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Modulatory Potentials of Voglibose Along With Probiotic in Diabetic Rats

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ABSTRACT – *Diabetesmellitusregarded as a chronicmetabolicdisordercharacterized by hyperglycemiaisprevailingrapidly all over the world and accompaniedwithvarious micro and macro vascular complications. The purpose of presentstudywas to examine the modulatoryeffect of probiotics on different doses of Voglibose in the type 2 diabetic (T2D) rat model. Sprague–Dawley rats (180-250gm) weresubjected to high Fat Diet (HFD) for a period of 2 weeksfollowed by Streptozotocin (STZ) (35mg/kg i.p.) till 21thday of experimentalprotocol. Then, diabetic rats weretreatedwithProbiotics (75 mg/kg, oral gavage), Voglibose (0.1 mg/kg or 0.3 mg/kg, oral gavage) and combination of different doses of voglibose and probiotics. SerumLipidparameterssuch as Total Cholesterol Estimation, Triglyceride estimation and estimation of blood glucose levelwasdone on 0, 14th, 21th and 42th day of the experimentalprotocol. However, TreatmentwithVoglibose (0.3mg/kg) and probiotics (75mg/kg) significantlyattenuates the body weight (BW) in a dose dependent manner when compared with normal animals. Further, whendrugs are given in combination i.e. Voglibose (0.3mg/kg) and probiotics (75mg/kg), significantlydecreased the blood glucose level, total cholesterollevel in comparison to diabetic group treatedwithVoglibose (0.3mg/kg). In addition, thereismarkedincrease in the level of high densitylipoprotein (HDL) with significant decrease in the level of LowDensitylipoprotein (LDL) and verylowdensitylipoprotein (VLDL) level in comparison to diabetic control. Thus, itmaybeconcludedthatsynergistic protective effect on T2D rats maybe due to combination of Probiotics and Voglibose.*

Keywords: Diabetes Mellitus, Probiotics, Voglibose.

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1. INTRODUCTION

Diabetes is one of the main threats to human health in the 21st century [1]. Diabetes mellitus (DM) is a disease in which homeostasis of carbohydrate, protein and lipid metabolism is improperly regulated by insulin hormone resulting in elevation of fasting and postprandial blood glucose levels [2], characterized by chronic hyperglycemia, glycosuria, hyperlipidemia, negative nitrogen balance and sometimes ketonaemia [3]. Type 2 diabetes (T2D) formerly labeled "maturity-onset diabetes" is characterized by insulin resistance or an abnormal secretion of insulin [4]. Impaired function of beta cell will cause deterioration in glucose homeostasis, at this point, insulin secretion cannot keep pace with the underlying insulin resistance and glucose intolerance, and ultimately T2DM occurs [5]. A large number of studies have identified a series of multiple risk factors such as genetic predisposition, epigenetic changes, unhealthy lifestyle, and altered gut microbiota that cause increased adiposity, β-cell dysfunction, hyperglycaemia, hypercholesterolemia, adiposity, dyslipidaemia, metabolic endotoxemia, systemic inflammation, intestinal permeability (leaky gut), defective secretion of incretins and oxidative stress associated with T2D [6]. Probiotics are lactic acid bacteria and live microbes which are used extensively in therapeutic preparations and added to foods [7]. Further, it has been documented that Probiotics particularly lactobacilli and bifidobacteria are known to decrease the serum level of glucose and glucose tolerance in diabetics and provide a higher quality of life to host [8] with proven efficacy in various in vitro and in vivo animal models adequately supported with their established multifunctional roles and mechanism of action for the prevention and disease treatment.

Out of several antihyperglycemic agents, Voglibose is a potent α-glucosidase inhibitor used for T2D, has shown anti-diabetic activity [9]. Voglibose acts by inhibiting the alpha-glucosidase enzymes present in the intestines and are involved in carbohydrate digestion. It decreases both postprandial hyperglycemia and hyperinsulinemia, and may improve insulin sensitivity and diminish the stress on pancreatic beta-cells [10]. Hence, the present study was designed to investigate the possible protective effect of probiotics on different doses of voglibose in T2D rat model.

2. MATERIALS & METHODS

The experimental protocol used in the present study was approved by the Institutional Animal Ethical Committee. Sprague Dawley rats weighing about 180–250 g were employed in the present study. They were fed on standard chow diet (Ashirwad Industries, Mohali, India) and water *ad libitum*. They were acclimatized in institutional animal

house and were exposed to normal cycle of day and night.

2.1 Drugs and Chemicals

Streptozotocin was purchased from Sigma-Aldrich Corporation, India. Voglibose was generously obtained as an ex-gratia sample from Zee laboratories Pvt.Ltd. (Paonta Sahib, HP) India. Marketed Probiotic mixture i.e. BIGLAC^{TM} (containing *lactobacilli* and *bifidobacterium*) was purchased from Foregen Healthcare Ltd. Blood glucose, total cholesterol, HDL & triglyceride estimation kits were purchased from Scientific Systems, Mohali. All other chemicals followed by reagents were employed in present study were of analytical grade.

2.2 Experimental Design

HFD was administered for 2 weeks followed by the single dose of STZ (35 mg/kg) was given in the present experimental study. The rats were administered STZ on 14th day of experimental protocol to produced T2DM after HFD for 2 weeks [11]. The selected dose did not cause any mortality. Experimental protocol consists of 6 groups and each group comprised 8 animals. On 42nd

day experiment protocol, animals sacrificed and parameters were carried out.

Group I (Normal Control): Animals were kept for 42 days without any treatment. Body weight, blood glucose and lipid profile levels were assessed on different time intervals, i.e., 0, 14, 21 and 42 days of experiment of protocol.

Group II (Diabetic Control): Animals were fed with HDF (2 weeks) followed by single dose of Streptozotocin (35mg/kg, i.p.).

Group III (Diabetic + Probiotics): On 21st day of protocol, rats were treated with probiotics (75mg/kg, p.o.) for three consecutive days in diabetic rats

Group IV (Diabetic + Probiotics + Voglibose (LD): On 21st day of protocol, rats were treated with probiotics (75mg/kg, p.o.) and low dose (LD) of Voglibose (0.1mg/kg, p.o.) in diabetic rats.

Group V (Diabetic + Probiotics + Voglibose (HD): On 21st day of protocol, rats were treated with probiotics (75mg/kg, p.o.) and high dose (HD) of Voglibose (0.3mg/kg p.o.) in diabetic rats.

Group VI (Diabetic + Voglibose (HD): Rats were treated with Voglibose (0.3 mg/kg, p.o.) on 21st day of protocol in diabetic rats.

Table 1: Diagrammatic Representation of Experimental Protocol

GROUPS	DAY	DAY 14	DAY 21	DAY 42
GROUP 1	NPD	NPD	NPD	SAC
GROUP 2	HFD	STZ	-----	SAC
GROUP 3	HFD	STZ	P	SAC
GROUP 4	HFD	STZ	P + VL	SAC
GROUP 5	HFD	STZ	P + VH	SAC
GROUP 6	HFD	STZ	VH	SAC

NPD- normal pellet diet, HFD- high fat diet,

STZ- streptozotocin, P- probiotics,

VL- voglibose low dose, VH- voglibose high dose,

SAC- sacrificed.

3. DIABETES EVALUATION PARAMETERS

Body Weight

Lipid Profile Estimation

Estimation of total cholesterol

Estimation of Triglyceride

Estimation of blood glucose level

3.1 Statistical Analysis

All data were presented as means ± SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. A p value <0.05 were taken into consideration for determining significance.

4. RESULTS

T2D was achieved by combination HFD which produce insulin resistance (IR) and a low dose of STZ (35mg/kg *i.p.*) single dose that cause the initial β cell dysfunction and hyperglycemia in SD rats. After STZ administration the animals having blood glucose level of greater than 250 mg/dl were considered as diabetic and selected for further pharmacological studies. The animals were allowed to continue to feed HFD until the end of the study. The drugs were administered to diabetic animals by orally and continued for 42 days.

4.1 Effect of Voglibose and Probiotics on body weight of HFD-T2D rats

Changes in body weight (BW) of animals are shown in Fig 1. Administration of HFD followed by STZ treatment significantly increased the body weight of rats as compared to normal rats. However, treated with combination of Voglibose and Probiotics significantly decreased the body weight dose dependently when compared with HFD diabetic rats (Fig.1).

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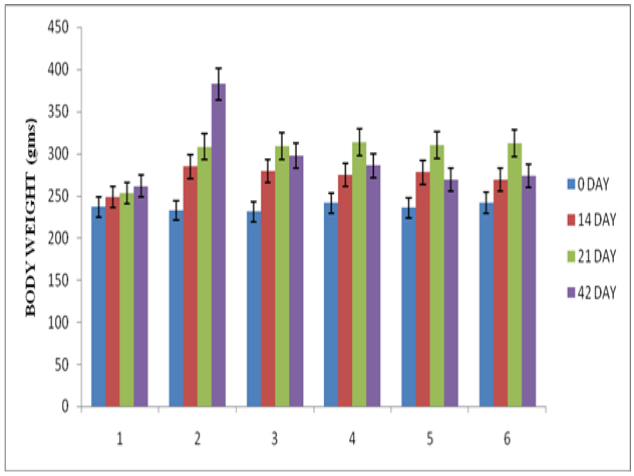


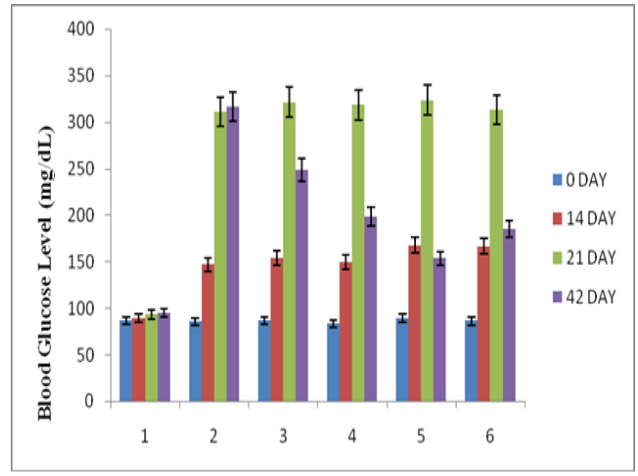


Figure: 2 Effect of Voglibose and Probiotics on blood glucose level of HFD-T2D rats.

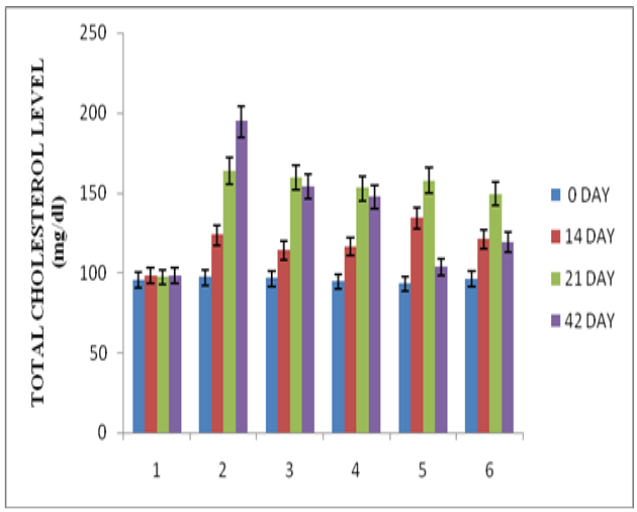
4.2 Effect of Voglibose and Probiotics on blood glucose level of HFD-T2D rats.

Administration of HFD-STZ significantly ($p<0.05$*, $p<0.01$** & $p<0.001$***) increased the blood glucose level of animals as compared to normal control. Treatment of diabetic animals with oral dose of Voglibose (0.3mg/kg), significantly $p<0.001$** decrease the blood glucose level as compared to normal animals. However, combined therapy of Voglibose (0.3mg/kg) and Probiotics (75mg/kg), showed significantly $p<0.05$* decreased the blood glucose level as compared to diabetic treated with Voglibose (0.3mg/kg) (Fig. 2).

4.3 Effect of Voglibose and Probiotics on total cholesterol level of HFD-T2D rats.

Administration of HFD-STZ significantly ($p<0.01$** & $p<0.001$***) increased the total cholesterol levelofdiabetic animals as compared to normal animals. Normal control animals fed on normal pellet diet in comparison to diabetic control animals fed (HFD + STZ 35mg/kg). Treatment with combined therapy of Voglibose (0.1mg/kg) and Probiotics (75mg/kg), significantly $p<0.05$* decreased the total cholesterol levelin comparison to diabetic control animals. Moreover, the treatment with Voglibose (0.3mg/kg) significantly $p<0.01$** decreased the total cholesterol levelas compared to

diabetic animals. However, combined therapy of Voglibose (0.3mg/kg) and Probiotics (75mg/kg) significantly p<0.001*** decreased the Total cholesterol levelas compared with diabetic control (Fig.3).



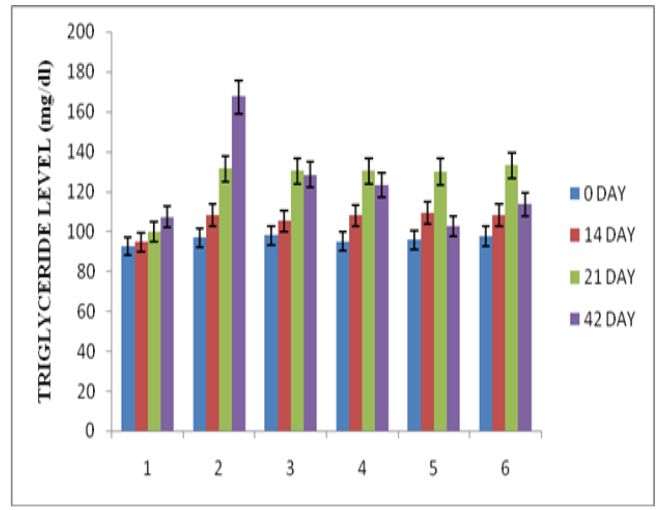
1. Normal Control
2. Diabetic Control
4. Diabetic + Probiotics (75mg/kg)
4. Diabetic + Probiotics (75 mg/kg) + Voglibose (0.1mg/kg)
5. Diabetic + Probiotics (75 mg/kg) + Voglibose (0.3mg/kg)
6. Diabetic + Voglibose (0.3mg/kg)

Values are expressed as mean ± SEM, n=8.*p<0.05, **p<0.01 & ***p<0.001. statistics coded at 21st & 42nd day of experimental protocol of all group, a*** vs normal control, b* vs diabetic control, c** vs diabetic + probiotic + voglibose (0.1mg), d*** vs diabetic + probiotic + voglibose (0.3mg)

Figure 3: Effect of Voglibose and Probiotics on total cholesterol level of HFD-T2D rats.

4.4 Effect of Voglibose and Probiotics on Triglyceride level of HFD-T2D rats.

Administration of HFD significantly (p<0.05* & p<0.001***) increased the triglyceride leveldiabetic animals as compared to normal pellet diet fed animals. Treatment of diabetic animals with oral dose of Probiotics (75mg/kg) significantly p<0.05* decreased the triglyceride levelas compared to diabetic control. (Fig. 4)



1. Normal Control
2. Diabetic Control
4. Diabetic + Probiotics (75mg/kg)
4. Diabetic + Probiotics (75 mg/kg) + Voglibose (0.1mg/kg)
5. Diabetic + Probiotics (75 mg/kg) + Voglibose (0.3mg/kg)
6. Diabetic + Voglibose (0.3mg/kg)

Values are expressed as mean ± SEM, n=8.*p<0.05, **p<0.01 & ***p<0.001. statistics coded at 21st & 42nd day of experimental protocol of all group, a*** vs normal control, b* vs diabetic control, c** vs diabetic + probiotic + voglibose (0.1mg), d*** vs diabetic + probiotic + voglibose (0.3mg)

Figure 4: Effect of Voglibose and Probiotics on Triglyceride level of HFD-T2D rats.

4.5 Effect of Voglibose and Probiotics on HDL level of HFD-T2D rats.

Administration of HFD significantly (p<0.01** & p<0.001***) decreased the HDL levelof diabetic animals as compared to normal animals. Treatment of diabetic animals with oral dose of Voglibose (0.3mg/kg) significantly p<0.01** increased the HDL levelin comparison to diabetic control animals. However, combined therapy of Voglibose (0.3mg/kg) and Probiotics (75mg/kg) significantly p<0.001*** increased

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the HDL levelin comparison to diabetic control animals (Fig. 5).

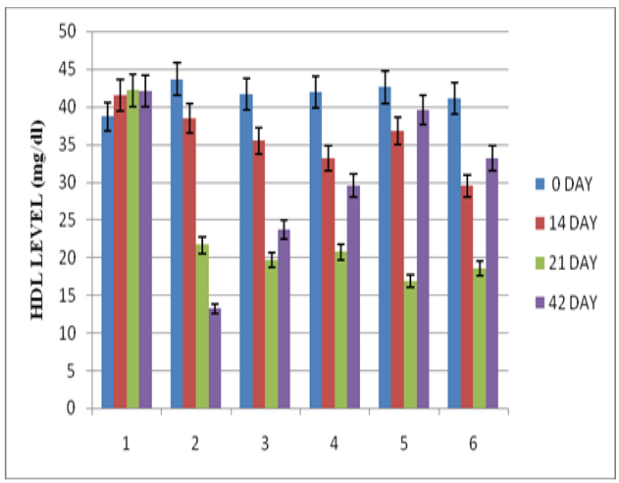


Figure 5: Effect of Voglibose and Probiotics on HDL level of HFD-T2D rats.

4.6 Effect of Voglibose and Probiotics on LDL level of HFD-T2D rats.

Administration of HFD significantly (p<0.05* & p<0.01**) increased the LDL level in diabetic animals as compared to normal animals. Treatment of diabetic animals with oral dose of Voglibose (0.3mg/kg) significantly p<0.01** decreased the LDL level as compared to diabetic control animals. However, combined therapy of Voglibose (0.3mg/kg) and Probiotics (75mg/kg), significantly p<0.001*** decreased in the LDL level in comparison to diabetic animals (Fig. 6)

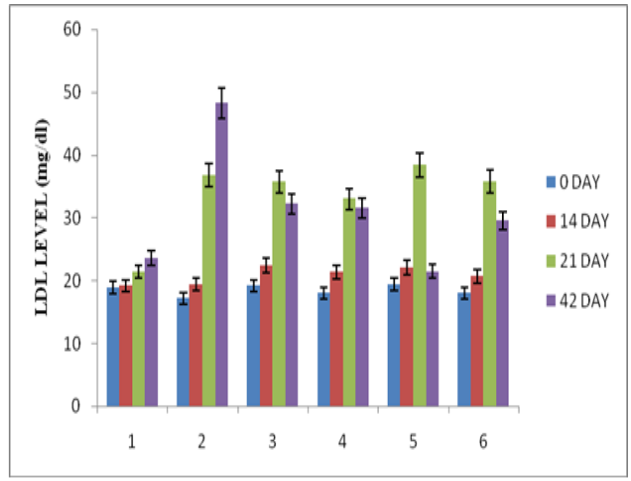
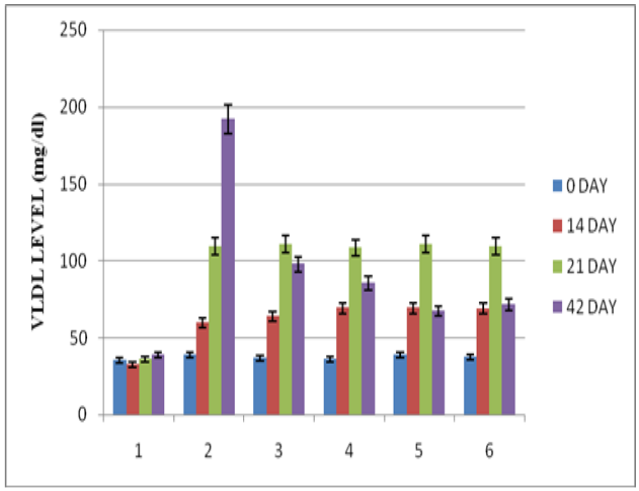


Figure 6: Effect of Voglibose and Probiotics on LDL level of HFD-T2D rats.

4.7 Effect of Voglibose and Probiotics on VLDL level of HFD-T2D rats.

Administration of HFD significantly (p<0.05* & p<0.01**) increased the VLDL level in diabetic animals as compared to normal animals. Treatment of diabetic animals with oral dose of Voglibose (0.3mg/kg) significantly p<0.01** decreased the VLDL level as compared to diabetic control animals. However, combined therapy of Voglibose (0.3mg/kg) and Probiotics (75mg/kg), significantly p<0.001*** decreased in the VLDL level in comparison to diabetic animals (Fig. 7)



1. Normal Control
2. Diabetic Control
4. Diabetic + Probiotics (75mg/kg)
4. Diabetic + Probiotics (75 mg/kg) + Voglibose (0.1mg/kg)
5. Diabetic + Probiotics (75 mg/kg) + Voglibose (0.3mg/kg)
6. Diabetic + Voglibose (0.3mg/kg)

Values are expressed as mean ± SEM, n=8.*p<0.05, **p<0.01 & ***p<0.001. statistics coded at 21st & 42nd day of experimental protocol of all group, a*** vs normal control, b* vs diabetic control, c** vs diabetic + probiotic + voglibose (0.1mg), d*** vs diabetic + probiotic + voglibose (0.3mg)

Figure 7: Effect of Voglibose and Probiotics on VLDL level of HFD-T2D rats.

5. DISCUSSION

DM is regarded as second largest disorder associated with significant morbidity and mortality associated with its micro-vascular and macro-vascular complications [12]. In our study, we have demonstrated the effect of High fat diet followed by STZ-induced T2D. HFD/STZ rat model explained either the early or the latent stage of Type 2 diabetes mellitus (T2DM) [12]. This contention is supported by results that rats having blood glucose level more than 250 mg/dl were considered diabetic and selected for further studies. In the present study HFD and STZ caused severe diabetic changes which were observed by increased body weight, high blood sugar level and altered lipid content. HFD is also associated with the development of obesity and that there is a direct relationship between the amount of dietary fat and the degree of obesity [13-14]. Obesity is another reason responsible for the pathogenesis of T2DM. This is supported by increase in total cholesterol and LDL/VLDL levels with marked decrease in the level of HDL. The pharmacological interventions employed in the study showed significant decreased in the level of blood glucose level and cholesterol biomarkers. Voglibose used in the present study is potent and tolerant α glycosisade inhibitor (α-GI) as compared to Acarbose and Miglitol thatselectively inhibits the alpha glucosidase enzyme activity in the gut [9]. Moreover, the probiotics may convert large polysaccharide into smaller monosaccharide by increasing the digestion process and maintain the glucose level. Therefore, present study shown protective effect on T2D may be due to the synergistic effect of probiotics on Voglibose. Our results too supported that diabetic rats when treated with oral dose of Voglibose (HD) and probiotics has significantly reduced body weight as compared to normal control. Similarly, there was decreased in blood glucose level on treatment with Voglibose (HD) and probiotics. Furthermore, Probiotics and Voglibose administration has significantly decreased the total cholesterol, triglyceride level of diabetic rats when administered with Probiotics. However, combined therapy of Voglibose (HD) and probiotics has significantly increased the HDL level in comparison to normal animals.

Furthermore, oral dose of Voglibose (HD) significantly decreased the LDL & VLDL in diabetic rats.

6. CONCLUSION

The findings of present study revealed that the combine therapy of probiotics with different doses of Voglibose showed the additive effect in T2DM. T2DM was produced in rats by administered HFD for 2 weeks and followed by single dose of STZ on 14th day of protocol [11]. Rats having blood glucose level more than 250 mg/dl were considered diabetic and selected for further studies. In the present study HFD and STZ caused severe diabetic changes which were observed by increased body weight, high blood sugar level and altered lipid content[12]. The treatment of diabetic rats with oral dose of Voglibose (HD) and probiotics has restored the loss of body weight. Similarly, Voglibose dose dependently with probiotics showed the antidiabetic effects. Furthermore, Probiotics and Voglibose produced protective potentials on lipid profile. However, combined therapy of Voglibose (0.3 mg/kg) and fix dose Probiotics has increased the HDL level. Hence, from the results obtained from the study we concluded that Voglibose when administered with Probiotic showed the protective and additive potential in attenuating T2DM may be due anti-inflammatory effects on gut flora.

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CONFLICT OF INTEREST

The authors has no conflicts of interests

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