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Effectiveness of Pumpkin Extract (Cucurbita moschata) Lowering Blood Glucose and Malondialdehyde Levels in Diabetic Model Rats

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A B S T R A C T

Hyperglycemia in uncontrolled diabetes mellitus (DM) can cause oxidative stress, among others, characterized by high levels of malondialdehyde (MDA). This situation continues to cause various complications. Pumpkin (Cucurbita moschata) is known to contain various antioxidants that can reduce blood glucose levels and oxidative stress levels in DM. This study aims to prove the effectiveness of pumpkin in the form of an extract to reduce fasting blood glucose (FBG)and MDA levels in DM rats.The study used 36 male Wistar rats which were divided into 6 groups, namely:KN:normal group;K(-):DM group;K(+):DM group was given glibenclamide 0.45 mg/kgBW/day;P1,P2,P3:DM group which was given the extract with a successive dose of 0.66mg/kgBW/day,1.32mg/200gBW/day, and 2.64mg/200gBW/day. The extract was administered for 21 consecutive days. The DM model was made by induction of streptozotocin (STZ) and nicotinamide (NA). FBG and MDA levels were measured serially 5 times and analyzed statistically. The results showed that the higher the dose of the extract and the longer the administration of the extract, the more effective the extract's ability to reduce blood FBG and MDA levels. Compared to glibenclamide, pumpkin extract was no less effective in reducing blood FBG and MDA levels. It was concluded that pumpkin extract was effective in lowering blood glucose and MDA levels in DM rats.

Kata kunci:

ekstrak labu kuning
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A B S T R A K

Hiperglikemia pada diabetes melitus (DM) yang tidak terkontrol dapat menyebabkan stres oksidatif antara lain ditandai dengan tingginya kadar malondialdehid (MDA). Keadaan ini terus menimbulkan berbagai komplikasi. Labu kuning (Cucurbita moschata) diketahui mengandung berbagai antioksidan yang dapat menurunkan kadar glukosa darah dan tingkat stres oksidatif pada DM. Penelitian ini bertujuan untuk membuktikan efektivitas labu kuning dalam bentuk ekstrak untuk menurunkan kadar glukosa darah puasa (GDP) dan MDA pada tikus DM. Penelitian menggunakan 36 ekor tikus Wistar jantan yang dibagi menjadi 6 kelompok, yaitu: KN: kelompok normal; K(-): grup DM; K(+): Kelompok DM diberikan glibenklamid 0,45 mg/kgBB/hari; P1, P2, P3 : Kelompok DM yang diberi ekstrak dengan dosis berturut-turut 0.66mg/kgBB/hari, 1.32mg/200gBB/hari dan 2.64mg/200gBB/hari. Ekstrak diberikan selama 21 hari berturut-turut. Model DM dibuat dengan induksi streptozotocin (STZ) dan nicotinamide (NA). Tingkat GDP dan MDA diukur secara serial sebanyak 5 kali dan dianalisis secara statistik. Hasil penelitian menunjukkan bahwa semakin tinggi dosis ekstrak dan semakin lama pemberian ekstrak, semakin efektif kemampuan ekstrak dalam menurunkan kadar GDP dan MDA darah.

Dibandingkan dengan glibenclamide, ekstrak labu tidak kalah efektif dalam menurunkan kadar GDP dan MDA darah. Disimpulkan bahwa ekstrak labu kuning efektif menurunkan kadar glukosa darah dan MDA pada tikus DM.

INTRODUCTION

Diabetes Mellitus (DM) is one of the unresolved nutritional problems in Indonesia. The prevalence of DM in Indonesia is 10.4 million people and every year it is increasing. The increasing prevalence of DM in various countries has an impact on increasing the prevalence of DM in the world. The prevalence in 2017 reached 327 million people, in 2019 it reached 463 million people and it is estimated that in 2045 it will reach 700 million people in the world. (Bowser & House, 2021; Ministry of Health RI, 2020) .

Diabetes Mellitus is a chronic metabolic disorder characterized by increased blood glucose levels (hyperglycemia) caused by a lack of insulin production, or insulin working inappropriately. Insulin is a hormone produced by the pancreas gland that functions to transport glucose from the bloodstream into the body's cells, where glucose is converted into energy. Lack of insulin production and lack of effectiveness of insulin action which is characterized by decreased cell response to insulin causes hyperglycemia. Hyperglycemia that is left for a long time can cause complications in various organs of the body, such as: Cataracts, retinopathy, neuropathy, nephropathy, and cardiovascular disorders (Karuranga, 2019).

In DM, uncontrolled hyperglycemia can also lead to increased production of *reactive oxygen species* (ROS). Excessive increase in ROS (which is one type of free radicals) can cause oxidative stress and damage to pancreatic beta cells(Decroli, 2019). The state of oxidative stress can cause lipid peroxidation in cell membranes which produces malondialdehyde (*malondialdehyde* | MDA). In the event of lipid peroxidation, a reaction occurs between free radicals and *polyunsaturated fatty acid (PUFA)* compounds in the cell membrane to produce MDA. Thus, an increase in blood glucose levels can cause MDA levels to increase (Hanum, 2013). In Setiawan (2016) also mentioned that high levels of MDA, which is a sign of oxidative stress, can be caused by an imbalance between the amount of antioxidants and free radicals, where there is a lack of antioxidants. Oxidative stress can be prevented through the consumption of antioxidant-rich foods.

Pumpkin (*Cucurbita moschata*) is one fruit that is known to contain lots of antioxidants. This fruit contains many active compounds such as: beta-carotene, flavonoids, vitamin C and vitamin E. These substances are antioxidants which in a state of oxidative stress can inhibit the activity of free radicals. Flavonoids can reduce insulin resistance, increase insulin sensitivity and have a hypoglycemic effect. The antioxidant content in pumpkin plays a major role in controlling blood glucose levels. In addition, the antioxidant content plays a role in controlling the occurrence of MDA accumulation *Flavonol* compounds in pumpkin are known to inhibit glucose absorption in the intestine and protect against damage to the pancreas (Loscalzo, 2011).

Research on pumpkin has been carried out by several researchers, including Fathonah et al., (2014) who showed that the aqueous extract of pumpkin (*Cucurbita moschata*) can significantly reduce blood glucose levels in alloxan-induced diabetic rats. Research on pumpkin fruit was also conducted by Monteiro (2014) who proved that giving pumpkin can significantly reduce triglyceride levels. In addition, research conducted by Rahayu *et al.,* (2020) showed that giving pumpkin has a significant effect in lowering blood glucose levels, increasing insulin expression, and improving lipid profiles in hyperglycemic rats. Although research on pumpkin fruit has been carried out by several researchers, research on the effectiveness of pumpkin extract (*Cucurbita moschata*) to improve diabetes, by monitoring blood glucose and MDA levels serially, in DM rats model with streptozotocin (STZ) and nicotinamide inducers (NA), has never been done. Based on the description above, the researchers were interested in proving the effectiveness of pumpkin extract (*Cucurbita moschata*) in lowering blood glucose and blood MDA levels in DM rats with streptozotocin (STZ) and nicotinamide (NA) inducers.

METHOD

Research characteristics and design

Animals

This study used 36 male Wistar rats *(Rattus novergicus)* as subjects, aged 2-3 months, body weight 170-250 grams and looked healthy (not physically disabled). The research was conducted at the Laboratory of Food and Nutrition Study Center of Gadjah Mada University, Yogyakarta. Rats before the study were adapted for 7 days. Then, the wistar rats were prepared by fasting them for 1 night. Rats were injected with a dose of *nicotinamide* (NA) 230 mg/kg dissolved in normal saline intraperitoneally 15 minutes before injection of *streptozotocin* (STZ) (65 mg/kg), with diabetes criteria if fasting blood glucose levels > 150 mg/dL after 72 hours injected with STZ-NA (Ortiz-Andrade et al., 2008). Diabetic rats were divided into 4 groups K- = rats modeled for DM and not treated with pumpkin extract, K+ = rats made up for DM model and treated with glibenclamide 0.45 mg/kgBW/day, P1, P2, and P3 each given a graded dose of 0.66mg/200gBW/day, 1.32mg/200gBW/day, and 2.64mg/200gBW/day. Rats were reared in a room at room temperature using a standard Comfeed AD2 and drink *ad libitum*. The intervention was given for 21 days.

Study Setting

This research was conducted in June – July 2022 at the Laboratory of Food and Nutrition Study Center of Gadjah Mada University, Yogyakarta (certified ISO/IEC 17025:2000). This research is a laboratory experimental research with *pre and post test control group design*. The research sample was taken from the population by means of *purposive sampling*.

This study used 36 rats, which were randomly divided into 6 groups, each group consisting of 6 rats, namely: KN = Normal control group, namely rats that were not modeled with DM and not treated with pumpkin extract, K- = Negative control group, namely rats that were modeled with DM and not treated with pumpkin extract, K+ = Positive control group, namely rats made up of DM model and treated with glibenclamide 0.45 mg/kgBW/day, P1 = Treatment group 1, ie rats made DM model and treated with pumpkin extract dose 1 of 0.66mg/200gBW/day, P2 = Treatment group 2, namely rats made up of DM model and treated with pumpkin extract dose 2 of 1.32 mg/200gBW/day, P3 = Treatment group 3, namely rats made DM model and treated with pumpkin extract dose 3 is 2.64mg/200gBW/day.

Sample size

The estimated sample size in each group was calculated using the formula according to Arifin and Zahirudin (2017) with a total of 36 rats as research subjects taking into account the *drop out* risk factor of 10% or 0.1.

Intervention research

Making of Type 2 DM Rats with Streptozotocin (STZ) *and Nicotinamide (NA)*

Making of type 2 DM rats performed by inducing mice using STZ-NA. Before being induced, fasted overnight. Induction of type 2 DM rat model was carried out according to the procedure of Ortiz-Andrade *et al*., (2008). Wistar rats were prepared by fasting them for 1 night. Mice were injected with nicotinamide (NA) 230 mg/kg dissolved in normal saline intraperitoneally 15 minutes before streptozotocin (STZ) injection (65 mg/kg), with diabetes criteria if fasting blood glucose level > 150 mg/dL after 72 hours injected with STZ-NA.

Pumpkin Extract

Pumpkin extract is made from pumpkin flesh. Pumpkin was taken from plantations in the Kopeng sub-district, Salatiga Regency, Central Java. Pumpkin extract was made at the Laboratory of Food and Nutrition Study Center of Gadjah Mada University, Yogyakarta. The pumpkin flesh was sorted, washed, cut into thin strips and dried using a cabinet *dryer* at 40 °C to dry. Then mashed with a blender. After that, the extraction process was carried out using the maceration method by soaking the simplicia powder in 96% ethanol solvent. The sample was homogenized until the solvent and sample were evenly mixed using a *shaker* for 24 hours. The pumpkin fruit filtrate was filtered using filter paper and carried out for 3 times. Furthermore, the filtered filtrate is concentrated in a *rotary evaporator* to form a thick extract.

This extract was then dissolved in 0.5% Na-CMC and given to white rats in 3 doses, namely: dose 1 (0.66 mg/200gBW/day) given to group P1 rats, dose 2 (1.32 mg/200gBW) /day) was given to rats in group P2, and dose 3 (2.64 mg/200gBW/day) was given to rats in group P3. The extract was administered for 21 consecutive days, starting after 72 hours the rats received STZ-NA injection (DM model was successfully created). The extract was given orally with a gastric probe.

Glibenclamide Dosage

In this study, glibenclamide was given to white rats in the positive control group (K+) at a dose of 0.45 mg/kgBW/day orally with a gastric probe for 21 days. The determination of the dose of glibenclamide in this study was based on the therapeutic dose of DM in humans, which was 5 mg per day. After conversion from a human dose with a body weight of 70 kg to a rat with a body weight of 200 grams, the dose of glibenclamide = 5 mg x 0.018 = 0.09 mg/ 200gBW /day = 0.45 mg/kgBW/day. The number 0.018 is the conversion rate from a human dose with a body weight of 70 kg to a rat with a body weight of 200 g.

Measures and covariates

Evaluation of Fasting Blood Glucose (FBG) and Malondialdehyde (MDA) Levels

Examination of fasting blood glucose (FBG) and MDA levels was carried out serially, namely: before the rats were induced by STZ-NA (H-), 3 days after STZ-NA induction but the rats had not been treated with extract (H0), after 7 days (H7), 14 days (H14), and 21 days (H21) of treatment with pumpkin extract. Blood collection and examination of blood FBG and MDA levels were carried out at the UGM PSPG Laboratory. On the night before the blood draw, the rats were fasted *overnight*. At 5 minutes before the white rats were drawn from the retroorbital vein, the rats were anesthetized with ketamine 45 mg/kgBW/day . Blood was taken from the retroorbital vein using a microcapillary tube and then the blood was collected in a test tube. Furthermore, the blood in the test tube was centrifuged at 4000 rpm for 15 minutes at room temperature to obtain blood serum, which was used for examination of FBG and MDA levels. Blood glucose levels were measured using the *Glucose Oxidase-Phenol Aminoantypyrin* (GOD-PAP) *test* (Subiyono et al., 2016). Measurement of blood MDA levels was carried out using the *thiobarbituric acid reacting substances* (TBARS) method (Nahwa, 2008).

Data analysis

In this study, to determine the differences in FBG and MDA levels between groups, the data were analyzed using the *One Way Anova* test and followed by the *Post Hoc test* (for normally distributed data). However, for data whose distribution was not normal, the data were analyzed using the *Kruskal-Wallis test* and continued with the *Mann-Whitney test*. As for knowing the differences in FBG and MDA levels from time to time in each group, the *Repeated Anova test was used* and followed by the *Post Hoc test.* The significance value used is = 0,05. This research was approved by the Research Ethics Committee, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia (Certificate *Ethical Clearance* no: 38/UN27.06.11/KEP/EC/2022).

RESULTS AND DISCUSSION

Giving pumpkin extract lowers fasting blood glucose (FBG) levels

Table 1 shows the average fasting blood glucose (FBG) levels of white rats at H-, H0, H7, H14, and H21 in each group.

Table 1.

Average FBG Levels of White Rats on H-, H0, H7, H14, and H21 in each group

Group	Mean ±SD (mg/dL)				
	H-	H0	H7	H14	H21
N	68.32±1.04	69.28±0.96	70.27±1.16	70.69±1.27	71.09±1.36
K-	69.60±2.30	269.16±2.90	271.17±2.49	272.26±2.87	274.67±2.42
K+	70.37±1.44	268.30±2.25	223.55±2.04	131.04±1.23	96.47±3.11
P1	69.28±3.54	268.30±2.76	243.62±2.05	150.30±2.11	120.51±2.07
P2	68.32±1.88	269.40±2.27	225.22±3.87	132.25±3.80	101.28±3.73
P3	70.43±1.98	269.40±2.27	214.73±2.34	119.31±2.12	90.51±2.12

Description: N=normal group; K-=DM; K+= DM and given glibenclamide 0.45 mg /kgBW/day; P1= DM rats were given extract dose 1 (0.66mg/200gBW/day); P2= DM rats were given extract dose 2 (1.32mg/200gBW/day); P3 = DM rats were given 3 doses of extract (2.64mg/200gBW/day).

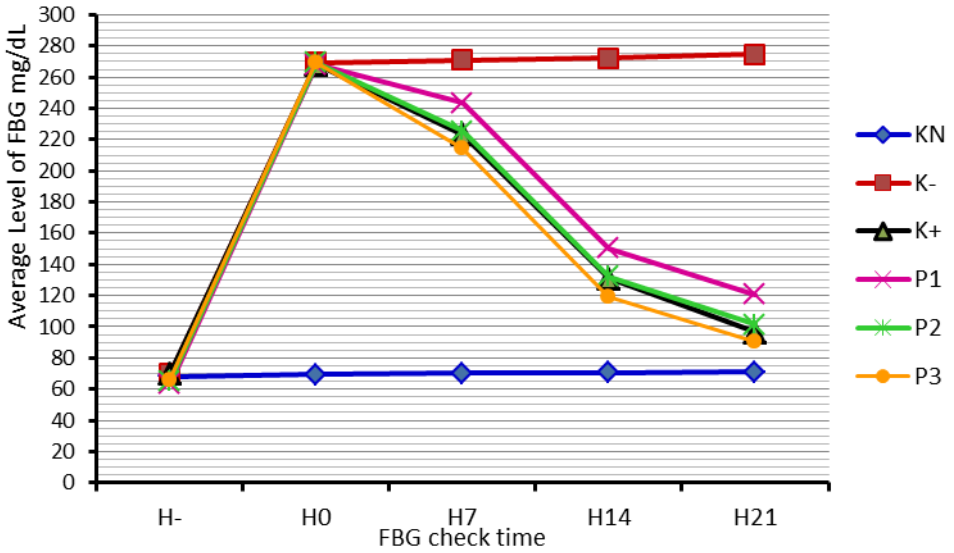

Figure 1. Graph of Average FBG Levels from Time to Time in Each Group

The results of statistical tests comparing FBG levels between KN, K(-), K(+), P1, P2, and P3 groups at each examination time can be seen in table 2.

Table 2.
Statistical Test Results Comparison of FBG Levels Between Groups at Each Time of Examination

Inspection time	Statistical test used	p value
H-	Kruskal Wallis	0.282
H0	*One Way Anova*	0.000*
H7	*One Way Anova*	0.000*
H14	*One Way Anova*	0.000*
H21	Kruskal Wallis	0.000*

Notes: *significant difference

Table 2 shows that the comparison of FBG levels between groups at H0, H7, H14, and H21 was significant, so the data were analyzed by *Post Hoc follow-up test*.

Repeated statistical test results *Anova* which compares FBG levels over time (between H0, H7, H14, H21) in each group can be seen in table 4.

Table 4 shows that the comparison of FBG levels over time (between H0, H7, H14, and H21) in each group was significant, so the data were analyzed by *Post Hoc follow-up test* (can be seen in table 5).

Table 3.
Summary of Post Hoc Test Results of FBG Levels as a Follow-up from One Way Anova and Kruskal-Wallis

Comparison between groups	*p* value			
	H0	H7	H14	H21
KN-K-	0.000 *	0.000 *	0.000 *	0.004 *
KN-K+	0.000 *	0.000 *	0.000 *	0.004 *
KN-P1	0.000 *	0.000 *	0.000 *	0.004 *
KN-P2	0.000 *	0.000 *	0.000 *	0.004 *
KN-P3	0.000 *	0.000 *	0.000 *	0.004 *
K- - K+	1,000	0.000 *	0.000 *	0.004 *
K- - P1	1,000	0.000 *	0.000 *	0.004 *
K- - P2	1,000	0.000 *	0.000 *	0.004 *
K- - P3	1,000	0.000 *	0.000 *	0.004 *
K+ - P1	1,000	0.000 *	0.000 *	0.004 *
K+ - P2	1,000	1,000	1,000	0.043
K+ - P3	1,000	0.000 *	0.000 *	0.010 *
P1 – P2	1,000	0.000 *	0.000 *	0.004 *
P1 – P3	1,000	0.000 *	0.000 *	0.004 *
P2 – P3	1,000	0.000 *	0.000 *	0.004 *

Note: *Significant difference

Table 4.

Repeated test results Anova FBG Levels Over Time in Each Group

Group	*p* value
KN	0.001 *
K-	0.000 *
K+	0.000 *
P1	0.000 *
P2	0.000 *
P3	0.000 *

Note: * Significant difference

In this study, the administration of STZ-NA succeeded in inducing an increase in FBG levels (hyperglycemia) in rats so as to produce a type 2 DM model. K+, P1, P2, P3) were significantly higher than the normal control group (KN) (each with p=0.000). FBG levels in the groups made up of DM showed values >150 mg/dL. This is in accordance with research conducted by Ortiz-Andrade *et al.*, (2008) which showed that rats were considered to have DM if their fasting blood glucose (FBG) level was > 150 mg/dL after 72 hours of STZ-NA injection.

Giving STZ-NA can induce hyperglycemia in rats resulting in a type 2 DM model. To reduce the number of pancreatic cells that were damaged by the administration of STZ, the administration of STZ was combined with NA, so that not all cells in the pancreas were damaged. Thus the DM model that occurs is similar to type 2 DM (Ghasemi et al., 2014).

Table 5.
Summary of Post Hoc Test Results of FBG Levels as Followed by Repeated Anova

Time comparison	*p* value					
	KN	K-	K+	P1	P2	P3
H- - H0	0.021 *	0.000 *	0.000 *	0.000 *	0.000 *	0.000 *
H- - H7	0.012 *	0.000 *	0.000 *	0.000 *	0.000 *	0.000 *
H- - H14	0.012 *	0.000 *	0.000 *	0.000 *	0.000 *	0.000 *
H- - H21	0.008 *	0.000 *	0.000 *	0.000 *	0.000 *	0.000 *
H0 – H7	0.017 *	0.009 *	0.000 *	0.000 *	0.000 *	0.000 *
H0 – H14	0.015 *	0.002 *	0.000 *	0.000 *	0.000 *	0.000 *
H0 – H21	0.008 *	0.000 *	0.000 *	0.000 *	0.000 *	0.000 *
H7 – H14	0.060	0.029 *	0.000 *	0.000 *	0.000 *	0.000 *
H7 – H21	0.010 *	0.000 *	0.000 *	0.000 *	0.000 *	0.000 *
H14 – H21	0.001 *	0.009 *	0.000 *	0.000 *	0.000 *	0.000 *

Note: *Significant difference

The results of this study indicate that the administration of pumpkin extract in three different doses (dose 1 of 0.66 mg/200gBW/day, dose 2 of 1.32 mg/200gBW/day, and dose 3 of 2.64 mg/200gBW/day) can significantly reduce FBG levels in DM model rats. This can be seen from the research data at H7, H14, and H21 FBG levels in the P1, P2, and P3 groups were significantly lower than the negative control (K-). The results of this study are in accordance with research conducted by Faqih (2017)which showed that administration of pumpkin water extract with the highest dose, namely 500mg/kgBW/day given at 0 and 120 minutes was able to significantly reduce blood glucose levels in DM model rats. . The results of this study are also in line with the results of Fathonah's research (2014)which showed that administration of pumpkin water extract at a dose of 200mg/kgBW/day given for 14 days could significantly reduce blood glucose levels in DM model rats.

Giving pumpkin extract can reduce blood glucose levels because pumpkin is known to contain various kinds of phytochemicals, such as: Beta-carotene, flavonoids, vitamin C and vitamin E. These substances are antioxidants which in conditions of oxidative stress can inhibit free radical activity. As antioxidants, these substances also play a role in controlling blood glucose levels (Novianti et al., 2019). In DM, uncontrolled high blood glucose levels can result in increased production of ROS free radicals. Excessive increase in ROS can cause oxidative stress. Oxidative stress can cause further damage to pancreatic cells in DM, so that high blood glucose levels in DM are increasingly uncontrollable (Decroli, 2019). Administration of antioxidants is expected to control oxidative stress. If oxidative stress can be controlled, further damage to pancreatic cells can be prevented, so that the increase in blood glucose levels can be controlled. Thus, antioxidants can control high blood glucose levels (Widowati, 2008, 2009).

Increasing the dose of pumpkin extract in this study can increase the effect of lowering blood glucose levels. This can be seen from the research data, both at H7, H14, and H21, FBG levels in P3 were significantly lower than P2, FBG levels in P2 were significantly lower than P1. In this study it was also seen that the longer the administration of pumpkin extract, the greater the effect on reducing blood glucose levels. This can be seen from the results of the study in groups P1, P2, and P3 when H21 FBG levels were significantly lower than during H14, and the results at H14 were significantly lower than H7.

Pumpkin extract with a dose of 3 (2.64 mg/ 200gBW /day) given for 21 consecutive days had the most optimal effect in lowering blood glucose levels compared to doses 1 and dose 2. H21, FBG at P3 has the lowest level when compared to P1 and P2. Pumpkin extract with a dose of 3 given for 21 days was also more effective than the administration of glibenclamide at a dose of 0.45 mg/kgBW/day for 21 days. This can be seen from the research data at H21, the level of FBG at P3 was significantly lower than at K+. Although administration of pumpkin extract dose 3 for 21 days showed the most optimal effect of reducing blood glucose levels, when compared to the normal control group (KN), the levels were still significantly higher. This provides an opportunity for further research with higher dosage variations and/or longer extract administration times.

Giving pumpkin extract lowers blood malondialdehyde (MDA) levels

Table 6 shows the average blood MDA levels of white rats at H-, H0, H7, H14, and H21 for each group.

The results of statistical tests comparing MDA levels between groups of KN, K(-), K(+), P1, P2, and P3 at each examination time can be seen in table 7.

Table 7 shows that the comparison of MDA levels between groups at H0, H7, H14, and H21 was significant, so the data were analyzed by *Post Hoc follow-up test*.

Table 6.
Average Blood MDA Levels of White Rats on H-, H0, H7, H14, and H21 in each group

Group	Mean ± SD (nmol/mL)				
	H-	H0	H7	H14	H21
N	1.34±0.15	1.44±0.14	1.66±0.15	1.77±0.12	1.87±0.15
K-	1.61±0.16	9.15±0.23	9.39±0.24	9.58±0.22	9.73±0.19
K+	1.59±0.17	9.33±0.17	7.76±0.19	4.13±0.16	2.30±0.16
P1	1.64±0.10	9.21±0.26	8.33±0.25	6.28±0.26	4.42±0.29
P2	1.50±0.29	9.55±0.35	7.95±0.34	4.57±0.32	2.69±0.33
P3	1.63±0.17	9.76±0.25	8.21±0.22	4.14±0.25	2.24±0.29

Description: N=normal group; K-=DM; K+= DM and given glibenclamide 0.45 mg /kgBW/day; P1= DM rats were given extract dose 1 (0.66mg/200gBW/day); P2= DM rats were given extract dose 2 (1.32mg/200gBW/day); P3 = DM rats were given 3 doses of extract (2.64mg/200gBW/day).

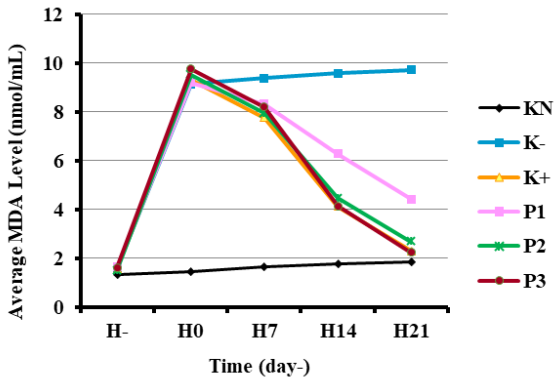

Figure 2. Graph of Average MDA Levels from Time to Time in Each Group

Table 7.
Statistical Test Results Comparison of MDA Levels Between Groups at Each Examination Time

Inspection time	Statistical test used	*p* value
H-	*One Way Anova*	0.071
H0	*One Way Anova*	0.000*
H7	*One Way Anova*	0.000*
H14	*One Way Anova*	0.000*
H21	*One Way Anova*	0.000*

Notes: * Significant difference

Table 8.
Summary of Post Hoc Test Results for MDA Levels as a continuation of One Way Anova

Comparison between groups	*p* value			
	H0	H7	H14	H21
KN-K-	0.000 *	0.000 *	0.000 *	0.000 *
KN-K+	0.000 *	0.000 *	0.000 *	0.095
KN-P1	0.000 *	0.000 *	0.000 *	0.000 *
KN-P2	0.000 *	0.000 *	0.000 *	0.000 *
KN-P3	0.000 *	0.000 *	0.000 *	0.239
K- - K+	1,000	0.000 *	0.000 *	0.000 *
K- - P1	1,000	0.000 *	0.000 *	0.000 *
K- - P2	0.133	0.000 *	0.000 *	0.000 *
K- - P3	0.003 8	0.000 *	0.000 *	0.000 *
K+ - P1	1,000	0.005 *	0.000 *	0.000 *
K+ - P2	1,000	1,000	0 0.047	0.168
K+ - P3	0.077	0.055	1,000	1,000
P1 – P2	0.380	0.173	0.000 *	0.000 *
P1 – P3	0.009 *	1,000	0.000 *	0.000 *
P2 – P3	1,000	1,000	0.057	0.065

Note: *Significant difference

Repeated statistical test results *Anova* which compares MDA levels over time (between H0, H7, H14, H21) in each group can be seen in table 9.

Table 9 shows that the comparison of MDA levels over time (between H0, H7, H14 and H21) in each group was significant, so the data were analyzed by *Post Hoc follow-up test*.

Repeated test results Anova Inter-Time MDA Levels in Each Group

Group	*p* value
KN	0.000 *
K-	0.000 *
K+	0.000 *
P1	0.000 *
P2	0.000 *
P3	0.000 *

Note: *Significant difference

Table 9.

Table 10.
Summary of Post Hoc Test Results for MDA Levels as Followed by Repeated ANOVA

Time comparison	*p* value					
	KN	K-	K+	P1	P2	P3
H- - H0	0.029	0.000 *	0.000 *	0.000 *	0.000 *	0.000 *
H- - H7	0.039	0.000 *	0.000 *	0.000 *	0.000 *	0.000 *
H- - H14	0.004 *	0.000 *	0.000 *	0.000 *	0.000 *	0.000 *
H- - H21	0.002 *	0.000 *	0.012 *	0.000 *	0.001 *	0.052
H0 – H7	0.136	0.030 *	0.000 *	0.000 *	0.000 *	0.000 *
H0 – H14	0.007 *	0.011 *	0.000 *	0.000 *	0.000 *	0.000 *
H0 – H21	0.003 *	0.005 *	0.000 *	0.000 *	0.000 *	0.000 *
H7 – H14	0.011 *	0.135	0.000 *	0.000 *	0.000 *	0.000 *
H7 – H21	0.001 *	0.031 *	0.000 *	0.000 *	0.000 *	0.000 *
H14 – H21	0.017 *	0.017 *	0.000 *	0.000 *	0.000 *	0.000 *

Note: *Significant difference

The effect of pumpkin extract in lowering blood MDA levels, shows a picture similar to the effect of the extract in reducing FBG levels. At H0, MDA levels in the groups modeled for DM (K-, K+, P1, P2, P3) showed a significantly higher value when compared to the normal control group (KN). This indicates that the administration of STZ-NA succeeded in inducing an increase in MDA (oxidative stress) levels in rats. STZ-NA induction can cause damage to some of the cells in the islets of Langerhans of the pancreas, causing a decrease in insulin levels in the blood and resulting in hyperglycemia (Szkudelski et al., 2013). Uncontrolled hyperglycemia can result in excessive ROS production, which can further cause oxidative stress (Decroli, 2019).The state of oxidative stress can cause lipid peroxidation in cell membranes. In the event of lipid peroxidation, a reaction occurs between free radical compounds and *polyunsaturated fatty acid* (PUFA) compounds present in the cell membrane so that MDA is produced. Thus hyperglycemia can cause MDA levels in the blood to increase (Hanum, 2013).

The results showed that administration of pumpkin extract dose 1, dose 2, and dose 3 was able to significantly reduce MDA (oxidative stress) levels in DM model rats. This can be seen from the research data at H7, H14, and H21, MDA levels in the P1, P2, and P3 groups were significantly lower than the negative control group (K-). Giving pumpkin extract can reduce oxidative stress because pumpkin is known to contain various kinds of phytochemicals that function as antioxidants. The antioxidants found in pumpkin include: beta-carotene, flavonoids, vitamin C and vitamin E (Suwanto et al., 2020). The presence of these various antioxidants can neutralize excessive free radicals in oxidative stress events (Fajrilah, 2016; Werdhasari, 2014). In Alam et al., (2012) stated that flavonoids can reduce insulin resistance, increase insulin sensitivity, prevent cells from oxidative stress, neutralize the toxic effects of free radicals and have a hypoglycemic effect by blocking the activity of alpha amylase and alpha glucosidase enzymes. According to Lismawati et al., (2021) beta-carotene has an antioxidant effect that has activity against free radicals. In addition, the mechanism of beta-carotene as an antioxidant is by protecting cell membranes so as to prevent lipid peroxidation (Kamilatussaniah et al., 2015; Latumahina, 2011; Lismawati et al., 2021). Vitamin C has an antioxidant effect that can prevent oxidation in pancreatic beta cells so that it can minimize damage, counteract and neutralize free radicals (Suwanto et al., 2020). Vitamin E can play a role in reducing free radicals, slowing oxidative damage, the main defense against damaging oxygen, lipid peroxide, and reducing oxidative stress (Akbari et al., 2016; Pathy, 2018; Rusiani et al., 2019).

In relation to the decrease in MDA levels (reduction in oxidative stress levels) this study is in line with research conducted by Florez et al., (2012), who succeeded in proving that administration of pumpkin extract at a dose of 200mg/kgBW/day for 30 days could improve lipid peroxidation in DM rats. In addition, research conducted by Dini (2015), also showed the antioxidant effect of pumpkin. In this study it was proven that administration of pumpkin fruit flour at a dose of 0.32 g/ 200gBW /day was effective in reducing lipid peroxidation for 21 days using male rats. The content of carotene can be used as an antioxidant to reduce singlet oxygen and *reactive oxygen species* (ROS) (Sayuti et al., 2015)

Increasing the dose of pumpkin extract in this study from doses 1 to doses 2 and 3 can increase the effect of reducing blood MDA levels. This can be seen from the research data, both at H14 and H21, MDA levels in P2 and P3 were significantly lower than P1. However, at H7 (after 7 days of extract administration), the variation in increasing the dose had no effect on the magnitude of the decrease in MDA levels. This can be seen in the research data at H7, where there are no significant differences between P1, P2, and P3. In this study, it means that the longer the administration of pumpkin extract, the greater the effect on decreasing MDA levels. This can also be seen from the results of the *Repeated Anova test* in groups P1, P2, and P3, where at H21 the MDA levels were significantly lower than during H14, and the results at H14 were significantly lower than H7.

At the end of the study, after 21 days of treatment, pumpkin extract doses 2 and 3 had the same ability to reduce blood MDA levels, and were more effective than dose 1. P2 and P3 have insignificant differences, but have significant differences with P1. At the end of the study (after 21 days of treatment), pumpkin extract with doses 2 and 3 also had the same ability as glibenclamide at a dose of 0.45 mg/kgBW/day in reducing MDA levels. This can be seen from the research data at H21, the MDA levels at P2 and P3 were not significantly different from the MDA levels at K+. Although administration of pumpkin extract dose 2 for 21 days showed an effect of reducing MDA levels which was equivalent to glibenclamide 0.45 mg/200gBW/day and was more effective than dose 1, when compared with the normal control group (KN), the MDA levels in the administration of extract dose 2 levels were still significantly higher. As for the extract dose 3 has the effect of reducing levels of MDA which is already equivalent to KN. This provides an opportunity for

further research to develop pumpkin extract for the treatment of DM, which is one of the diseases associated with improper diet (nutrition).

LIMITATION

This study only used three variations of the dose and the time of the study was 21 days, the results of which decreased glucose levels were not the same as normal controls. The parameters measured in this study were limited to a decrease in blood glucose levels and blood MDA in relation to the pathogenesis mechanism of DM.

CONCLUSION AND SUGGESTION

It can be concluded that the administration of pumpkin extract (*Cucurbita moschata*) is effective in reducing blood glucose and malondialdehyde levels in diabetes mellitus model rats. It is still necessary to do further research with more varied extract dosages and longer administration times so that more optimal results can be achieved as well as normal conditions. The need for further research with parameters other than blood glucose and malondialdehyde.

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ETHICAL CONSIDERATIONS

This research protocol was approved by the Research Ethics Committee of the Faculty of Medicine, Sebelas Maret University (No: 38/UN27.06.11/KEP/EC/2022).

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Conflict of Interest Statement

All authors declare there is no conflict of interest in this manuscript

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