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ORIGINAL ARTICLE

Effect of Different Melatonin-Rich Extract of Emprit Ginger (*Zingiber Officinale Var. Amarum*) Doses on Biochemical Parameters in Streptozotocin-Induced Diabetic Rats

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Abstract

Background/Aim: Emprit ginger (*Zingiber officinale var. amarum*) is an Indonesian natural plant with various bioactivities, including antidiabetic properties. Several studies have shown that bioactivities can be attributed to the presence of flavonoids and also melatonin which playing a role in carbohydrate metabolism and blood sugar levels regulation. Ginger also has an effect on the lipid profile in studies in experimental animals. Therefore, this study aimed to determine the *in vivo* antidiabetic activity of melatonin-rich extract of Emprit ginger using blood sugar and lipid profiles parameters.

Methods: The study procedure comprised 30 male white rats of Wistar strain, which were divided into 5 groups. These included: I - K-: negative control; II - K+: injected with streptozotocin (STZ) - nicotinamide (NA); III - P1: given STZ-NA + 100 mg/kg body weight (BW)/day ginger extract; IV - P2: given STZ-NA + 200 mg/kg BW/day ginger extract; and V - P3: injected with STZ-NA + metformin 300 mg/kg BW/day. In addition, the treatment was carried out for a total of 21 days, followed by the measurement of random blood sugar (RBS) levels and lipid profiles.

Results: The highest pre-treatment RBS, triglyceride, cholesterol, HDL and LDL levels were 426.8 ± 55.8 mg/dL (P2), 142 ± 39.8 mg/dL (P2), 130.8 ± 21.7 mg/dL (K+), 53.4 ± 4.92 mg/dL (P1) and 61.67 ± 17.69 mg/dL (K+), respectively. The results showed that the largest decrease in RBS was obtained in P3 by 262.8 ± 70.6 mg/dL, while P2 experienced the highest decrease in triglyceride levels by 83.66 ± 52.04 mg/dL. In addition, the largest decrease in cholesterol was in P3 by 50.2 ± 26.30 mg/dL, with P1 possessing the highest reduction in HDL by 15.4 ± 15.88 mg/dL. In terms of LDL, the largest decrease was obtained in P3, 20.44 ± 16.68 mg/dL. Based on the results, changes in biochemical parameters levels between all groups showed significant differences except for HDL.

Conclusion: STZ-NA injection caused an increase in RBS and cholesterol in rats. Administration of melatonin-rich ginger extract led to a decrease in RBS, cholesterol and LDL and was more effective in the group given metformin. Melatonin-rich ginger extract at a dose of 200 mg/kg BW/day was more effective in reducing triglycerides compared to other clinical parameters.

Key words: Melatonin; *Zingiber officinale*; Diabetes mellitus; Glucose; Cholesterol; Triglycerides.

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Introduction

Diabetes mellitus (DM) is a non-communicable disease (NCD) with a high prevalence in several countries around the world, particularly, in Indonesia.[1] In addition, it is a metabolic disease characterised by hyperglycaemia caused by insulin insufficiency due to pancreatic beta cell damage (type 1 DM/T1DM) or insulin resistance and secretion defects (type 2 DM/T2DM). In Central Java Province, DM is ranked as the second most common non-communicable disease, affecting 618,546 people (10.7 %) in 2021.[2] According to World Health Organization (WHO), the number of T2DM patients in Indonesia is also expected to increase from 8.4 million in 2000 to 21.3 million by 2030.[3] Consequently, DM remains a significant concern in public health, necessitating concerted prevention and management efforts.

Patients with T2DM typically experience abnormalities in the homeostasis of blood glucose levels characterised by hyperglycaemia. These patients also experience disturbances in the function of the hormone insulin, leading to fat metabolism diseases. Several studies showed the role of melatonin hormone in carbohydrate metabolism and regulation of blood sugar levels in humans or mice.[4, 5] Melatonin has also been identified in several plant species, including coffee, goji, almond, walnut, cherry, tomato and ginger.[6] In addition, melatonin bioactivities can be attributed to constituent compounds, namely alkaloids. These compounds have antioxidant effects, act as free radical scavengers, regulate antioxidant enzymes, reduce mitochondrial electron leakage and interfere with proinflammatory signalling pathways.[6] Arnao stated that ginger contained 583.7 pg/g[7] melatonin, while Badria reported a high content of 142.3 ng/100 g.[8] Study exploring the isolation process of melatonin in cherries typically used a solid/liquid extraction method with acetyl acetate followed by melatonin detection through liquid chromatography have been reported.[9]

The role of melatonin in carbohydrate metabolism is proven by other study that revealed individuals with low melatonin secretion were at significant risk of T2DM.[10] A genomic report analysing variants in the melatonin receptor coding gene found a significant association between the rs10830963 polymorphism of the MTNR1B gene and the obesity status of Javanese T2DM patients.[11] The results indicate that the hormone has a significant effect on the incidence of DM. The administration of melatonin to streptozotocin (STZ) - nicotinamide (NA) - injected experimental animals for 6 weeks caused an increase in insulin and superoxide dismutase activity as well as decreased hepatic malondialdehyde (MDA) levels.[12] The results support the theory that it affects glucose homeostasis and antioxidant agents.

The management of DM is often carried out through medical nutrition therapy accompanied by the use of anti-hyperglycaemic drugs. At present, there is still ongoing development of different drugs, including those from medicinal plant as an alternative treatment. A typical Indonesian natural plant that has widely been studied for antidiabetic potential is ginger (*Zingiber officinale Rosc*), containing several compounds, including melatonin. Ginger has been reported to contain various compounds with antioxidant, immunomodulatory and anti-inflammatory properties. The study by Maqsood et al showed that the administration of ginger powder with compositions of 1 %, 3 % and 5 % for 1 month in rats reduced blood sugar levels from an average of 362.35 ± 25.61 to 117.94 ± 10.96 mg/dL.[13] In addition, Nirvana et al, using ginger extract at doses of 200 mg/kg body weight (BW) and 350 mg/kg BW for 42 days in *Rattus norvegicus* rats reported a decrease in LDL by 32.7 % and 31.4 %, respectively.[14] The antidiabetic activity of ginger can be attributed to the inhibition effect of the enzyme α-glucosidase, which is related to other active phenolic components, such as gingerol and shogaol.[15] The activity of (S)-[8]-gingerol is related to an increase in the surface distribution of GLUT4 on the plasma membrane of L6 myotubes.[16] Increased glucose uptake in L6 cells (rat skeletal muscle cells) supports the potential of ginger in preventing and managing hyperglycaemia in T2DM patients.[17]

Ginger is widely known to have antidiabetic effects, primarily attributed to gingerol and shogaol. Despite the available literature, there are no reports on the role of melatonin compounds in contributing to antidiabetic properties other than gingerol and shogaol found in ginger. Therefore, this study aimed to determine the *in vivo* antidiabetic activity of melatonin-rich extract of Emprit ginger using blood sugar parameters and lipid profiles.

Methods

Study design

This experimental study was conducted using 30 male albino Wistar rats (*Rattus norvegicus*) aged 2 months with BW of 200-300 g obtained from the experimental animal laboratory of Muhammadiyah University Semarang, Indonesia. Rats were divided into 5 groups, including:

K-: Negative control group without STZ and NA injection;
K+: The positive control group was given STZ dose 45 mg/kg BW and NA dose 230 mg/kg BW;
P1: Group given STZ dose 45 mg/kg BW, NA dose 230 mg/kg BW and ginger extract 100 mg/kg BW/day;
P2: Group given STZ dose 45 mg/kg BW, NA dose 230 mg/kg BW and ginger extract 200 mg/kg BW/day;
P3: Group given STZ dose 45 mg/kg BW, NA dose 230 mg/kg BW and metformin 300 mg/kg BW/day.

Hyperglycaemia model

Rats were adapted from days 1 to 5, then NA was injected at a dose of 230 mg/kg BW on day 6 and then given a one-day break for STZ injection at a dose of 45 mg/kg BW. RBS measurements were taken after 3 days of STZ-NA injection in rats with RBS > 150 mg/dL as DM rats.[18]

Preparation of Emprit ginger extraction

The base material for ethanol extract was made using 5 kg each of fresh Emprit gingers, which were separated from damaged and sprouted pieces before dicing to remove any soil and dirt. The rhizomes were sliced thinly with a 1-2 mm thickness and dried using an oven at 80 °C. After drying, the rhizomes were blended to form a fine powder of dry Simplicia. Subsequently, a total of 150 g ginger powder was taken and placed into a glass jar. Maceration was then carried out using 70 % ethanol solvent in a ratio of 1:20 for 2 h at room temperature. The results of maceration were then filtered, macerated again and filtered. The macerate was collected and evaporated using a rotary evaporator at 400 °C. Emprit ginger extract obtained was administered by sonde for 21 days.

Melatonin qualitative examination

Identification of melatonin was performed by thin layer chromatography (TLC) method using silica F254 with standard melatonin comparator. The ethanol solution was bottled in a silica gel GF254 plate for preparative purposes and eluted using BAW mobile phase (n-butanol: acetic acid: water) in the ratio of 12:3:5.

Blood sampling and blood biochemical examination

The procedure of blood collection at the retroorbital plexus was used. Blood sugar examination was carried out using GOD PAP method, cholesterol and HDL levels were assessed with CHOD PAP method and triglycerides were analysed with GPO PAP. Normal values of triglycerides, cholesterol, LDL and HDL were 26-145 mg/dL,[19] 10-54 mg/dL, < 100 mg/dL and > 40 mg/dl,[20] respectively.

Data analysis

Bivariate analysis was used to examine differences in clinical chemistry parameters between treatments. To determine differences in blood sugar levels between groups before treatment, after treatment and the difference between before and after treatment was tested with One-way ANOVA with a p-value < 0.05.

Results

This study assessed melatonin compounds contained in ginger extract using TLC method, which was positive for melatonin when it had a difference price between standard Rf and extract Rf ≤ 0.2. The results showed that the difference of Rf Emprit ginger was 0.1429, indicating the presence of melatonin compounds in ginger extract.

Rats' BW was weighed every 2 days starting from the first day. Based on Figure 1, before STZ-NA injection, BW of rats in all groups tended to increase. However, after STZ-NA injection, BW in all groups tended to decrease. In rats that were not injected with STZ-NA, body weight increased. Weighing on the 29th day, compared to 14th day, BW of rats that were not injected with STZ-NA significantly decreased.

Figure 1 showed that the average BW of rats at the beginning of the treatment ranged from 241.8-243.2 g, with the variable showing a decreasing trend during the experiment. After statistical

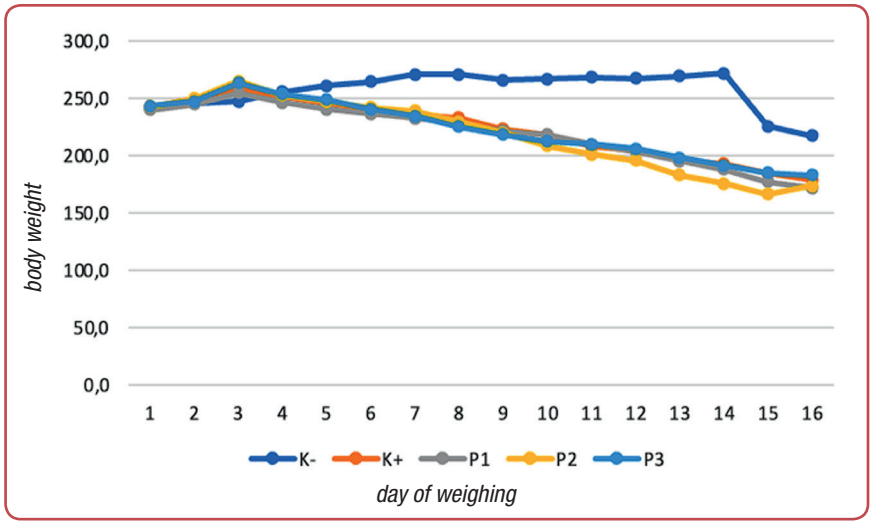

Figure 1: Average body weight of rats during the study

Table 1: Comparison of biochemical parameters in groups before intervention

Variable (mg/dL)	K-	K+	P1	P2	P3	p-value
RBG	109.4 ± 23.3	411.2 ± 114.5	418.4 ± 99.3	426.8 ± 55.8	410.0 ± 74.1	< 0.001 *
Triglycerides	64.8 ± 19.1	108.3 ± 46.6	79.2 ± 29.6	142 ± 39.8	132.0 ± 31.7	0.007 *
Cholesterol	104.0 ± 11.3	130.8 ± 21.7	121.2 ± 22.0	122.8 ± 9.6	128.4 ± 13.1	0.112
HDL	46.6 ± 10.7	47.5 ± 8.4	53.4 ± 4.9	47.3 ± 5.0	41.6 ± 10.7	0.302
LDL	44.6 ± 13.9	61.7 ± 17.7	52.0 ± 27.1	47.1 ± 11.1	60.4 ± 17.7	0.433

**Significant result, p ≤ 0.05; RBG: random blood glucose; HDL: high density lipoprotein cholesterol; LDL: low density lipoprotein cholesterol; K-: Negative control group without streptozotocin (STZ) and nicotinamide (NA) injection; K+: The positive control group was given STZ dose 45 mg/kg BW and NA dose 230 mg/kg BW; P1: Group given STZ dose 45 mg/kg BW, NA dose 230 mg/kg BW and ginger extract 100 mg/kg BW/day; P2: Group given STZ dose 45 mg/kg BW, NA dose 230 mg/kg BW and ginger extract 200 mg/kg BW/day; P3: Group given STZ dose 45 mg/kg BW, NA dose 230 mg/kg BW and metformin 300 mg/kg BW/day.*

Table 2: Comparison of biochemical parameters in groups after intervention

Variable (mg/dL)	K-	K+	P1	P2	P3	p-value
RBG	110.8 ± 15.8	399.2 ± 102.0	216.6 ± 39.9	176.5 ± 39.5	147.2 ± 15.8	< 0.001 *
Triglycerides	57.8 ± 24.5	150.8 ± 33.5	57.8 ± 24.5	58.3 ± 22.6	50.2 ± 25.3	< 0.001 *
Cholesterol	92.8 ± 8.4	166.5 ± 33.5	91.4 ± 33.3	82.7 ± 24.8	78.2 ± 19.8	< 0.001 *
HDL	40.8 ± 8.2	55.7 ± 9.5	38.0 ± 14.8	42.7 ± 13.7	35 ± 9.6	0.057
LDL	40.9 ± 9.2	80.7 ± 21.6	41.8 ± 26.7	35.5 ± 13.9	33.2 ± 10.6	0.001 *

**Significant result, p ≤ 0.05; RBG: random blood glucose; HDL: high density lipoprotein cholesterol; LDL: low density lipoprotein cholesterol; K-: Negative control group without streptozotocin (STZ) and nicotinamide (NA) injection; K+: The positive control group was given STZ dose 45 mg/kg BW and NA dose 230 mg/kg BW; P1: Group given STZ dose 45 mg/kg BW, NA dose 230 mg/kg BW and ginger extract 100 mg/kg BW/day; P2: Group given STZ dose 45 mg/kg BW, NA dose 230 mg/kg BW and ginger extract 200 mg/kg BW/day; P3: Group given STZ dose 45 mg/kg BW, NA dose 230 mg/kg BW and metformin 300 mg/kg BW/day.*

Table 3: Comparison of changes in biochemical parameters before and after treatment

Variable (mg/dL)	K-	K+	P1	P2	P3	p-value
RBG	1.4 ± 31.8	-12.0 ± 16.8	-201.8 ± 84.8	-250.3 ± 66.1	-262.8 ± 70.6	< 0.001 *
Triglycerides	-9.4 ± 20.1	42.5 ± 36.5	-21.4 ± 37.3	-83.7 ± 52.0	-81.8 ± 62.8	< 0.001 *
Cholesterol	-11.2 ± 8.5	35.7 ± 43.9	-29.8 ± 22.6	-40.2 ± 28.8	-50.2 ± 26.3	0.001 *
HDL	-5.6 ± 11.8	8.2 ± 17.2	-15.4 ± 15.9	-4.7 ± 16.8	-6.6 ± 10.6	0.170
LDL	-3.7 ± 8.1	19.0 ± 32.1	-10.1 ± 18.8	-11.6 ± 8.7	-20.4 ± 16.7	0.028 *

**Significant result, p ≤ 0.05; RBG: random blood glucose; HDL: high density lipoprotein cholesterol; LDL: low density lipoprotein cholesterol; The value (-) means there is a decrease; K-: Negative control group without streptozotocin (STZ) and nicotinamide (NA) injection; K+: The positive control group was given STZ dose 45 mg/kg BW and NA dose 230 mg/kg BW; P1: Group given STZ dose 45 mg/kg BW, NA dose 230 mg/kg BW and ginger extract 100 mg/kg BW/day; P2: Group given STZ dose 45 mg/kg BW, NA dose 230 mg/kg BW and ginger extract 200 mg/kg BW/day; P3: Group given STZ dose 45 mg/kg BW, NA dose 230 mg/kg BW and metformin 300 mg/kg BW/day.*

testing with ANOVA repeated measure test on the output between subjects, a p-value of 0.079 was obtained. This indicated that BW of rats between treatments during the study was not significantly different.

Administration of STZ-NA was proven to increase the blood sugar level in the injected animals. The first RBS measurement was 3 days after STZ-NA injection with RBS levels being ≥ 400 mg/dL. The highest pre-treatment RBS levels were observed in P2 with 426.8 ± 55.8 mg/dL, while the lowest was in group K- (negative control) with 109.4 ± 23.3 mg/dL. Meanwhile, based on lipid profile levels before treatment, the highest triglyceride level was 142 ± 39.8 mg/dL (P2), the highest cholesterol level was 130.8 ± 21.7 mg/dL (K+) and the highest HDL was 53.4 ± 4.92 mg/dL (P1). The highest LDL obtained was 61.67 ± 17.69 mg/dL (K+), as shown in Table 1. After comparing the levels of clinical chemical parameters between groups before treatment, there were significant differences in the levels of RBS ($p < 0.001$) and triglyceride ($p = 0.007$). The results also showed that the cholesterol, HDL and LDL levels did not significantly differ with p-values of 0.112, 0.302 and 0.443, respectively.

The treatment was carried out for 21 days and RBS levels and lipid profile were re-examined. The results after treatment showed changes with the highest RBS levels (399.2 ± 102.0 mg/dL), highest triglycerides (150.83 ± 33.52 mg/dL), highest cholesterol (166.5 ± 33.5 mg/dL), highest HDL (55.66 ± 9.52 mg/dL) and highest LDL levels (80.67 ± 21.6 mg/dL) in K+. All the highest clinical chemistry parameters were obtained in K+ group, which was only given STZ-NA. When compared to other groups, all parameters were significantly different except for HDL, as shown in Table 2.

This study also analysed changes in the data of parameter levels before and after treatment. The results showed an increase and decrease in parameters that varied, namely (1) the largest decrease in RBS was in P3 by 262.8 ± 70.6 mg/dL, (2) the largest decrease in triglycerides was in P2 by 83.66 ± 52.04 mg/dL, (3) the largest decrease in cholesterol was in P3 by 50.2 ± 26.30 mg/dL, (4) the largest decrease in HDL was in P1 by 15.4 ± 15.88 mg/dL and (5) the largest decrease in LDL was in P3 by 20.44 ± 16.68 mg/dL. Changes in parameter levels between the groups were also analysed, with results showing significant differences except in HDL, as presented in Table 3. When reviewing the results of changes in clinical parameters that occurred in the group given ginger extract, the one that had the highest reduction effect was in triglyceride parameters at a dose of 200 mg/kg BW/day (group P2).

Discussion

The results showed all groups of rats injected with STZ-NA tended to experience a significant decrease in BW. The results were in line with Rias where BW of rats after 72 h given STZ decreased.[21] In hyperglycaemia conditions, insulin deficiency typically caused protein and fat metabolism diseases by inhibiting the function of insulin, which caused a decrease in muscle mass, leading to weight loss.[22] The result was also consistent with other studies, which tested the effect of weight loss on rats. Weight loss in DM rats was due to protein degradation, also known as muscle wasting.[23, 24] This process typically occurred due to the body's lack of glucose and lipids, thereby positioning protein as the main source of energy. Structural proteins, which played an important role in maintaining BW, were degraded in DM condition. This led to increased energy requirements by oxidising proteins and fatty acids. The loss of tissue protein often caused decreased muscle mass, leading to weight loss.[25] The decrease in body weight also can lead to a decrease in the number of calories stored.[26] The decline observed in body weight in the group given ginger extract could also be due to the content of melatonin, which had hypocholesterolaemic effects. Studies analysing changes in body weight of rats given melatonin showed that the melatonin treatment group experienced significant reductions. Weight loss is marked in 2 ways, due to an increase in circulating irisin levels and faecal cholesterol excretion results from melatonin administration.[27] Weight loss in the treatment group was also consistent with other studies, where the group given red ginger extract at doses of 200, 350 and 500 mg/kg BW experienced weight loss of 21.9 %, 22.5 %, 22.1 % and 21.0 %, respectively.[14]

Experimental animals in this study were treated by injecting STZ-NA to cause hyperglycaemia. The highest RBS levels before treatment were obtained in P2, while the lowest was in K-. The lowest RBS levels before injection were observed

in K-, which received no treatment and were only given standard food. The first RBS measurement was carried out 3 days after STZ-NA injection with an average rat blood sugar level of > 400 mg/dL. The administration of nicotinamide before STZ administration could protect pancreatic cells from the toxic effects of STZ, preventing DM development. Streptozotocin formed highly reactive free radicals, damaging cell membranes, proteins and DNA, which disrupted insulin production by Langerhans beta cells in the pancreas.[28] Studies using Wistar rats modelled hyperglycaemia by injecting NA dose 230 mg/kg BW and STZ dose 65 mg/kg BW with a break for 15 min. The results showed that fasting sugar levels ranged from 228-280 mg/dL on day 15.[29] In this study, a 1-day pause was given between the administration of NA and STZ because, in the preliminary study, a 15-minute pause led to the death of the experimental animals. This was possibly due to hypoglycaemic shock and ketoacidosis.[30] Nicotinamide injection inhibited the cytotoxic effects of STZ through the formation of nitric oxide, which served as a protection against apoptosis. In addition, a 15-minute interval was suspected to be insufficient to provide protective effects against the effects of STZ, indicating the occurrence of rat mortality. This was caused by hypoglycaemic shock that occurred in the first 6-12 h after STZ injection.[30]

Based on the lipid profile levels before treatment, the highest triglyceride levels were obtained in P2, the highest cholesterol levels in K+, the highest HDL in P1 and the highest LDL in K+. This indicated that STZ-NA injection affected the lipid profile levels of rats. Fitri et al also showed that STZ injection increased triglycerides, cholesterol and LDL but lowered HDL levels in DM rats.[31] A significant increase in cholesterol in STZ-NA injected rats but not in HDL was also in line with Rani et al which obtained similar results.[32] The presence of insulin resistance increased sensitive hormones in adipose tissue, thereby increasing triglyceride lipolysis. This caused an increase in blood levels of free fatty acids. The increased production of fatty acids due to insulin resistance led to an increase in triglyceride levels and very low-density lipoprotein (VLDL) density. In addition, it increased cholesterol ester transfer protein (CETP) activity, enriched triglycerides (TG) and lowered HDL levels.[33] Increased plasma lipid levels were associated with lipid peroxidation in DM rats, leading to oxidative stress reactions. Lipid peroxidation was reported to cause oxidative stress reactions, which contributed to the development of DM complications, including retinopathy.[34]

Clinical parameter levels were analysed for changes before and after the treatment. The results showed that the biggest decrease in RBS was observed in P3, which was given metformin, followed by P2. In addition, the largest cholesterol reduction was obtained in P3. Metformin, a standard DM drug, was proven to reduce RBS and cholesterol levels, as reported by Rani et al.[32] According to previous studies, metformin was a first-line drug for adults or elderly with T2DM by reducing liver gluconeogenesis function and increasing insulin sensitivity to control blood glucose.[35] The decrease in RBS also occurred in rats given 200 mg/kg BW ginger extract, followed by a further decrease in rats given 100 mg/kg BW ginger extract. The results were consistent with Andi, where the administration of 2000 mg/mL of Emprit ginger juice for 4 days could reduce blood sugar from 464 ± 127.99 mg/dL to 160 ± 43.37 mg/dL, representing a decrease of 304 mg/dL.[36] The bioactive compounds in ginger, such as gingerol, shogaol, zingerone and zingiberene had antidiabetic properties believed to increase insulin secretion through modulation of K_{ATP} channels in pancreatic beta cells.[37] This study also proved that the administration of steamed ginger extract was more effective in reducing hyperglycaemia compared to ginger extract. In addition, the antidiabetic effect of ginger, particularly 6-shogaol, effectively inhibited α-glucosidase and increased glucose uptake through GLUT 2 transporters. The results revealed that it delayed glucose absorption in the intestinal cavity, thereby reducing postprandial blood glucose levels.[15]

This study also proved the presence of melatonin in Emprit ginger. This result is in line with a study which found that the melatonin content in ginger was around 583.7 ng/g.[38] Melatonin had been shown to significantly affect glucose uptake and metabolism in various cells. The effects of melatonin on glucose metabolism could be affected by genetic variations, as evidenced by studies investigating the effect of melatonin-related gene variants on the risk of T2DM.[11] In addition, it had the potential to ameliorate impaired endothelial progenitor cell mobilisation and circulation recovery in response to ischaemic injury caused by chronic hyperglycaemia. This suggested the potential therapeutic use

in treating hyperglycaemia-related vascular complications.[39]

Ginger extract 200 mg/kg BW/day could reduce cholesterol although the decrease was lower compared to the metformin group. The results were consistent with a study, where red ginger extract given to experimental animals at 200 mg/kg BW for 14 days reduced cholesterol by 47.9 %.[14] The results obtained were in line with a study where the administration of 3.2 mL/kg BW/day red ginger drink for 21 days reduced the average total cholesterol level by 8.64 %. The improvement in levels of total cholesterol, LDL and triglycerides in rats fed a high-fat diet was related to the potent antioxidant ability of ginger extract tested *in vitro* and *in vivo*.[40] Ginger could counteract free radicals in the body, have anti-atherogenic effects, reduce cholesterol and prevent adverse cardiovascular effects.[41, 42] The content of zingerone in ginger had also been proven to reduce the risk of atherosclerosis by regulating lipid profile levels (including reducing total cholesterol, triglycerides and LDL), antioxidant status and gene expression associated with lipid metabolism. Zingerone could result in the upregulation of gene expression associated with lipid regulation, such as fatty acid synthase (FAS), sterol regulatory element-binding protein-c (SREBP-c), acetyl-CoA synthetase (ACS), acetyl-CoA carboxylase (ACC) and others.[42] Melatonin's hypocholesterolaemia ability was through increased excretion of cholesterol through faces. A previous report showed that faecal cholesterol excretion could be increased by stimulating biliary cholesterol secretion and reducing intestinal cholesterol absorption. The cholesterol-lowering mechanism was thought to inhibit LDL receptor activity without affecting fatty acid synthesis.[27] Melatonin inhibited the activation of sterol element-binding transcription factor 1c, fatty acid synthase and lipogenesis-related stearoyl-CoA desaturase one, as well as increased peroxisome proliferator-activated receptor-α-dependent lipolysis.[43, 44] In addition, high cholesterol could induce reactive oxygen species (ROS) and apoptosis. This study indicate that melatonin administration could reduce ROS, apoptosis and intracellular cholesterol by increasing ABCA1 expression, suggesting the ability to provide a protective effect against the adverse effects of high cholesterol.

The largest LDL reduction was observed in P3, followed by P2. According to research by Murad et al which gave 5 g of ginger for 3 months to patients showed a reduction in LDL cholesterol of 17.4 %.[41] The decrease in LDL levels in rats was related to the content in ginger, which could increase LDL receptors and reduce HMG-CoA activity.[45] Regulation of HMG-CoA reductase and LDL receptors affected circulating LDL cholesterol, total cholesterol and triglycerides by increasing LDL cholesterol uptake and reducing cholesterol biosynthesis. By reducing the cholesterol biosynthesis process, increasing the number of LDL receptors could accelerate the removal of LDL cholesterol from circulation, thereby reducing cholesterol concentration in the plasma. Melatonin in ginger also affected the maintenance of LDL levels as well as inhibited the accumulation of LDL and LDL receptors. The results revealed that administering melatonin 20 mg/kg in STZ-injected rats increased HDL levels by 10 %.[46]

The results showed that the largest decrease in triglycerides was found in group P2. This revealed that the administration of Emprit ginger extract rich in melatonin at a dose of 200 mg/kg BW/day had a greater effect on reducing triglycerides compared to other clinical parameters. The findings were consistent with Adeniyi Paulina et al which treated male albino rats with a high-fat diet to induce DM. The test animals were treated with raw and cooked ginger extract for 4 weeks. The results showed that the groups given raw and cooked ginger extract, as well as metformin, experienced a 36.35 % decrease in triglycerides.[47] Based on the findings of a study analysing the ability of immature ginger to reduce triglycerides, it was reported to have the ability to inhibit lipogenesis by limiting the distribution of glucose carbon in fatty acid synthesis. The mechanism was through the inhibition of the expression of acetyl-CoA carboxylase and reduction of glycerol production by inhibiting the expression of phosphoenolpyruvate carboxy kinase (PEPCK). This caused inhibition of triglyceride formation.[48] Despite the results presented in this current study, it had not identified the quantitative levels of melatonin. This indicated that it was necessary to analyse the differences between the 2 groups to determine the variation in parameter levels.

Conclusion

In conclusion, STZ-NA injection caused an increase in RBS and cholesterol in rats. Furthermore, the administration of melatonin-rich ginger extract led to a decrease in RBS, cholesterol and LDL and was more effective in the group given metformin. Melatonin-rich ginger extract at a dose of 200 mg/kg BW/day was more effective in reducing triglycerides compared to other clinical parameters.

Ethics

The study was approved by the Ethics Committee of the Medicine Faculty, Universitas Muhammadiyah Semarang, decision No 078/EC/KEPK-FK/UNIMUS/2023, dated 1 November 2023.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

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