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Young khalaal dates (*Phoenix dactylifera*) effect on blood glucose in alloxan-induced wistar male rats

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ABSTRACT

This study analyzed young dates (*Phoenix dactylifera*) effect on blood glucose in alloxan-induced male wistar rats. It used a completely randomized design with pre-post test and control group design. The research samples were 25 male white wistar rats that divided into 5 groups: (1) negative control group with no treatment (K1); positive control group with alloxan induction only (K2); treatment group 1 with alloxan induction and 0.25 g/day of young dates treatment (P1); treatment group 2 with alloxan induction and 0.5 g/day of young dates treatment (P2); and treatment group 3 with alloxan induction and 1 g/day of young dates treatment (P3). The result were tested by Kolmogorov-Smirnov normality test ($p>0.05$), Levene's homogeneity test ($p>0.05$), followed by General Multivariate Manova test by Wilks' Lambda, between-subject effect test, and LSD ($p<0.05$). The results indicated that young dates could effectively reduced the blood glucose levels ($p = 0.000$). The fasting blood glucose level in positive control group (K2) was different from negative control group (K1, $p = 0.000$) and all treatment groups (P1, $p = 0.000$, P2, $p = 0.000$ and P3, $p = 0.000$). 0.25 g/day of young dates treatment could effectively reduced the fasting blood glucose level in alloxan-induced male wistar rats.

Keywords: young dates, blood glucose levels, alloxan

INTRODUCTION

Diabetes Mellitus was an increase on blood glucose levels due to decreased peripheral tissue response to insulin (insulin resistance). It was also the response of pancreatic β cell dysfunction in insulin secretion that is inadequate due to insulin resistance. Both of these might aggravate hyperglycemia condition[(1)].

Diabetes Mellitus (DM) prevalence continues to increase in every year. According to the International Diabetes Federation (IDF) data in 2013, the numbers of DM sufferers worldwide reached 382 million people and was expected to increase until 529 million in 2035. Indonesia was the 7th largest country for DM prevalence. WHO stated that DM sufferers prevalence in Indonesia would increased by 152%, from 8.4 million in 2000 to about 21.3 million in 2030[(2)]. It was also commensurate with Basic Health Research data for 2013 that 6.9% of Indonesians with aged ≥15 years old had been diagnosed with DM[(3)].

Diabetes mellitus (DM) is divided into 2 main types, type 1 and type 2. DM type 1, commonly was called by insulin dependent diabetes mellitus (IDDM), was caused by a lack of insulin secretion produced by pancreatic β cells[(4)]. DM type 2, called non-insulin dependent diabetes mellitus (NIDDM), was caused by decreased peripheral tissue response to insulin (insulin resistance) and pancreatic β cells dysfunction as inadequate insulin secretion that was resulted by insulin resistance[(1)]. American Diabetes Association (ADA) stated that most of the incidence rate of DM worldwide was DM type 2 with 90-95%.

Insulin resistance in Diabetes Mellitus sufferers were caused by decreased activation of insulin receptors or IRS (Insulin Receptor Substrate), whereas pancreatic β cell dysfunction is inadequate insulin secretion that was caused by the accumulation of free radicals in the body that can directly damage DNA of pancreatic β cells. Free radicals in the body could be characterized by high Reactive Oxygen Species (ROS)[(5)].

The young dates of Khalaal (*Phoenix dactylifera*) was type of dates that had not been through perfect maturation process. Dates contain antioxidant flavonoids that could make free radicals in the body became more stable. In addition to containing flavonoids, young dates was also contained by vitamin A and vitamin E which was a kind of secondary antioxidants. In its work, beta-carotene, which was the most active pro-vitamin A, could bind oxygen singuel (O_2), counteracted the peroxyl radical, and inhibited lipid oxidation so that the amount of ROS could be lowered and improved the performance of pancreatic β cells. Increased vitamin A performance

could be aided by the presence of vitamin E and other antioxidants[6]. On the other hand, magnesium might help increased insulin sensitivity by activating insulin receptors[7].

Many other fruits in Indonesia may contain flavonoids, vitamin A, vitamin E, magnesium, and low glucose but, so far, it was rarely found fruit containing various vitamins and minerals in one fruit, which its content can help reduce hyperglycemia in normal limits in people with Diabetes Mellitus. Therefore, it was necessary to did research on the effect of young khalaal dates (Phoenix dactylifera) to blood glucose level.

METHODS

This research was an experimental research with randomized design method, completed with pre-post test and control group design. The research was conducted at the Laboratory of Faculty of Medicine, Airlangga University, Surabaya in July-August 2017.

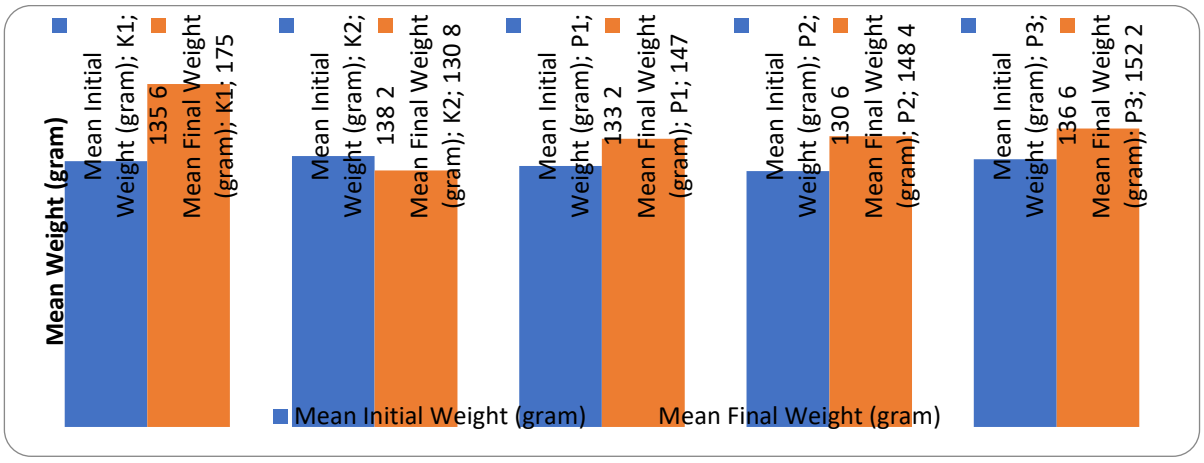
The research samples were 25 male white wistar-strain rats that divided into 5 groups at random: (1) negative control group with no treatment (K1); positive control group with alloxan induction only (K2); treatment group 1 with alloxan induction and 0.25 g young date treatment (P1); treatment group 2 with alloxan induction and 0.5 g young date treatment (P2); and treatment group 3 with alloxan induction and 1 g of young dates treatment (P3). All groups, excepted for the K1 group, were conditioned to be diabetic by alloxan induced dose with 125 mg alloxan per kilograms body weight. The determination of alloxan dose was refered to Prameswari et al.[8]. Aloxan was injected intraperitoneally with a single dose then hyperglycemic effect would appear after approximately 72 hours.

The principle of measuring rats blood glucose levels was took blood from the lateral vein (tail tip) and then measured it well with a glucose meter kit. Taking was done by inserting a rat into a jar containing-alcohol-ether. After a decrease in consciousness, rats were removed from the jars. Disinfection was done with alcohol swab at the rat tail and wait a while until it was dry. The tip of the mouse tail was slashed using a razor blade and measured blood glucose levels using a glucose meter kit.

The research data were analyzed by using General Linier Model Multivariate Mannova test and looking at Wilk's Lambda value. The effect was said to be significant if the value of $p < 0.05$. Furthermore, it was also analyzed by the differences of each group and the value of LSD, with the difference was said significant if the value of $p < 0.05$.

RESULTS

The data that obtained from the results of this study were pre-post weight and fasting blood glucose (FBG) levels in beginning, pre, and post. The results could be described as follows. The characteristics of experimental animal based on weight gain were shown in Figure 1.



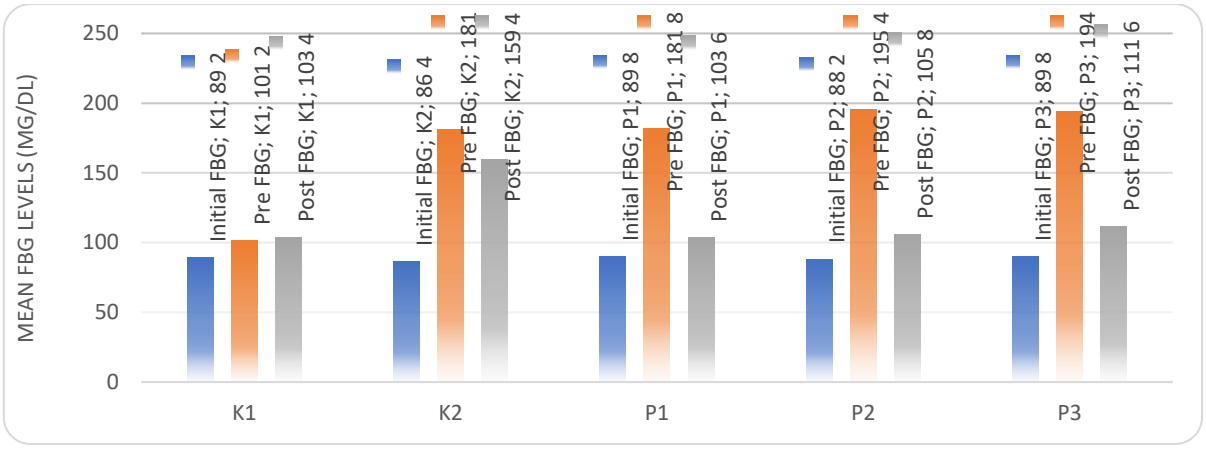
Information:

K1 = group without treatment; K2 = positive control group with alloxan induction only; P1 = group with alloxan induction and 0.25 g young date treatment; P2 = group with alloxan induction and 0.5 g of young dates treatment; and P3 = treatment group 3 with alloxan induction and 1 g of young dates treatment.

Figure 1. Mean Initial and Final Weight Between Control Groups and Treatment Groups

Based on figure 1 it could be seen that there was a decrease of mean weight in K2 group, while K1, P1, P2, and P3 had weight gain. The highest increase occurred in the K1 group (without treatment).

Characteristics of experimental animal which based on fasting blood glucose was shown in Figure 2.



Information:
Initial FBG = initial FBG baseline examination; FBG-pre = examination of FBG levels after alloxan induction; FBG-post = examination of blood glucose levels after given young date treatments for 14 days

Figure 2. Mean Initial, FBG, FBG-Pre and FBG-Post Levels Between Control Groups and Treatment Groups

Based on Figure 2, it was known that the average distribution of fasting blood glucose (FBG) in the P1, P2, and P3 groups was decreased. The highest decrease in FBG was occurred in the P1 group which got 0.25 g/day young dates treatment.

The influence between control group and treatment groups were known by General Linear Test of Multivariate Manova Model. The test results obtained the value from Wilks' Lambda with 0.000 ($p < 0.05$) which meant that there was a significant influence from young dates treatments with different doses in fasting blood glucose level. The p-value of between-subject effect also showed that the difference after treatment was $p = 0.000$ (<0.05), which means there were difference between groups.

To knowing which groups differ significantly, the LSD test were taken. The difference was said to be significant if $p < 0.05$. The results of LSD test on fasting blood glucose level (FBG) for all control and treatment groups were shown in Table 1 as follows:

Table 1. P Value of LSD Test in Fasting Blood Glucose Level (FBG) After Treatment Between Control Groups and Treatment Groups

Group	K1	K2	P1	P2	P3
K1	-	0.000	0.985	0.817	0.432
K2	0.000	-	0.000	0.000	0.000
P1	0.985	0.000	-	0.832	0.443
P2	0.817	0.000	0.832	-	0.577
P3	0.432	0.000	0.443	0.577	-

Table 2 showed the different blood glucose levels in each pair of groups with $p < \alpha$ values (0.05) were between K1 with K2 ($p = 0.000$), K2 with P1 ($p = 0.000$), K2 with P2 ($p = 0.000$), and K2 with P3 ($p = 0.000$). For more details, the LSD test results of the post treatment blood glucose levels were shown by the following bar diagram.

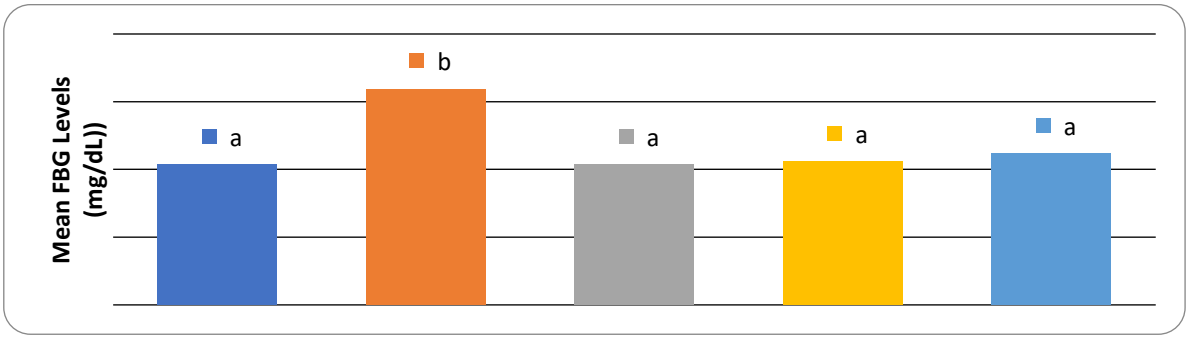


Figure 3. Differences on FBG Levels (mg/dL) After Post Treatment of Young Dates Between Control Groups and Treatment Groups

Based on Figure 3 it could be seen that there was a difference between K1 with K2 and K2 with all treatment groups (P1, P2, and P3). The letters "a" and "b" above showed a significant difference based on the LSD test at the p value of 0.05.

DISCUSSION

The results showed a significant effect on post blood glucose levels in treatment groups with different doses, with Wilks' Lambda value 0.000. This meant that the active substances that contained in young dates, such as magnesium, flavonoids, vitamin A, and vitamin E, could worked maximally in the process of decreasing blood glucose levels into normal limits in diabetic rats.

For the initial blood glucose measurement, all experimental animals were still within normal limits (<126 mg/dl). Furthermore in pre-blood glucose measurements, the alloxan-induced groups had elevated blood glucose levels (K2, P1, P2 and P3). The alloxan induction performed on pretreatment could lead into elevated blood glucose levels in experimental animals. Alloxan caused the elevated blood glucose levels because it was selectively toxic to pancreatic β-cells that produced insulin due to the accumulation of alloxan in particular through the glucose transporter GLUT 2[9]. The alloxan was reacted by weakening the essential substance in pancreatic β cells, causing the reduction of insulin-carrying granules in pancreatic β cells[10].

The blood glucose levels measurement was found decreased into normal levels in all three treatment groups (P1, P2 and P3) after being given young dates treatment. Flavonoid acted as a natural antioxidant that could inhibited ROS formation. In the formation of ROS, oxygen would bound free electrons that came out due to electron chains leakage. The reaction between oxygen and free electrons was produced ROS in mitochondria. Antioxidants in flavonoids could donated hydrogen atoms. Flavonoids would oxidized and bound free radicals so the free radicals became more stable compounds[5].

In addition to the antioxidants of flavonoids, vitamin A and vitamin E also had the same role. Vitamin A in young dates acted as a free radical stabilizer through its action in binding oxygen singular (O_2) or non-paired oxygen to made it less reactive and more stable. In addition, beta carotene which was a precursor/pro-vitamin A might inhibited lipid peroxide activity which was free radicals main source[6]. Increased vitamin A performance could be aided by vitamin E and other antioxidants[11]. β-carotene could improved lipid metabolism in diabetics by lowering total cholesterol synthesis, LDL and VLDL[12].

Free radicals had unpaired electrons, while oxidants were compounds that could accept electrons. Free radicals had the property of converting nonradical compounds to radicals[13]. Free radicals could be linked to metabolic abnormalities in the body. An increase in free radicals in the body was proportional to an increase blood glucose levels that could ultimately lead to multiple complications[10]. For that, the reduction of free radicals in people with Diabetes Mellitus also needed to be attempted so that possible complications could be prevented.

Magnesium (Mg) content in young dates was a co-factor for various enzymes, involving glucose metabolism, especially those that used high-energy phosphate bonds. Mg was very important as a cofactor in all ATP transfer reactions. This indicated that Mg had a very important role in insulin receptor phosphorilation, where an intracellular Mg depletion could caused a defective tyrosine kinase function on insulin receptors and was associated with a decrease in the ability of insulin to stimulated glucose uptake in insulin-sensitive tissues[14].

In the process of performance, magnesium could produce ATP bonds on the intracellular component of the β subunits that were insulin receptors. This ATP bond would trigger the phosphorylase of subunit β through tyrosine kinase enzyme. Tyrosine phosphorylation on these intracellular substlevels was called by IRS (Insulin Receptor Substrate). IRS might bound other signal molecules to activate insulin[7]. The performance of IRS in binding molecular signals was a performance boost from insulin receptors that could directly improved insulin ability to respond glucose to enter into the blood. If the insulin worked optimally, insulin resistance in hyperglycemia conditions could be prevented. So, the tissue glucose uptake could increased and glucose levels in the blood remained normal[7].

In this study, among the three treatment groups, it was found that in the P1 group (0.25 g/day dates treatment) experienced the highest decrease in blood glucose when compared with P2 (0.5 g/day dates treatment) and P3 (1 g/day dates treatment). This could be happen because the young dates contained variety of antioxidants. Excessive levels of antioxidants in the body could made the original antioxidant to be pre-oxidant. Pre-oxidants were compounds that could oxidize excess lipid compounds in cell membranes. Its product might caused some degenerative diseases[15].

The principle of experimental type research, especially one that provides treatment in the form of therapy, was to find the smallest dose that gave better impact than other doses. The principle of therapy was intended to reduce the risk or negative impact that might arise if given treatment with larger doses. The result of this study, in accordance with the principle of therapy, was showed that 0.25 g/day of young dates treatment in P1 group could reduced blood glucose leveled up to 103.4 mg/dl. This value was lower when compared with P2 group (0,5 g/day young dates treatment) with 105,8 mg/dl of decreasing blood glucose level and P3 group (1 g/day young dates treatment) with decreasing blood glucose level equal to 111.6 mg/dl.

CONCLUSION

The 0.25 g/day of young dates treatment could effectively reduced the fasting blood glucose level in alloxan-induced male wistar rats.

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