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Research Article

The Potential Effect of Honey-derived D-Allulose in Counteracting Hyperglycemia by Time and Dose Dependent Manner in Diabetes Mellitus

Flori R. Sari[1],*

[1]*Department of Pharmacology, Faculty of Medicine, Universitas Islam Negeri Syarif Hidayatullah, Ciputat, Banten, 15419, Indonesia*

Email: florirsari@uinjkt.ac.id

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Abstract
Diabetes mellitus has become a worldwide burden due to its persistent, chronic hyperglycemia. D-allulose, a monosaccharide sugar with a 180.16 molecular weight, is widely used as a low-calorie sweetener, is not involved in glucose-related metabolism, and thus does not alter insulin and pancreatic function. This study aimed to evaluate the potential role of honey-derived D-allulose in acute and sub-chronic diabetes mellitus. Diabetic Sprague-Dawley rats were divided into 9 groups and treated with 0.1, 0.2, and 0.4 g/kg BW honey-derived D-allulose for 28, 56, and 84 days. Post-prandial blood glucose levels and body weight were measured every 4 weeks. Significant reductions in post-prandial blood glucose levels were observed on days 56 and 84 treatment with 0.1 g/kg BW D-allulose. More significant reductions were observed on days 28, 56, and 84 of treatment with 0.2 or 0.4 g/kg BW D-allulose. Eighty-four days of treatment with 0.4 g/kg BW D-allulose significantly reduced post-prandial blood glucose levels compared to all groups. We identified that honey-derived D-allulose reduced post-prandial blood glucose levels in a dose- and time-dependent manner. Thus, honey-derived D-allulose may provide beneficial support for diabetic conditions not only as a sweetener but also as a pharmacological treatment.

Keywords: D-allulose, honey, diabetes mellitus, blood glucose level, sweetener

1. INTRODUCTION

Diabetes mellitus is a worldwide burden due to its endemic nature. Characterized by chronic, prolonged hyperglycemia, diabetes mellitus may cause high morbidity due to its complications, including macrovascular and microvascular disease.[1] Normal glycemic control, compliance with anti-diabetic or insulin treatment, and controlling sugary foods are some of the main strategies for controlling diabetes mellitus.[2]

D-allulose, a rare monosaccharide sugar, has a molecular weight of 180.16 and a molecular formula of $C_6H_{12}O_6$. Categorized as low-energy sugar, it is present in natural products, including fruits and honey, in a very small amount. D-allulose is a C-3 epimer of D-fructose and has a different structure in the paired C_2-C_3 atoms compared to D-fructose sugar.[3,4] Generally, D-allulose can be produced through the isomerization of D-fructose through the enzymes D-tagatose 3-epimerases or D-allulose 3-epimerases.[5] The appearance of D-allulose includes a white crystalline form, low hygroscopicity, being odorless, being highly soluble in water, and a melting point of 96°C).[6] Several evidences have validated the synthesis of D-allulose, including chemical methods from D-fructose catalytic action and boiling D-fructose in ethanol and triethylamine; however, the result is insufficient in terms of high production.[7] Mass productions of D-allulose were achieved by using the D-tagatose-3 epimerase bioreactor. The result of this process is crystallized D-psicose.[7]

D-allulose is poorly absorbed by the digestive system,[3] does not have the same metabolic pathway as D-fructose, and is not

metabolized in the liver.[4,8] Thus, D-allulose is unlikely to be involved in energy production and will probably provide zero energy.[9] Chemically, D-allulose is a rare sugar that has 70% of the sweetness of sucrose and dissolves easily, making it easy for the cooking process.[10] However, D-allulose is not involved in glucose metabolism, is stable in gastric fluid, cannot be metabolized, and cannot raise blood sugar.[11] One study reported that 98% of injected or consumed D-allulose was excreted in the form of urine. Only a small part of D-allulose is excreted in faecal form.[12] Thus, D-allulose is an ideal choice of natural, safe sweetener and has recently been used worldwide as an artificial sweetener since it is not only low in calories but also metabolized to a lesser extent than other normal sugars.[7,13,14]

Despite its role as a sweetener, D-allulose was also found to have a direct anti-diabetic property. Matsuo T et al., 2006 found that D-allulose resembles an alpha-glucosidase inhibitor by inhibiting the intestinal alpha-glucosidase enzyme, which further suppressed the postprandial glycemic response in the rat.[15] Iida et al further validated the role of acute D-allulose administration in decreasing glycemic responses induced by maltodextrin in normal adults.[16] Conclusively, the potential medicinal role of D-allulose varies from its role as an insulin mimetic and modulator of some insulin-responsive genes,[17] as an anti-atherogenic property,[17] as the metabolic regulator of glucose and lipid metabolism,[18,19] as a regulator of postprandial glucose metabolism[20] and as a regulator of muscular glucose disposal.[21]

Considering recent advances, D-allulose may play a beneficial role as a sweetener or sugar substitute as well as a pharmacologic treatment for diabetic conditions. To fully elucidate the effective dose and optimum duration of D-allulose in counteracting hyperglycemia, we applied several doses and time durations of D-allulose treatment in the acute and chronic protocols of diabetes mellitus.

2. RESEARCH METHODS

Animal handling

All rats were maintained in our facilities of animal house (Animal Laboratory, Faculty of Medicine, Universitas Islam Negeri Syarif Hidayatullah) and freely access the water and the food (Hi-Pro-Vite 511B, Charoen Phokphand Indonesia). Experiment protocols were designed in accordance with the Declaration of Helsinki for animal research.

Ethical approval

Experimental research protocol was approved by the Ethics Committee of Faculty of Medicine Universitas Islam Negeri Syarif Hidayatullah with the protocol number of 3674022P211132019072200005 and registry number of B-004/F12/KEPK/TL.00/8/2019

Diabetes model

Diabetic condition was elicited by injecting a single dose of 60 mg/kg BW streptozotocin (Sigma Aldrich, USA). In brief, streptozotocin was diluted in freshly prepared citrate buffer (0,1 M, pH 4.5, made from combination of citric acid and sodium citrate) and injected to the male Sprague Dawley rat according to the body weight. Five days after the streptozotocin injection, post-prandial blood glucose level of each rat was analyzed by taking blood samples from the tail. Diabetic rats were defined as rats having blood glucose level more than 250 mg/dL.[22] Day one of the protocol was defined as the first day of treatment after diabetic confirmation.

D-allulose treatment dose

D-allulose was extracted by Mr. Budi Saksono, M.Sc, Ph.D at the Center of Biotechnology, The Indonesian Academy of Sciences (LIPI), Bogor. In general, 200 mL honey was mixed with 800 mL alkaline water then enzymatically fermented with encapsulated DPEase enzyme (Center of Biotechnology, The Indonesian Academy of Sciences) for 9 hours at the temperature of 35 ^{o}C. The fermentation reaction was terminated by separating the encapsulant from the honey. The final result of fermentation, liquid D-allulose, was then pasteurized for 30 minutes at the temperature of 70 ^{o}C. The final concentration of D-allulose was determined to estimate the oral preparation for the rats. Liquid D-allulose freshly prepared every week. As a treatment, D-allulose was given daily through the decoction method in

diabetic rats at the dose of 0.1 (D-Al 0.1 group), 0.2 (D-Al 0.2 group) and 0.4 g/kg body weight (D-Al 0.4 group). Each dose of the treatment was given for 28 days (D28 protocol group), 56 days (D56 protocol group) and 84 days (D84 protocol group) to fully elucidate the effective dose and treatment duration. Diabetic Sprague-Dawley rats (DM group) were applied as the positive control rat as well as the normal (N) Sprague-Dawley rats were applied as the negative control, without diabetic condition. At the end of each protocol, each rat was sacrificed to collect the plasma samples and the organ tissues for further experiment. D-allulose was extracted by Mr. Budi Saksono, M.Sc, Ph.D at the Center of Biotechnology, The Indonesian Academy of Sciences (LIPI), Bogor. In general, 200 mL honey was mixed with 800 mL alkaline water then enzymatically fermented with encapsulated DPEase enzyme (Center of Biotechnology, The Indonesian Academy of Sciences) for 9 hours at the temperature of 35 ^{o}C. The fermentation reaction was terminated by separating the encapsulant from the honey. The final result of fermentation, liquid D-allulose, was then pasteurized for 30 minutes at the temperature of 70 ^{o}C. The final concentration of D-allulose was determined to estimate the oral preparation for the rats. Liquid D-allulose freshly prepared every week. As a treatment, D-allulose was given daily through the decoction method in diabetic rats at the dose of 0.1 (D-Al 0.1 group), 0.2 (D-Al 0.2 group) and 0.4 g/kg body weight (D-Al 0.4 group). Each dose of the treatment was given for 28 days (D28 protocol group), 56 days (D56 protocol group) and 84 days (D84 protocol group) to fully elucidate the effective dose and treatment duration. Diabetic Sprague-Dawley rats (DM group) were applied as the positive control rat as well as the normal (N) Sprague-Dawley rats were applied as the negative control, without diabetic condition. At the end of each protocol, each rat was sacrificed to collect the plasma samples and the organ tissues for further experiment.

Blood glucose and body weight measurement

Post-prandial blood glucose of each rat was measured every 4 weeks on day 1, day 28, day 56 and day 84 by taking the blood from the rat' tail and using blood glucose strip test (Nesco glucose strip, China). Body weight of each rat was regularly measured every 4 weeks by using the animal weight scale from our animal house.

Statistical analysis

All data were shown as descriptive data. Analytical data was assessed by ANOVA wherever applicable with the statistical software. Significance was defined with probability value less than 0.05.

3. RESULTS AND DISCUSSION

Diabetic animal model

Five days after streptozotocin injection, post-prandial blood glucose levels were measured in each rat. Diabetic animal models were confirmed in the diabetic groups as well as the diabetic with D-allulose treatment groups on day 1 of all dose protocols (0.1, 0.2, and 0.4 g/kg BW), as shown by the high glucose level above 400 mg/dL (Table 1). Significant hyperglycemia were persistently observed in all diabetic groups of all dose protocols (0.1, 0.2, and 0.4 g/kg BW) during the study, confirming the animal model of acute and sub-chronic diabetes mellitus. To evaluate the efficacy of some compounds, researchers often use an acute model of diabetes mellitus for 28 days. However, to fully elucidate the effective dose and the optimum duration of treatment for the potential new compound, we applied both acute and sub-chronic models of diabetes mellitus since a single dose and short treatment may not be sufficient to evaluate the potencies of the compound.

Role of D-allulose treatment on body weight

Despite its role as a sweetener, D-allulose also possesses the property of an anti-obese. Several pieces of evidence validated that intervention with a 5% D-allulose significantly decreases adipose tissue and total body fat[9] and decreased the metabolic status of diet-induced rats.[23] In patients, consuming a diabetic diet with allulose not only improved glycemic status but also improved body weight and body mass index.[24,25] Different results were observed in our study. As depicted in Figure 1, the body weight of the normal rats was significantly increased compared to the day 1 body weight. Contrary to the normal rats,

Table 1. Blood glucose level in all groups from day 28, day 56 and day 84 protocols

Blood glucose level	D1	D28	D56	D84
D-allulose 0,1 g/kg BW protocol				
Normal (n=4)	103 ± 13	100 ± 7	106 ± 22	118 ± 93
DM (n=5)	566 ± 40**	536 ± 90**	533 ± 82**	557 ± 52**
DM-Al 0.1 (n=5)	564 ± 49	476 ± 88	360 ± 100$^{\#}$	356 ± 82$^{\#\#}$
D-allulose 0.2 g/kg BW protocol				
Normal (n=4)	94 ± 5	103 ± 20	95 ± 12	96 ± 16
DM (n=5)	591 ± 13**	555 ± 42**	536 ± 47**	518 ± 41**
DM-Al 0.2 (n=4)	519 ± 65	297 ± 146$^{\#}$	280 ± 148$^{\#\#}$	224 ± 157$^{\#\#}$
D-allulose 0.4 g/kg BW protocol				
Normal (n=4)	114 ± 19	111 ± 16	113 ± 16	109 ± 16
DM (n=4)	569 ± 36**	494 ± 41**	584 ± 20**	555 ± 40**
DM-Al 0.4 (n=4)	469 ± 59	191 ± 64$^{\#\#,\&\&}$	164 ± 53$^{\#\#,\&\&}$	155 ± 33$^{\#\#,\&\&}$

D1=Day 1, D28=Day 28, D56=Day 56, D84=Day 84. **p<0.01 vs. normal group, #p<0.05 and ##p<0.01 vs. DM groups, $^{\&\&}$p<0.01 vs. 0.1 g/kg BW protocol

diabetic rats in all protocols suffered from significant body weight reduction compared to the normal rats in the same protocols. These results enhance the condition that clinical symptoms of diabetes mellitus involve body weight reduction due to the inability of glucose to enter muscular deposit through the GLUT4 receptor. Interestingly, D-allulose treatment prevented body loss in diabetic rats. Body weight improvements were observed in the diabetic rats receiving D-allulose 0.1 g/kg BW on day 84 compared to day 1 in the same protocol. Earlier body weight improvements on day 56 were observed in the higher doses of 0.2 and 0.4 g/kg BW D-allulose treatment compared to their diabetic counterpart rats. These results were quite controversial compared to previous findings that D-allulose possesses anti-obesity properties. However, a possible mechanism of body weight improvement by the D-allulose treatment may indirectly come from the other properties of D-allulose to ameliorate skeletal muscle insulin resistance and increase GLUT4 uptake.[26]

Role of D-allulose treatment on blood glucose level

Chronic diabetes mellitus is characterised by persistent hyperglycemia due to an absolute deficiency of insulin or relative resistance of the insulin receptor. As depicted in Figure 2, significant and persistently high post-prandial blood glucose levels were observed in the diabetic rats without treatment on day 28, day 56, and 84 compared to their control normal rats. Pancreatic damage in the streptozotocin-induced diabetic model is irreversible; thus, there will be no reversible improvement of the diabetic condition in the rat. Consistent with an irreversible diabetic model, patients with diabetes mellitus tend to develop chronic hyperglycemia once diagnosed with diabetes mellitus. Good glycemic control, high compliance with anti-diabetic or insulin treatment, and controlling sugary foods have become the main strategies for controlling diabetes mellitus.[2]

D-allulose (molecular formula : $C_6H_{12}O_6$) is a monosaccharide sugar that has a molecular weight of 180.16. Structurally, D-allulose has a different structure in the paired C_2-C_3 atoms compared to D-fructose sugar,[3] so D-allulose does not have the same metabolic pathway as D-fructose.[8] Additionally, D-allulose is not involved in glucose metabolism, is not metabolized in hepar, does not alter pancreatic and insulin function, and does not influence post-prandial blood glucose levels.[7,11,13,14] Most of the D-allulose was excreted in the form of urine. Only a small part of D-allulose

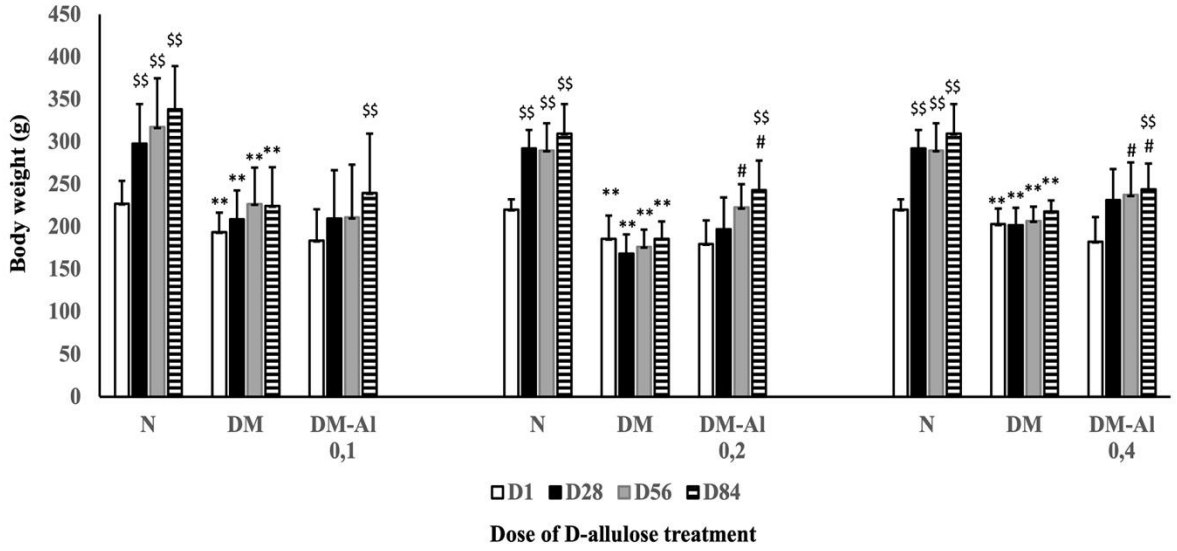

Figure 1. Body weight of all groups from day 28, day 56 and day 84 protocols. N=normal; DM=diabetes mellitus; DM-Al 0.1=DM with D-allulose treatment 0.1 g/kgBW; DM-Al 0.2=DM with D-allulose treatment 0.2 g/kgBW; DM-Al 0.4=DM with D-allulose treatment 0.4 g/kgBW. **p<0.01 vs. N group; #p<0.05 vs. DM group on the same protocol; $^{\$\$}$p<0.01 vs. D1 on the same group.

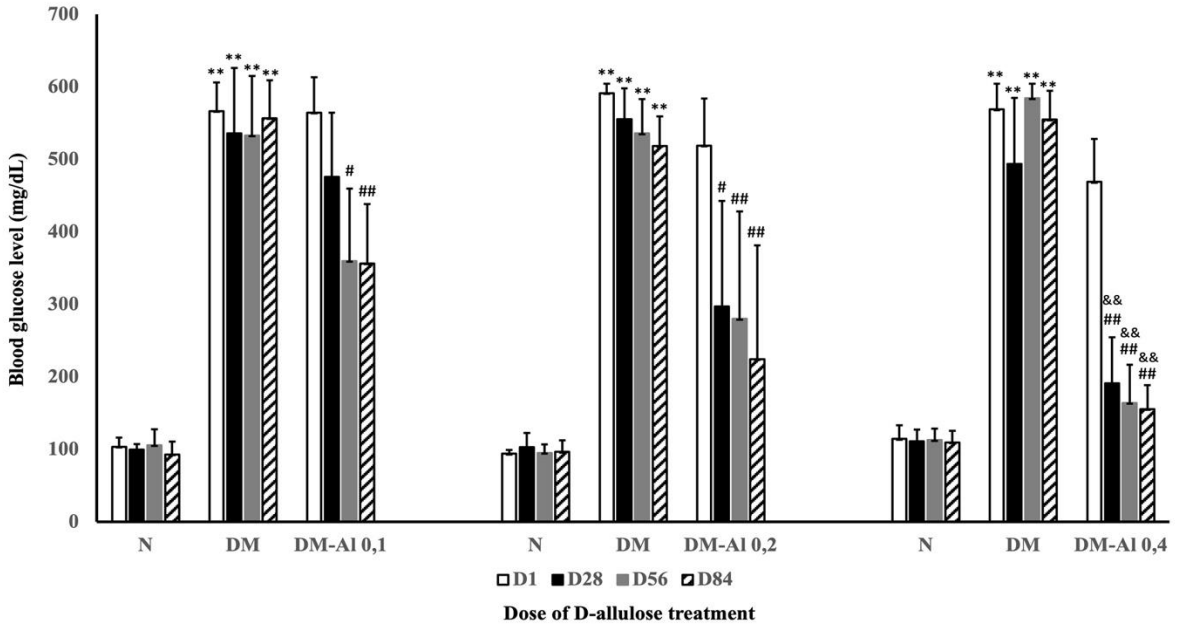

Figure 2. Blood glucose level of all groups from day 28, day 56 and day 84 protocols showing the time and dose dependent manner of D-allulose treatment on chronic diabetes mellitus. N=normal; DM=diabetes mellitus; DM-Al 0.1=DM with D-allulose treatment 0.1 g/kgBW; DM-Al 0.2=DM with D-allulose treatment 0.2 g/kgBW; DM-Al 0.4=DM with D-allulose treatment 0.4 g/kgBW. **p<0.01 vs. N group; #p<0.05 and ##p<0.01 vs. DM group on the same protocol; $^{\&\&}$p<0.01 vs. 0.1 g/kg BW protocol

is excreted in faecal form.[11] As a rare sugar, D-allulose has 70% of the sweetness of sucrose and dissolves easily, making it easy for the cooking process.[10] It is also stable in gastric fluid and has a wide range of toxicity doses.[11] In brief, giving 2 g/kg BW D-allulose to rats did not cause any adverse hazardous effects.[27] Thus, D-allulose is an ideal choice of natural, safe sweetener and has recently been used worldwide as an artificial sweetener since it is not only sweet but also low in energy. Furthermore, it is not influencing pancreatic function nor altering the blood glucose level.[7,11,13,14]

Recently, despite its role as a safe sweetener, evidence has elucidated potential medicinal role of D-allulose as an anti-diabetic through its role as an insulin mimetic and modulator of some insulin-responsive genes.[11] Our

result confirmed previous findings that once daily treatment of 0.1 g/kg BW D-allulose significantly reduces post-prandial blood glucose levels in the diabetic rats on days 56 and 84 compared to their diabetic counterparts without treatment, though the blood glucose level reduction did not reach normal levels (Table 1). We further confirmed that a higher dose and longer duration of D-allulose treatment significantly reduced post-prandial blood glucose levels to a greater extent, though the reduction did not yet reach a normal level. In brief, once daily treatment of 0.2 g/kg BW D-allulose significantly reduces post-prandial blood glucose levels in the diabetic rats on days 28, 56, and 84 compared to their diabetic without treatment counterparts. Earlier, a significant reduction in blood glucose level was observed in the 0.2 g/kg BW compared to the 0.1 g/kg BW. Interestingly, once daily treatment of 0.4 g/kg BW D-allulose gave the best result compared to the other dose group. Treatment with 0.4 g/kg BW D-allulose did not only significantly reduce post-prandial blood glucose levels but also reached the normal level of blood glucose earlier on day 28 (below 200 mg/dL) compared to the other dose groups. Additionally, post-prandial blood glucose levels were significantly reduced on days 28, 56 and 84 on the DM-Al 0.4 protocol compared to the days 28, 56, and 84 on the DM-Al 0.1 protocol. Overall, our result supports another evidence that D-allulose plays a beneficial role not only as a chemical sweetener but also gives pharmacological support as an anti-diabetic, probably through its chemical structure as a mimetic sugar bypassing the pancreatic and insulin functions and through its functional structure as an insulin mimetic and modulator of some insulin-responsive genes.[17] Furthermore, D-allulose possesses anti-atherogenic property,[17] a metabolic regulator of glucose and lipid metabolism,[18,19] a regulator of postprandial glucose metabolism[20] and is a regulator of muscular glucose disposal.[21] In addition, administration of 25 g of D-allulose did not change blood lipids, uric acid, or hsCR, making D-allulose an excellent, effective, and safe sugar substitute.[28] Conclusively, the novel finding of our result is that D-allulose may play a medicinal agent with pharmacologic properties when it is applied in the proper dose and exact duration, since in our study we found that D-allulose reduced post-prandial blood glucose levels in a dose- and time-dependent manner.

4. CONCLUSIONS

D-allulose is widely known as a rare monosaccharide sugar, a safe sweetener, low in calories, and not metabolized by the liver and pancreas. Recently, D-allulose was reported to possess anti-diabetic and anti-obesity properties. We identified that D-allulose 0.1 g/kg BW reduced post-prandial blood glucose levels on days 56 and 84. Earlier and higher reductions of blood glucose were observed in the treatment of D-allulose 0.2 g/kg BW on days 28, 56, and 84 when compared to the lower dose group. The best reductions of blood glucose level, reaching the normal blood glucose level, were achieved in the treatment of D-allulose at 0.4 g/kg BW compared to all lower doses. Thus, D-allulose may have beneficial medicinal properties to counteract hyperglycemia in a time- and dose-dependent manner in acute and sub-chronic diabetes mellitus, at least in part through its chemical and functional structures.

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