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Red dragon fruit juice in reducing ros levels and insulin resistance In rats with type 2 diabetes mellitus model

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ABSTRACT

Background: *The peel of red dragon fruit (Hylocereus polyrhizus) had been proven to have a total polyphenol content and total flavonoids 2 to 3 times more than its flesh. These components could reduce oxidative stress and maintain the function of pancreatic beta cells, which could affect blood sugar levels.*

Objectives: *This study aimed to test the red dragon fruit juice using peel and flesh to reduce oxidative stress and insulin resistance in T2DM model rats.*

Materials and Methods: *This study was a true experimental study with a randomized controlled trial, with a Matching Pretest Post-test Control Group Design. We used 21 white male rats (Rattus norvegicus) Wistar strain which was divided into three groups: (P1) negative control group (induced Streptozotocin + Nicotinamide induction), (P2) positive control group (given Streptozotocin + Nicotinamide and given Metformin HCl induction 0,9 mg/kg BW, and (P3) Red Dragon fruit group (induced Streptozotocin + Nicotinamide and given Red Dragon Fruit juice 3.6 ml / 200 g BW / day given for 14 days. The data were analyzed using a one-way ANOVA test, paired t-test, and Post Hoc.*

Results: *After 14 days of intervention, the average HOMA-IR levels were as follows: negative control group (Mean=8.32; SD=0.26), positive group (Mean 4.89; SD=0.29), and the Red Dragon Fruit intervention group (Mean=4.65; SD=0.30). The average MDA levels were as follows: control group (Mean = 9.08; SD = 0.68), positive group (Mean=3.34;SD=0.22), and the red dragon fruit intervention group (Mean = 3.05; SD = 0.47). Both the Metformin group and the Red Dragon Fruit group had low HOMA-IR and MDA levels compared to the negative control group.*

Conclusions: *When administered alone, red dragon fruit and metformin effectively reduced HOMA-IR and MDA levels in rats with type 2 DM. Red dragon fruit can be used as an alternative to metformin because of its effectiveness in reducing plasma HOMA-IR and MDA.*

Keywords: *HOMA-IR; Red Dragon Fruit; Type 2 Diabetes Mellitus*

BACKGROUND

Red Dragon fruit (RDF) is a fruit source that is rich in natural antioxidants, namely betacyanin, flavonoids, polyphenols, ascorbic acid, and also fiber [1]. The main antioxidant content in RDF is flavonoids. Flavonoids have a polyphenolic structure which is found in many fruits [2]. Increasing RDF flesh consumption leaves the skin that is currently not used optimally. Apart from the flesh, RDF skin can also be used as an alternative because of its nutritional content and antioxidant effects [3]. The total content of polyphenols and flavonoids from 80% methanol extract of RDF skin is three times higher than RDF flesh. The total phenolic content extracted from the skin and flesh is 14.82 ± 1.07 and 4.91 ± 0.55 mg Gallic Acid Equivalent (GAE) / 100g [4].

In type 2 diabetes mellitus (T2DM), hyperglycemia is caused by the inability of Insulin to mobilize blood glucose into cells due to insulin receptor resistance [5]. Hyperglycemia increases the auto-oxidation of glucose from free radicals. In hyperglycemia conditions, the formation of free radicals or Reactive Oxygen Species (ROS) comes from glucose oxidation, on-enzymatic glycosylation of proteins, and oxidative degradation of glycolic proteins [6]. The increase in intracellular glucose causes an abundance of electron donors to be generated during the Kreb cycle, thereby pushing the potential of the inner mitochondrial membrane upward - a condition associated with mitochondrial dysfunction and increased production of ROS [7]. In addition, ROS will increase the expression of Tumour Necrosis Factor-α (TNF-α) and exacerbate oxidative stress. TNF-α can result in insulin resistance through decreased autophosphorylation (auto-phosphorylation) of insulin receptors [8,9]. These oxidative stress markers can be measured using Malondialdehyde (MDA) [10].

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Insulin resistance impairs the ability of muscle cells to take up and store glucose and triglycerides, which results in high levels of glucose and triglycerides circulating in the blood [11]. One of the biomarkers used to measure insulin resistance is the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR). HOMA-IR measures insulin resistance (IR) based on the value of fasting blood glucose and plasma insulin levels [12].

Hypoglycemic effects of flavonoids are by regulating carbohydrate digestion, insulin signaling, insulin secretion, glucose uptake, and adipose deposition [13]. Flavonoids work by targeting many molecules involved in regulating multiple pathways, such as increasing β cell proliferation, promoting insulin secretion, reducing apoptosis, and increasing hyperglycemia by regulating glucose metabolism in the liver [14]. Red dragon fruit skin makes up 22% of all RDF, usually thrown away.

Two-thirds of the fruit consumed is whole fruit, and one-third is 100% fruit juice. One hundred percent of consumption of fruit juice treatments with several health benefits, such as reduced levels of lipid profiles and reduced obesity [15]. A meta-analysis study has proven that an alternative way to consume the right amount of fruit is by drinking, such as consuming fruit juices, especially on glycemic control [16]. Therefore, in this study, the authors are interested in using both skin and flesh of RDF to determine their effectiveness in reducing ROS and insulin resistance in T2DM model rats.

MATERIALS AND METHODS

Experiment Protocols

The Red Dragon Fruit (*Hylocereus polyrhizus*) is obtained from Tawangmangu and the same plantation to maintain variety homogeneity. The maintenance and treatment of experimental animals were carried out at the Laboratory of the Center for Food and Nutrition Studies, Gadjah Mada University, Yogyakarta. The antioxidant test was conducted at the Sebelas Maret University Nutrition and Food Lab. Preparation and observation of examination of levels of fasting blood glucose, serum insulin, and plasma MDA were carried out at the integrated Research and Testing Institute of Gajah Mada University, Yogyakarta. All chemicals used have met lab analysis standards. The ethics committee approved this study of The Health Research Ethics Committee of Faculty Medicine Universitas Sebelas Maret for medical research, protocol number 465/UN27.06/KEPK/EC/2019.

Sample Size and Study Design

This study is a true experimental study with a randomized controlled trial, with a Matching Pretest Post-test Control Group Design. This study used white male rats (*Rattus norvegicus*) Wistar strain as research objects with three treatment groups: 1 negative control group, one positive control group, and one treatment group. The sample size for each group was determined based on the provisions of the Institutional Animal Care and Usse Committee (IACUC) (2002): at least six rats in one study group. Each group added 20% for the probability of dropping out. The sampling technique was simple random sampling to obtain seven rats in each group. This study used three treatment groups, so that the total sample of this study was 21 rats.

Dosages

The dose of juice therapy used in humans corresponds to 1 glass of juice consumed daily by adult individuals with an average weight of 200 ml [17]. Higher fruit and vegetable juices consumption was associated with higher-quality diets and better compliance with the French National Plan for Nutrition and Health. Making 200 ml of RDF juice requires 274 grams (both flesh and peel). The conversion dose for a rat was 0.018, so the dose for the sample was 3.6 ml/200g BW/day, which was given by sonde for 14 days. The maximum volume of fluid administration for white rats weighing 200 grams is 5ml so that the volume of juice given is appropriate.

The reference material used in this study was metformin HCl. The usual dose of metformin HCl used to reduce blood glucose levels is 500 mg - 1700 mg per day in humans with a bodyweight of 70 kg given orally in a single dose [18]. Metformin is a biguanide compound that is still used as an oral hypoglycemic drug in Indonesia which works to reduce blood glucose levels by improving glucose transport to muscle cells. In addition, this drug can improve glucose uptake by 10-40%). In this study, there are three groups: (P1) negative control group (induced Streptozotocin + Nicotinamide induction), (P2) positive control group (given Streptozotocin + Nicotinamide and given Metformin HCl 0 induction, 9 mg/kg BW, and (P3) Red Dragon fruit group (induced Streptozotocin + Nicotinamide and given Red Dragon Fruit juice 3.6 ml / 200 g BW / day given for 14 days.

Biological Experiment Protocols

Rats were obtained from Inter-University Center (IUC) Nutrition of Gadjah Mada University in pre-clinical service and experimental animal development. Rats were kept in a particular room placed in clean polypropylene cages with seven rats

per large cage which were then given transparent dividers so that one rat occupied one small cage. The food was a standard Comfeed feed consisting of 70% corn starch, 10% casein corn oil, 4% salt mixture, 1% vitamin mixture, and 5% cellulose. The study was started by preparing 24 male Wistar rats aged 8-10 weeks, body weight ± 180 grams and adapted for seven days in the cage, then randomized into three groups. Diabetic rats were induced by giving 230 mg/kg Nicotinamide (NA), then 15 minutes later given 65 mg/kg Streptozotocin (STZ) in cold citrate buffer, pH 4.5, intraperitoneally to male rats, which previously did not need to be fastened. Hyperglycemia confirmed after 48 hours of STZ-NA administration was characterized by an increase in fasting blood glucose levels. Therefore, rats with blood glucose levels of 180 mg/dL were considered diabetic and were included in the study.

Measurement Instruments

A sampling of test animal blood is part of a series of in vivo studies. In this study, blood sampling in rats used the Plexus Retro-Orbital method in the eyes [19]. Measurement of MDA levels from blood samples of Wistar rats was examined quantitatively using the thiobarbituric acid-reactive substance (TBARS) kit. Measurement of plasma insulin levels from blood samples of Wistar rats that were examined quantitatively. Rat Enzym-Linked Immunosorbent Assay (ELISA) Insulin kit DRG brand no EIA catalog 2048. r. The amount of glucose contained in the blood of Wistar rats was examined quantitatively by the Enzymatic Colorimetric Test GOD-PAP (Glucose Oxidase Phenol 4-Aminophenazone) method, which was carried out before being given treatment (pre-test) and at the time after being given treatment (day 14). The homeostasis model assessment-insulin resistance (HOMA-IR) is a validated and widely used method to measure insulin resistance from fasting glucose and Insulin.

HOMA-IR : $\frac{Fasting\ blood\ glucose\ levels\ \left(\frac{mg}{dL}\right) x\ insulin\ level\ \left(\frac{ng}{mL}\right)}{405}$ 20

Statistical Analysis

The data were coded and analyzed using SPSS for Windows version 20. This study used paired T-test for normal data with 95% significance. Data that were not normally distributed were analyzed using Mann Whitney U Test. If the value of p <0.05, there was a significant difference between variables, and if p> 0.05 means that there was no statistical difference in the effect before and after the intervention. The different effects of those three groups were analyzed using the parametric statistical test, one-way ANOVA for normally distributed data, and homogeneous data, then continued with the Post Hoc test .

RESULT

Tabel 1. Chemical Compositions of Red Dragon Fruit Juice per 3,6 ml

Sample	Test	Analysis Parameters			
		Reducing Sugar [1)] (% wb)	**Antioxidants [2)] (% wb)**	**Total Phenol [3)] (% wb)**	**Anthocyanins [4)] (ppm wb)**
RDF	I	8.29	2.29	0.064	147.84
	II	8.46	2.50	0.061	135.90
	Average	8.37	2.39	0.063	141.87

Source: Primary Data (2019)

1) *Nelson Somogyi;* 2) *Spektrofotometri;* 3) *Spektrofotometri;* 4) *Giusti & Worlstad*

The chemical and antioxidant composition of Red Dragon Fruit juice has been investigated, and the results are recorded in Table 1. Table 1 shows the reduced sugar content of 8.37% wb; Antioxidants 2.39% wb; Total Phenol as much as 0.063% wb and anthocyanin content in 3.6 ml of RDF juice as much as 141.87 ppm wb.

Tabel 2. The Effect of RDF Juice on Fasting Blood Glucose Levels

Group	Duration		Δ Fasting Blood Glucose (mg/dL)	p^a
	Day-0 (*mean* ± *SD*) mg/dL	**Day-14 (*mean* ± *SD*) mg/dL**		
P1	272.69 ± 9.53	275.03 ± 8.69	2.33 ± 2.92	0.080

P2	282.49 ± 7.35	116.95 ± 7.32	-165.54 ± 8.89	< 0.001*
P3	276.25 ± 8.84	112.42 ± 6.69	-163.82 ± 13.65	< 0.001*
p [b]	0.090	< 0.001*	< 0.001*	

Source: Primary Data (2019)
*) There is a significant difference; [a]) (p < 0.05) *Paired T-Test ;* [b]) (p < 0.05) *One Way Anova*

One Way-Anova is a comparative test used to test the difference in the mean (average) of data for more than two groups. The difference in the mean effect of RDF juice on Fasting blood glucose (FBG) levels can be seen in Table 2. Table 2 shows the mean FBG after being tested with One Way-Anova and Paired T-Test. Before administering the intervention, mean values of FBG levels were compared among the three study groups, and it was not statistically significant (Day-0), indicating that randomization had achieved the intended goal. However, both the Metformin group (P2) and the RDF group (P3) had lower FBG levels (p=<0.01) than the negative control group (P1), and the mean difference in their partners was statistically significant. After the One Way-Anova test, the three groups were statistically significant (Day-14; Δ Fasting Blood Glucose). Therefore, a further test was carried out to determine which group was different (Table 6).

Tabel 3. The Effect of RDF Juice on Insulin Levels

Group	**Duration**		**Δ Insulin Plasma (pg/ml)**	***p*** [a]
	Day-0 (*mean* ± *SD*) pg/ml	**Day-14 (*mean* ± *SD*) pg/ml**		
P1	414,01 ± 6,07	409,90 ± 2,92	-4,10 ± 5,47	0,095
P2	413,55 ± 2,97	549,88 ± 4,90	136,32 ± 6,48	< 0,001*
P3	414,92 ± 4,33	548,66 ± 7,70	133,74 ± 6,22	< 0,001*
p [b]	0,160	< 0,001*	< 0,001*	

Source: Primary Data (2019)
*) There is a significant difference ; [a]) (p < 0.05) *Paired T-Test ;* [b]) (p < 0.05) *One Way Anova*

Table 3 shows the difference in mean Insulin after being tested by One Way-Anova and Paired T-Test. Before administering the intervention, the mean scores of insulin levels were compared among the three study groups. It was not statistically significant (Day-0), indicating that randomization had achieved the intended goal. Both the Metformin group (P2) and the RDF group (P3) had lower Insulin levels than the negative control group (P1), and the mean difference in their partners was statistically significant. After the One Way-Anova test, the three groups were statistically significant (Day-14; Δ Insulin Plasma). A further test was carried out to find out which group was different (Table 6).

Tabel 4. The Effect of RDF Juice on MDA Level

Group	**Duration**		**Δ MDA Plasma (nmol/ml)**	***P*** [a]
	Day-0 (*mean* ± *SD*) pg/ml	**Day-14 (*mean* ± *SD*) pg/ml**		
P1	8,98 ± 0,69	9,08 ± 0,68	0,09 ± 0,10	0,061
P2	8,77 ± 0,66	3,34 ± 0,22	-5,43 ± 0,62	< 0,001*
P3	9,25 ± 0,32	3,05 ± 0,47	-6,20 ± 0,50	< 0,001*
P [b]	0.658	< 0.001*	< 0.001*	

Source: Primary Data (2019)
*) There is a significant difference ; [a]) (p < 0.05) *Paired T-Test ;* [b]) (p < 0.05) *One Way Anova*

Table 4 shows the difference in mean MDA after being tested with One Way-Anova and Paired T-Test. Before administering the intervention, mean MDA levels were compared across the three study groups, and it was not statistically significant, indicating that randomization had achieved its intended goal. Both the Metformin group (P2) and the RDF group (P3) had lower MDA levels than the negative control group (P1), and the mean difference in their partners was statistically significant. After the One Way-Anova test, the three groups were statistically significant (Day-14; Δ MDA Plasma). A further test was carried out to find out which group was different (Table 6).

Tabel 5. Effect of Red Dragon Fruit Juice on HOMA-IR Level

Group	Duration		Δ HOMA-IR	*P* [a]
	Day-0 (*mean* ± *SD*) pg/ml	Day-14 (*mean* ± *SD*) pg/ml		
P1	8.35 ± 0.19	8.32 ± 0.26	-0.03 ± 0.12	0.460
P2	8.65 ± 0.25	4.89 ± 0.29	-3.75 ± 0.09	< 0.001*
P3	8.49 ± 0.28	4.65 ± 0.30	-3.83 ± 0.08	< 0.001*
p [b]	0.067	< 0.001*	< 0.001*	

Source: Primary Data (2019)
*) There is a significant difference; [a]) (p < 0.05) *Paired T-Test* ;[b]) (p < 0.05) *One Way Anova*

Table 5 shows the difference in mean HOMA-IR after being tested with One Way-Anova and Paired T-Test. Before administering the intervention, the mean scores of HOMA-IR levels were compared among the three study groups. It was not statistically significant, indicating that randomization had achieved its intended goal. Both the Metformin group (P2) and the RDF group (P3) had lower HOMA-IR levels than the negative control group (P1), and the mean difference in their partners was statistically significant. After the One Way-Anova test, the three groups were statistically significant (Day-14; Δ HOMA-IR). A further test was carried out to find out which group was different (Table 6).

Tabel 6. Effect of Red Dragon Fruit Juice on Mean Difference Group Pair

Group Pair		Mean Different			
Group I	Group II	FBG	Insulin	MDA	HOMA-IR
P1	P3	< 0.001*[a]	< 0.001*[a]	0,002*[b]	< 0.001*[a]
P2	P3	0,655 [b]	1.000 [a]	0.229 [a]	1.000 [a]

Source: Primary Data (2019)
*) There is a significant difference; [a]) (p < 0.05) *Post hocTest* ; [b]) (p < 0.05) *Mann-Whitney*

Table 6 shows a difference with the mean at P1 and P2 with a significance of <0.001. This indicates that both Metformin (P1) and RDF juice (P3) can reduce GDP, MDA, HOMA-IR and increase Insulin compared to P1. When the mean P2 is compared with the mean P3 group, the result is >0.005, which means that RDF dose (P2) had the same effect as Metformin (P3) in reducing GDP, MDA, HOMA-IR, and increasing Insulin in T2DM model rats.

DISCUSSION

One way-ANOVA only provides conclusions about whether there are differences between three or more data groups, while which groups are different cannot be concluded. To solve which group has differences in one-way ANOVA, a Post Hoc follow-up test is carried out. The result shows that the mean difference is not statistically significant between P2 and P3 in FGB (p=0,655), Insulin (p=1.000), MDA (p=0.229), and Insulin level (p=1.000). This study proves that the provision of red dragon fruit (peel and flesh) with the dose of 3.6 ml / 200 gr BW / day had the same effect as Metformin HCl in reducing FGB, MDA, HOMA-IR also increase Insulin level (table 6). The assessment of insulin resistance is complex and challenging to apply. The Homeostasis

Model Assessment-Insulin Resistance (HOMA-IR), which uses fasting glucose and Insulin parameters, is a validated and widely used insulin resistance index.

Fasting Blood Glucose Level

The FBG levels in the T2DM model rats after STZ-NA induction increased above 200 mg/dl in all groups. This illustrates the condition of hyperglycemia due to the provision of STZ causing cell damage β Langerhans pancreas which results in decreased insulin secretion resulting in T2DM (21). In comparison, Nicotinamide (NA) is a vitamin B3 (niacin) derivative with antioxidant capacity that reduces the cytotoxic action of STZ and protects β cells against STZ. STZ is transported into the B-cells via the GLUT2 glucose transporter and causes DNA damage leading to increased activity of poly (ADP-ribose) polymerase (PARP-1) to repair DNA (22). However, the overactivity of this enzyme results in depletion of intracellular NAD (+) and ATP, and insulin-secreting cells undergo necrosis. Therefore, the protective action of NA is to inhibit PARP-1 activity. Therefore, NA inhibits this enzyme, preventing the depletion of NAD (+) and ATP in STZ-exposed cells [23].

The hypoglycemic effect of RDF is obtained from the main antioxidant components in RDF peel and flesh, namely flavonoids. Flavonoids, especially quercetin, are potent inhibitors of GLUT 2 in the intestinal mucosa, a pathway for glucose and fructose absorption in the intestinal membrane. This inhibitory mechanism is noncompetitive. A recent systematic review and meta-analysis of animal studies showed that quercetin decreases serum glucose levels at doses of 10, 25, and 50 mg/kg of body weight [24]. This results in a reduction in the absorption of glucose and fructose from the intestine to decrease blood glucose levels [25].

Apart from Quentin, one of the flavonoid compounds that play a role in the mechanisms involved in hypoglycemia and its protective activity against diabetes complications is the Rutin compound [26]. This compound is proven to be in the RDF, and its content is higher than white dragon fruit (4). In testing on T2DM model rats that STZ insulated, oral administration at a dose of 5-10 mg/kg significantly decreased FBG levels (27,28). Nature et al. reported that common effects (50 and 100 mg/kg) on FBG and glycosylated hemoglobin were comparable to pioglitazone. This is due to receptor agonists activated by proliferation [29]. Jadhav and Puchchakayala [30] observed that among rutin, boswellic acid, ellagic acid, and quercetin, rutin was the most active flavonoid in increasing glucose tolerance and lowering FBG. Furthermore, they found that rutin (100 mg/kg), comparable to glibenclamide (10 mg/kg), lowered plasma glucose in diabetic and normoglycemic rats [31]. Rutin's mechanism in reducing glucose absorption from the small intestine is by inhibiting α-glucosidase and α-amylase, which are involved in carbohydrate digestion [30–32].

Insulin Level

Increased blood glucose (hyperglycemia) and free fatty acids stimulate the formation of reactive oxygen species (ROS), reactive nitrogen species (RNS), and oxidative stress. This can interfere with pancreatic beta-cell function and insulin resistance to worsen diabetes conditions [33]. The increase in plasma insulin levels is caused by the antioxidant flavonoids present in the peel and flesh of RDF. Flavonoids have a mechanism in inhibiting phosphodiesterase so that cAMP levels in pancreatic β cells increase. This will stimulate insulin secretion through the Ca pathway. In addition, this increase in cAMP levels will cause the closure of the K + ATP channels in the plasma membrane of β cells. This situation causes membrane depolarization and opens Ca channels depending on the voltage, thereby accelerating the entry of Ca ions into the cell. The increase in Ca ion in the cytoplasm of β cells will cause insulin secretion by β cells of the pancreas [34–36].

One of the flavonoid compounds that play a role in the mechanism involved in the antihyperglycemic effect and its protective activity against diabetes complications is the Rutin compound (vitamin P) [26]. Routine can stimulate insulin secretion from beta cells and increase glucose uptake by tissues. In isolated mouse pancreatic islets, the routine significantly increased insulin secretion [37.] In mouse beta cells, rutin has been shown to increase glucose-induced insulin secretion and maintain glucose-sensing ability in high glucose conditions [38]. In rat beta cells, rutin increased glucose-induced insulin secretion, and rutin also demonstrated a role for insulin-mimetics in rat soleus and diaphragm muscles [32,38]. It stimulates glucose transport into muscle via activation of the synthesis and translocation of the GLUT-4 transporter. Like the insulin signaling pathway, phosphoinositide 3-kinase (PI3K), protein kinase C, and mitogen-activated protein kinase (MAPK) are involved in routine intracellular transduction, leading to a stimulatory effect on tissue glucose uptake [38]. Rutin also increases PPARγ expression, increasing insulin resistance and glucose uptake in skeletal muscle and adipose tissue [32].

Insulin Resistance (HOMA-IR)

Insulin resistance is an abnormal physiological condition that occurs when Insulin from pancreatic β cells cannot trigger signal transduction pathways in target organs such as the liver, muscle, and adipose tissue. Loss of insulin sensitivity is commonly associated with persistent hyperglycemia (diabetes) [39]. Insulin resistance impairs PI3K / Akt activation of skeletal muscle and adipose tissue, leading to decreased expression and translocation of GLUT4, resulting in impaired glucose uptake. Deficits in hepatic insulin signaling release FOXO1 back into the nucleus to promote expression of PEPCK and G6P genes, promoting gluconeogenesis and reducing activation of Glucokinase/Glycogen Synthase Kinase (GK and GSK), which suppress glycogen synthesis [40]. Based on the research, flavonoids can increase the expression of Akt, AMPK, GLUT4, and adiponectin in skeletal muscle tissue [41] and increase levels of IRS1 and GLUT4 mRNA in skeletal muscle tissue [42] resulting in increased insulin resistance in skeletal muscle tissue. Also can increase GK activity in liver tissue [43] and increase GSK mRNA levels in liver tissue resulting in an increase in insulin resistance in the liver [42].

The HOMA-IR value was inversely proportional to plasma insulin levels and directly proportional to FBG levels. The results showed the value of insulin resistance (HOMA-IR), then the uptake and use of glucose by the body's cells were disrupted. As a result, the glucose levels in the blood increased. Flavonoids can reduce insulin concentration and improve glucose tolerance through adipocytokine regulation, including increased serum adiponectin [44]. Flavonoids have also been shown to stimulate adipocyte differentiation and increase glucose transport in adipocytes by inducing PPARg-mediated adiponectin expression and translocation of GLUT4 in 3T3-L1 adipocytes [45]. Previous research also revealed that intake of flavonoids could reduce insulin resistance levels [46].

MDA Level

MDA levels in the T2DM model rats after STZ-NA induction increased in all groups compared to controls. This indicates that the induction of STZ-NA succeeded in increasing Reactive Oxygen Species (ROS) levels in the T2DM model rats. The source of oxidative stress in T2DMs is due to a shift in the balance of redox reactions due to changes in carbohydrate and lipid metabolism, which will increase ROS formation from glycation and lipid oxidation reactions, thereby reducing the antioxidant defense system [47].

The effect of decreasing plasma MDA levels can be caused by antioxidant flavonoids in the RDF peel and flesh. One of the flavonoids content, namely betacyanin, which is a pigment of RDF, has a high antioxidant effect and plays a role in reducing ROS levels to provide a protective effect on diabetic rat pancreatic cells. [25].

Hyperglycemia in T2DM can produce intracellular reactive oxygen/nitrogen species (ROS / RNS) excess. Experiments on diabetic animals prove that giving rutin can increase antioxidant status in various tissues by increasing non-enzymatic antioxidant status (reducing glutathione) and enzymatic antioxidant status (Superoxide dismutase and catalase) [29,48,49]. Rutin contains many OH substitutions so that it has a sufficient effect in reducing free radical levels [26]. Analysis of the structure-function relationship shows the importance of Bring and 3'-OH and 4'-OH groups to free radicals that work on the effect of Rutin [50]. Because of this group, rutin tends to give electrons to free radicals, converting them into more stable radical intermediates and inhibiting further free radical reactions [50].

CONCLUSIONS

The administration of RDF fruit juice intervention for 14 days significantly reduced FBG, MDA, HOMA-IR levels and significantly increased plasma insulin levels in the T2DM rats model. RDF fruit juice can be an alternative therapy to reduce ROS and insulin resistance in the T2DM rats model. Long duration follow-up studies are required before application in diabetic patients.

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