2021). Globally, approximately 589 million adults aged 20 to 79 years are estimated to be living with diabetes mellitus in 2024. In Indonesia, an estimated 20.4 million adults are projected to have diabetes mellitus in 2024. Roughly 90% of diabetes mellitus patients worldwide have Type 2 Diabetes mellitus (International Diabetes Federation, 2025)

Obesity and overweight are major risk factors that significantly contribute to the development of type 2 diabetes mellitus. It is estimated that approximately 80–90% of individuals with type 2 diabetes mellitus are overweight or obese (Nianogo & Arah, 2022). Obesity poses a risk for adipose tissue dysfunction, leading to an excessive increase in free fatty acids (FFAs), reactive oxygen species (ROS), and pro-inflammatory cytokines, all of which provoke insulin resistance (Ahmed et al., 2021). An important part of managing diabetes mellitus is medical nutrition therapy. This includes dietary regulation, such as consuming complex carbohydrate sources with a low glycemic index and increasing the intake of high-fiber foods (Soviana & Maenasari, 2019).

Rice bran contains approximately 15% dietary fiber, largely composed of insoluble dietary fibers such as cellulose, hemicellulose, and arabinoxylan. However, it also possesses soluble dietary fibers like pectin and β-glucan (Sapwarobol et al., 2021). The fiber content in rice bran has beneficial effects in improving both fasting and postprandial blood glucose levels in patients with diabetes mellitus (Silva et al., 2005). Additionally, rice bran also contains other bioactive components, including antioxidants such as ferulic acid, tricin, β-sitosterol, γ-oryzanol, tocotrienols/tocopherols, and phytic acid(Henderson et al., 2012). The primary antioxidant in rice bran is γ-oryzanol (62.9%), which can relieve endoplasmic reticulum stress in pancreatic β-cells and improve β-cell (Kozuka et al., 2015).

Another useful food that is rich in antioxidants is tempeh. Genistein, a member of the isoflavonoid group included in tempeh, is one of them. It has the ability to inhibit α-glucosidase activity, which can act as an antidiabetic mechanism by slowing postprandial carbohydrate absorption(Astawan, 2019). Furthermore, it can also enhance the activity of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase in the liver, which contributes to improved insulin sensitivity(Amanat et al., 2021).

Some research indicates that administering a combination of tempeh flour and rice bran flour at a dose of 33.7g did not significantly reduce triglyceride levels in dyslipidemic patients (Bintanah & Mufnaetty, 2021). Elevated triglycerides will eventually exacerbate insulin resistance and also accelerate beta-cell dysfunction (Ma et al., 2020). However, adding functional food ingredients like Javanese ginger, which contains the compounds curcumin and xanthorrhizol, can lower triglyceride levels and thereby improve insulin resistance (Xia et al., 2020).

Javanese ginger is a local food ingredient that contains antioxidant components (Rosidi et al., 2014). Curcumin, an antioxidant found in Javanese ginger, can ameliorate insulin resistance and

alleviate levels of free fatty acids, leptin, resistin, interleukin 6 (IL-6), Interleukin-1 beta (IL-1β), and tumor necrosis factor-α (TNF-α) (Marton et al., 2021).

High-fat diet (HFD) administration leads to mitochondrial dysfunction and oxidative stress in adipose tissue (Li et al., 2023). A diet rich in fat or carbohydrates can increase Reactive Oxygen Species (ROS), ultimately causing oxidative stress. This process involves lipid peroxidation, which then generates ROS. In addition, adipose tissue contributes to the production of various adipokines, including pro-inflammatory cytokines such as leptin, TNF-α, and IL-6. Both TNF-α and IL-6 induce ROS production and the occurrence of oxidative stress (Jiang et al., 2021). Oxidative stress has an important role in developing insulin resistance by disturbing the insulin signaling pathway and causing the imbalance of adipocytokine regulation (Li et al., 2023). The insulin resistance is known as the initial cause of hyperglycemia in type 2 diabetes mellitus (International Diabetes Federation, 2025).

Previous studies have shown that each component of Bekatewak has antidiabetic potential through different mechanisms. Rice bran has been reported to reduce blood glucose levels by increasing GLUT4 expression and through the antioxidant activity of γ-oryzanol (Kozuka et al., 2015; Sapwarobol et al., 2021). Tempeh contains the isoflavone genistein, which can enhance insulin sensitivity and decrease levels of IL-6 and TNF-α (Amanat et al., 2021; Jain et al., 2022). Meanwhile, Javanese ginger (Curcuma xanthorrhiza) is rich in curcuminoids and xanthorrhizol, compounds that have been shown to alleviate oxidative stress and improve insulin resistance (Kim et al., 2014; Xia et al., 2020).

This study contributes novelty by the formulation of a combination of these three local food ingredients rice bran, tempeh, and Javanese ginger into Bekatewak flour as a natural antioxidant-based functional food. This combination is expected to produce synergistic effects in reducing fasting blood glucose levels in type 2 diabetic rats induced by a high-fat diet. Such a combined approach has been rarely explored in previous studies, particularly in high-fat diet–induced diabetic animal models. Therefore, it is necessary to investigate how bekatewak (a combination of rice bran, tempeh, and Javanese ginger) flour influences fasting blood glucose levels (FBG) in type 2 diabetes mellitus Wistar rats fed a high-fat diet. This research assumes that the administration of Bekatewak flour can reduce fasting blood glucose levels in type 2 diabetic rats induced by a high-fat diet.

METHODS

Research Design, Time, and Location

This research employed a true experimental design with a randomized pretest and posttest with a control group design. The animal study was conducted from June to July 2023 at the Integrated Research and Testing Laboratory, Gadjah Mada University, Yogyakarta. The use of animal subjects adhered to the 3R principles (Replacement, Reduction, and Refinement). Throughout the study, rats were housed in clean cages and provided with standard feed and water ad libitum. Procedures with the potential to cause pain were administered with anesthesia at recommended doses, and euthanasia procedures followed established animal research ethics protocols. This research was conducted after obtaining approval from the Health Research Ethics Committee (KEPK), Faculty of Medicine, University of Muhammadiyah Semarang, with No: 099/EC/KEPK-FK/UNIMUS/2023.

Blood glucose levels were determined using the Glucose Oxidase-Peroxidase Aminoantipyrine (GOD-PAP) method. This method is commonly employed in laboratories due to its high precision and ability to yield accurate data. The instrument utilized in this method was a spectrophotometer. In this process, glucose was oxidized to gluconic acid, while oxygen was reduced to hydrogen peroxide through the activity of the glucose oxidase enzyme. Subsequently, hydrogen peroxide was broken down by the peroxidase enzyme into water and reactive oxygen. This reactive oxygen then reacted with 4-aminoantipyrine and phenol, forming a colored quinoneimine compound. The intensity of the color produced was directly proportional to the glucose concentration in the sample and was analyzed colorimetrically at a wavelength of 505 nm against a similarly processed standard comparator(Subiyono et al., 2016).

Research Materials and Equipment

Table 1

Research Materials and Equipment

Materials	Equipment
Bekatewak Flour Ingredients: Rice bran flour, tempeh flour, Javanese ginger flour.	**Equipment for Bekatewak Flour Production:** Cabinet dryer, digital scale, disc mill, steamer pot, knife blender mixer, and 100-mesh sieve.
GOD-PAP Examination Materials: Glucose reagent, standard reagent, ketamine, distilled water, and serum samples. **High-Fat Diet Preparation Materials:** Beef brain porridge.	**Equipment for GOD-PAP Examination:** Photometer, micropipettes (10 µL and 1000 µL), microhematocrit tubes and blood collection tubes, blue and yellow pipette tips, centrifuge water bath/incubator, test tubes, cuvettes, vortex mixer, timer, syringes (3 mL).

Population and Sample

The study population consisted of male Wistar rats (Rattus norvegicus), 8-12 weeks old, weighing 150-200 g, active, healthy, and without anatomical abnormalities or deformities. Rats were excluded if they died during the study, exhibited unusual or stressed behavior, or refused to eat. This study included three groups: a negative control group (K-), a positive control group (K+), and a treatment group (P). The minimum sample size was determined based on World Health Organization (WHO) guidelines, which recommended at least 5 animals per group. An additional 10% (or 1 animal per group) was added to account for potential dropouts, resulting in a total sample size of 18 rats. Sample selection was performed using simple random sampling.

Research Procedures

Each rat was acclimatized to similar conditions for 3 (three) days in separate individual cages within a room with adequate circulation and lighting, and a room temperature ranging from 28-32°C. During the acclimatization period, rats were provided with standard adult chicken feed II (AD II) at 10% of their body weight or 20 grams per day, along with water ad libitum.

Following the acclimatization period, blood samples (± 1 µL) were collected from the rats via the retro-orbital sinus. The blood was then centrifuged to obtain serum. Initial fasting blood glucose (FBG) levels were subsequently measured after the rats had been fasted for 8-12 hours and anesthetized with ketamine at 60 mg/kg BW. FBG levels were analyzed using the glucose oxidase (GOD-PAP) method. Steps for Blood Glucose Level Examination were as follows:

1. Prepare three tubes, each of which is filled with the components as detailed in Table 2.
2. Vortex for ten seconds.
3. Incubate for ten minutes at 37°C.
4. Read the absorbance at 500 nm using a photometer within 60 minutes.

Table 2

Components of the GOD-PAP Method

Component	Blank (µL)	Sample (µL)	Standard (µL)
Serum	-	10	-
Standard	-	-	10
Distilled water	10	-	-
Reagent	1000	1000	1000

Glucose concentration (mg/dL)=

$$\frac{\text{Absorbance of sample} - \text{absorbance of blank}}{\text{Absorbance of standard} - \text{Absorbance of blank}} \times \text{Concentration of glucose standard (mg/dL)}$$

The concentration of the glucose standard used was 100 mg/dL.

Subsequently, the K+ and the P group were provided with a standard diet and water ad libitum. Additionally, HFD consisting of bovine brain porridge (prepared with a 2:1 ratio of distilled water to bovine brain porridge) was administered via oral gavage at 3 mL/day for 15 days (Naufalina & Nuryanto, 2014). Conversely, the K-group received only a standard diet and water ad libitum. Following this, blood samples were collected for the analysis of FBG levels before any intervention. Rats were considered to have developed diabetes if their FBG levels were ≥ 126 mg/dL (International Diabetes Federation, 2025; Jung et al., 2011). Bekatewak flour was administered to the treatment group at a dose of 540 mg/200g body weight/day via oral gavage for 30 days. In contrast, the K- and the K+ groups received only a standard diet and water ad libitum. After 30 days, blood samples were recollected from the rats to assess FBG levels post-intervention.

Data Analysis

Data on fasting blood glucose (FBG) levels were collected at three time points: before the high-fat diet administration, after the high-fat diet administration, and after the intervention with bekatewak flour. The collected data underwent editing, coding, processing, and cleaning. Data normality was assessed using the Shapiro-Wilk test, and the homogeneity of variances was evaluated using Levene's test. Subsequently, the data were analyzed using paired sample t-tests, one-way ANOVA, and Bonferroni post hoc tests. After data entry, all data were re-checked to ensure accuracy and to identify any errors.

RESULTS AND DISCUSSION

Effect of High-Fat Diet (HFD) Administration on Fasting Blood Glucose (FBG) Levels

Normality test results, presented in Table 3, indicated that all data distributions were normal (p>0.05), allowing for data presentation as mean ± standard deviation. Mean of fasting blood glucose (FBG) levels before and after high-fat diet (HFD) administration are shown in Table 4. Statistical analysis of Table 4 revealed that before HFD administration, the K-group exhibited the highest mean FBG level at 70.61 mg/dL, while the P group had the lowest at 70.13 mg/dL. Following HFD administration, Group K+ and Group P experienced significant increases in FBG levels, rising by 66.73 mg/dL (95.39%) and 65.33 mg/dL (93.15%), respectively. Paired sample t-tests confirmed a significant growth in FBG levels for both these groups (p<0.05). Conversely, the K-group, which only received a standard diet, showed a minor FBG increase of 1.52%, but this increase was not statistically significant (p>0.05).

Table 3

Results of the Shapiro-Wilk normality test

Treatment Group	Before HFD (*p*-value)	After HFD (*p*-value)	After Intervention (*p*-value)
K-	0.882*	0.989*	0.871*
K+	0.153*	0.837*	0.805*
P	0.757*	0.606*	0.892*

*) Normal data distribution ($p > 0.05$)

The findings of this study are largely consistent with prior research. Specifically, a study conducted by Novita & Sehatman (2019) similarly demonstrates that rats fed an HFD exhibit an increase in FBG levels exceeding normal thresholds and leading to hyperglycemia (Novita & Sehatman, 2019). High-fat diet (HFD) administration is associated with weight gain/adiposity, dyslipidemia, peripheral insulin resistance, and glucose intolerance(Montgomery et al., 2013). Fat accumulation leads to adipocyte damage, which subsequently raises the production of cytokines like TNF-α. It consequently generates reactive oxygen species (ROS) within tissues and elevates the rate of lipid peroxidation (Li et al., 2023). It also systemically induces oxidative stress in both humans and rats by intensifying reactive oxygen species (ROS) production, coupled with elevated nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity and decreased antioxidant enzyme activity(Kesh et al., 2015).

Oxidative stress plays a crucial role in developing insulin resistance by disrupting insulin signalling pathways and causing an imbalance in adipocytokine regulation. Besides, it results in a decrease of glucose transporter type 4 (GLUT4) expression, ultimately weakening insulin sensitivity (Li et al., 2023). Rats fed a high-fat diet showed increased ROS production in the liver and adipose tissue, leading to insulin resistance that provokes the beginning of hyperglycemia in type 2 diabetes mellitus (T2DM) (International Diabetes Federation, 2025; Tangvarasittichai, 2015).

Table 4

Mean FBG levels in rats before and after HFD administration

Treatment Group	Before Mean ± SD	After Mean ± SD	Δ Mean ± SD	*p-value*
K-	70.61 ± 1.77	71.69 ± 1.79	1.08 ± 0.28	0.141
K+	69.95 ± 2.13	136.68 ± 3.15	66.73 ± 4.56	<0.001*
P	70.13 ± 2.02	135.46 ± 2.32	65.33 ± 3.71	<0.001*

*) Statistically significant data (p<0.05)

Effect of Bekatewak Flour Administration on FBG Levels

Mean fasting blood glucose (FBG) levels before and after intervention are presented in Table 5. Statistical analysis of Table 5 revealed that before intervention, the K+ group exhibited the highest mean FBG level at 136.68 mg/dL, whereas the K- group had the lowest at 71.69 mg/dL. Following the intervention, the K+ group showed a tendency towards a rise in FBG levels by 2.28 mg/dL (1.66%). Paired sample t-test results indicated a significant increase in this group (p=0.009). The K-group also displayed a slight increase in FBG levels by 1.39 mg/dL (1.93%), but this change was not statistically significant (p=0.141). Conversely, the P group demonstrated a significant decrease in FBG levels of 43.69 mg/dL (32.26%) after the intervention, as confirmed by a paired sample t-test (p<0.001).

Table 5

Mean FBG levels in rats before and after intervention

Treatment Group	Before Mean ± SD	After Mean ± SD	Δ Mean ± SD	*p-value*
K-	71.69 ± 1.79	73.07 ± 1.54	1.39 ± 0.52	0.141
K+	136.68 ± 3.15	138.95 ± 3.53	2.28 ± 1.33	0.009*
P	135.46 ± 2.32	91.76 ± 1.77	-43.69 ± 3.73	<0.001*

*) Statistically significant data (p<0.05)

The comparison of FBG levels for each group after intervention is presented in Table 6. The results of the one-way ANOVA in Table 5 indicated that there was at least one statistically significant difference in mean FBG levels among the groups (p=0.000). Consequently, a Bonferroni post hoc test was performed. Based on the results of the post hoc test in Table 7, significant differences in FBG levels were observed between the K- and K+ groups, the K- and P groups, and the K+ and P groups.

Bekatewak flour was administered to the P group for 30 days, alongside consistent provision of standard feed to the rats. The K+ group, which received only standard feed, served as a comparison for the P group. After four weeks of bekatewak flour administration, the mean fasting blood glucose (FBG) levels in the P group decreased, reaching a normal condition (<126 mg/dL). Statistical analysis confirmed a significant difference in FBG levels after Pre-Treatment Levels (PTL) compared to after intervention in both the P and K+ groups. However, the FBG levels in the K+ group remained above the normal limit (≥126 mg/dL), showing a propensity to rise. This growth is likely attributed to the

high-fat diet provided, which prevented the insulin response in the body from recovering to normal levels. This situation can lead to a sustained increase in blood glucose levels, even after the high-fat diet is discontinued (Rahmawati & Kusumastuti, 2015). Statistical analysis also revealed a significant difference in post-intervention fasting blood glucose levels between the K+ group and the P group.

Table 6

Comparison of FBG levels after intervention

Treatment Group	Mean ± SD (mg/dL)	*p-value*
K-	73.07 ± 1.54	
K+	138.95 ± 3.53	<0.001*
P	91.76 ± 1.77	

*) Statistically significant data (p<0.05)

Table 7

Post-hoc analysis of FBG levels after intervention

Treatment Group		*p-value*
K-	K+	<0.001*
	P	<0.001*
K+	K-	<0.001*
	P	<0.001*
P	K-	<0.001*
	K+	<0.001*

*) Statistically significant data (p<0.05)

The formation of Reactive Oxygen Species (ROS) under hyperglycemic conditions can induce oxidative stress in β-cells. This oxidative stress, consequently, disrupts insulin production by β-cells and also triggers insulin resistance. Antioxidants, on the other hand, can protect β-cells against apoptosis caused by oxidative stress, maintain β-cell function, and potentially reduce diabetes-related complications (Kanwugu et al., 2022). Furthermore, several research findings also indicate that antioxidant administration can enhance insulin sensitivity (van der Schaft et al., 2019).

In many studies, rice bran, tempeh, and Javanese ginger contain antioxidants that can improve blood glucose profiles. Rice bran contains antioxidants, including ferulic acid, tricin, β-sitosterol, γ-oryzanol, tocotrienol/tocopherol, and phytic acid, that aid in the treatment and management of diseases associated with oxidative stress. It can also activate glucose uptake into cells by inducing the expression of glucose transporter messenger ribonucleic acid (mRNA), glucose transporter-1 (GLUT1), and glucose transporter-4 (GLUT4), and simultaneously stimulate insulin release in rat insulinoma cell line (INS-1) cells (Sapwarobol et al., 2021). A study by Parklak (2017) finds that rice bran extract at doses of 2.205 and 4.410 mg/kg/day for 4 weeks significantly decreases fasting blood glucose levels in rats fed a high-fat diet compared to rats given Pre-Treatment Levels (PTL) that receive only standard feed (Parklak et al., 2017). A similar finding by Nurroshima and his colleagues shows that rice bran extract at doses of 330 and 660 mg/kg/day for three weeks significantly lowers fasting blood glucose levels in rats (Nurrohima et al., 2022) .

Tempeh contains antioxidant compounds, primarily isoflavones, with genistein being one prominent form. Notably, tempeh has a higher genistein content than other soy products. Genistein is an antioxidant capable of regulating oxidative stress and inflammation in individuals with diabetes (Prasetyastuti & Ghozali, 2021). It can reduce the levels of IL-6 and TNF-α in rats, thereby acting as an anti-inflammatory and antioxidant agent that can enhance insulin sensitivity(Jain et al., 2022). It can also alleviate hepatic insulin resistance and stimulate pancreatic β-cells to raise insulin production, ultimately improving glucose uptake by cells (Wicaksono et al., 2021). Besides, there is a significant difference in fasting blood glucose levels between rats fed a high-fat diet supplemented with

1.9g/200g/day of tempeh flour for 3 weeks and rats that obtain only a high-fat diet(Dewi et al., 2020). Likewise, administering tempeh flour at a dose of 1.8 g/day for 4 weeks significantly minimizes fasting blood glucose levels in streptozotocin-induced diabetic rats (Bintari et al., 2015) .

Javanese ginger contains bioactive compounds such as curcuminoids, camphor, geranyl acetate, zerumbone, β-curcumene, zingiberene, ar-curcumene, and xanthorrhizol (Kim et al., 2014). Curcumin can inhibit the formation of ROS, which leads to preventing cellular apoptosis and mitigating oxidative stress. The curcumin compounds found in Javanese ginger can strengthen insulin sensitivity by improving insulin signalling in adipose tissue and hepatocytes, as well as by decreasing hepatic lipogenesis and inflammation in adipose tissue (Xia et al., 2020).

Administering Javanese ginger extract can reduce triglyceride secretion and free fatty acid release in serum, thereby mitigating hyperglycemia induced by a high-fat diet (Kim et al., 2014). Additionally, the extract also ameliorates the expression of pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-6 in insulin target tissues, including adipose tissue, liver, and muscle, which ultimately can decrease the level of insulin resistance. The administration of Javanese ginger extract at 50 or 100 mg/kg/day for 16 weeks significantly lowers fasting blood glucose levels in high-fat diet-fed rats compared to rats that only eat a high-fat diet. However, another research by Rahmayani and her colleagues shows a different result, that the Javanese ginger extract at 100 mg/kg/day does not affect the fasting blood glucose levels of streptozotocin-induced diabetic rats (Rahmayani et al., 2015).

The limitation of this study is that the treatment group consisted of only one group receiving a combination of three types of flour (rice bran, tempeh, and Javanese ginger), making it impossible to determine which individual flour was the most effective in improving blood glucose levels in rats. Additionally, this study did not measure other variables such as insulin levels, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) to assess insulin sensitivity, or Homeostasis Assessment Model of Beta-cell function (HOMA-β) to evaluate beta-cell function. To address the limitation of unmeasured variables, this study employed an alternative approach by using fasting blood glucose (FBG) levels as an indicator of glycemic status, which could better represent insulin resistance and pancreatic beta-cell dysfunction. Furthermore, the rats were induced using a high-fat diet, which can trigger insulin resistance, ultimately leading to hyperglycemia due to impaired insulin action.

CONCLUSION

The administration of beef brain porridge at a 2:1 ratio, given via oral gavage at a dose of 3 ml/day for 15 days, significantly increases the fasting blood glucose (FBG) levels in rats. Conversely, the administration of Bekatewak flour (a combination of rice bran, tempeh, and Javanese ginger) at a dose of 540 mg/200gBW/day for 30 days significantly reduces FBG levels in hyperglycemic rats. Thus, rice bran, tempeh, and Javanese ginger are food ingredients rich in antioxidants, which can help lower FBG levels and therefore may serve as alternative functional ingredients to improve blood glucose profiles.

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Author Contribution Statement

Sufiati Bintanah: Conceptualization**;** Methodology; Validation; Investigation; Resources; Writing, Review & Editing; Supervision; Funding Acquisition. **Rijzal Rusyidie:** Software; Formal Analysis; Investigation; Data Curation; Writing Original Draft; Writing, Review & Editing. **Ika Dyah Kurniati:** Conceptualization; Validation.

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