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Functional properties of glycoside synthesized using polyphenolic extract of *Moringa oleifera* catalyzed through transglycosylation reaction

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ABSTRACT
The present study was designed to investigate gallic acid-4-O-β-glucopyranoside (GAGP) in normal and alloxan-induced diabetic rats by a single dose administration of alloxan (150 mg/kg, i.p.). Blood glucose levels measured were significantly ($p < 0.05$) increasing in all diabetic rats as compared with normal control rats. Three different dosages of GAGP (100, 200, and 400 mg/kg, body weight) showed significantly decreasing ($p < 0.05$) blood glucose levels of the diabetic rats (<3%) as compared with untreated diabetic group (>66%). All the hyperglycemic groups which were treated with various dosages of GAGP recorded lower biochemical parameters of blood serum as compared with untreated diabetic group. A significant reduction in catalase activity (<40%–60%) of the diabetic groups was observed as compared with control group, while the diabetic groups showed elevation as compared with untreated diabetic group and catalase activity was quite near to normal rats. Glutathione levels of the diabetic groups were reduced (<2%–3%) but diabetic treated groups with different dosage showed improvement near to normal rats. In diabetic rat tissue, significant elevation (>45%) in lipid peroxidation was observed as compared with normal rats. In contrast, treatment with GAGP restores back near towards normal level (>5%–15%).

INTRODUCTION

Moringa oleifera Lam. Syn. (Moringaceae) is well known for their pharmacological actions and is used for the traditional treatment of diabetes mellitus (Babu and Chaudhuri, 2005). Its leaves, fruits, and stem bark have been scientifically examined for their use in hypercholesterolemia (Ghasi *et al*., 2000; Mehta *et al*., 2003). Fruits and stem bark have been reported to have anti-diabetic action (Kar *et al*., 2003). It was found to contain many essential nutrients, for instance, vitamins, minerals, amino acids, beta-carotene, antioxidants, and anti-inflammatory nutrients (Kasolo *et al*., 2010). Due to the presence of several sorts of phenolics compound, it was able to extend the period of food containing fats regarding with its antioxidant activity (Dillard and German, 2000; Siddhuraju and Becker, 2003). However, there are no reports on hypoglycemic and anti-diabetic actions of its leaves.

Nevertheless, polyphenols are of restricted use because they are easily spoiled, degraded in an aqueous solution following-on in rapid browning. Nevertheless, although the constituents might have such beneficial properties, however, their use is restricted due to their low stability and solubility and they are easily degraded in an aqueous solution following in rapid browning reaction (Nayak *et al*., 2015). For that reason, their antioxidant properties were influenced or reduced. It has been found that a number of physical properties of the polyphenols were enhanced through enzymatic transglycosylation reaction (Sulistyo *et al*., 2001; 2014). Glycosylation of polyphenols into glycosides has a number of advantages in contrast to chemical synthesis, for example, low-cost production and its enzyme, which is derived from microbial culture as a source of enzyme with transglycosylation capacity, can also be produced for the purpose of synthesis of several bioactive compounds due to its easiness and simplicity procedure.

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On the other side, the chemical synthesis of glycosides is not only complex and complicated but also expensive. Moreover, it is not as simple as enzymatic reaction since it generates a complex mixture of products with α- and β-configuration. Enzymatic transglycosylation permits insoluble and less stable organic bioactive compounds to be changed into the resultant soluble and more stable compounds during suitable single-step glycosylation (Shimoda *et al*., 2006). The clinical importance of polyphenol glycosides is one of the pharmacological interests (Satoh *et al*., 2001).

The aim of the study was to investigate a purified and identified gallic acid-4-O-β-D-glucopyranoside (GAGP) as enzymatically synthesized polyphenol glycoside that had been obtained by application of Cyclodextrin glucano transferase (CGTase) derived from *Trichoderma viride* in the presence of polyphenolic extract of *M. oleifera* leaves, dealing with its capability as antioxidative, hypolipidemic agent, and antidiabetic agent using animal model experiment.

MATERIALS AND METHODS

Extraction of polyphenols

Extraction of polyphenolic compounds was done by following the method of Charoensin (2014) with slight modification. The leaves of *M. oleifera* (1.0 kg dried base weight) which had been purchased from a local market were dried in hot air oven at 50°C for 72 hours and ground to powder (approximately 600 g) and preserved overnight at 4°C until extraction. Fifteen grams of the powder was extracted with 350 ml of methanol and the liquid extract was filtered through Whatman No. 1 filter paper. Solvent was removed by using rotary evaporator and the extract was freeze-dried to obtain crude polyphenol (approximately 30 mg).

Isolation and purification of synthesized glycosides

A reaction mixture (200 ml) containing prepared polyphenolic constituents of *M. oleifera* used as substrate-acceptor (2.5%, w/v) and a commercial Nona wheat flour (5.0%, w/v) as substrate-donor were incubated with *T. viride* CGTase at 40°C for 24 hours. The reaction mixture was extracted with diethyl ether to remove residual unreacted polyphenolic constituents and separated. The aqueous phase was concentrated and the crude mixture was then subjected to column chromatography for separation which was eluted with a gradient solvent of methanol in 1% formic acid (v/v). Fraction solutions resulted by flushing the column that is exhibited single spots on thin layer chromatography plate where their retention factor values were parallel to the spot of arbutin as an authentic reference of commercial polyphenol glycoside were then collected and concentrated (Hashmi *et al*., 2014).

HPLC analysis and structure identification by NMR spectroscopy

The purified transfer products (TPs) were also analyzed by using high performance liquid chromatography (HPLC). The HPLC/ Ultra violet-visible (UV-VIS) system was comprised of Agilent HPLC system provided with a pump, an automatic injector, an UV-VIS detector, and a degasser. Separations were carried out using Apollo C18 reverse-phase column at room temperature. Acetonitrile (A) and 0.1% aqueous H_3PO_4 (B) were used as a mobile phase with a gradient elution of 24% (A) at 0–12 minutes, 24–50% (A) at 12–22 minutes, 50%–24% (A) at 22–40 minutes, and 24% (A) at 40–50 minutes. The separation was monitored through absorbance at 254 nm at a flow rate of 0.5 ml/minute (Chiang *et al*., 2012). The ^{1}H-nuclear magnetic resonance (NMR), ^{13}C-NMR spectra were measured using a Varian XL-400 spectrometer in dimethyl sulfoxide (DMSO) solution. The purified transfer products were furthermore identified by using proton and carbon-13 nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR) spectroscopy using a Varian XL-400 spectrometer in DMSO solution (Shimoda and Hamada, 2010)

Animal Model Experiment

Male Sprague-Dawley rats weighing about 100–200 g, obtained from Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, were used. The animals were housed in colony plastic cages at an ambient temperature of 25°C–27°C under a 12 hour light/dark cycle and free admittance to standard rat diet and tap water. The rats were allowed to adapt to the laboratory environment for 1 week before starting the experiment. All the experimental procedures were performed according to the ethical guidelines for the use and care of laboratory animals.

Induction of diabetes

Diabetes mellitus was induced by a single intraperitoneal injection of alloxan monohydrate freshly prepared at a dose of 150 mg/kg, body weight (BW). It was observed that 4 hours after the alloxan injection, tap water was changed with a 5% glucose solution for 24 hours (Prince *et al*., 2004). Also, 1 week after the alloxan injection, the blood glucose level of the overnight fasted animals was checked by the use of glucometer. The animals that displayed glucose levels higher than 200 mg/dl were considered as diabetic rats and furthermore, they were included in the study (Manickam and Periyasamy, 2013).

Assessment of catalase activity

Catalase activity was determined by following the method of Pari and Latha (2004). The percent catalase inhibition was determined by monitoring decrease in absorbance at 620 nm. The pancreas was homogenized and centrifuged at 5,000 rpm in 0.01 M phosphate buffer (pH 7.0). The reaction mixture comprised of 0.4 ml of H_2O_2 (0.2 M), 1 ml of 0.01 M phosphate buffer having pH 7.0, and 0.1 ml of pancreas homogenate (10% w/v). The reaction was stopped by the addition of 2 ml reagent (5% $K_2Cr_2O_7$ prepared in glacial acetic acid). The changes in the absorbance were observed at 620 nm. Percent inhibition was analyzed as follows; % catalase inhibition = [(normal activity − inhibited activity)/(normal activity)] × 100.

Assessment of reduced glutathione (GSH) activity

Modified method of Oyedemi *et al*. (2010) was used to determine the reduced GSH. 1.0 ml supernatant of pancreas homogenate was treated with 0.5 ml of Ellman's reagent (19.8 mg of 5,5'-dithiobisnitro benzoic acid in 100 ml of 0.1% sodium nitrate) and 3 ml of phosphate buffer (0.2 M, pH 8.0). The absorbance was recorded at 412 nm and percent inhibition of reduced GSH was calculated as % GSH inhibition = $(A_0 - A_1)/A_0 \times 100$, where

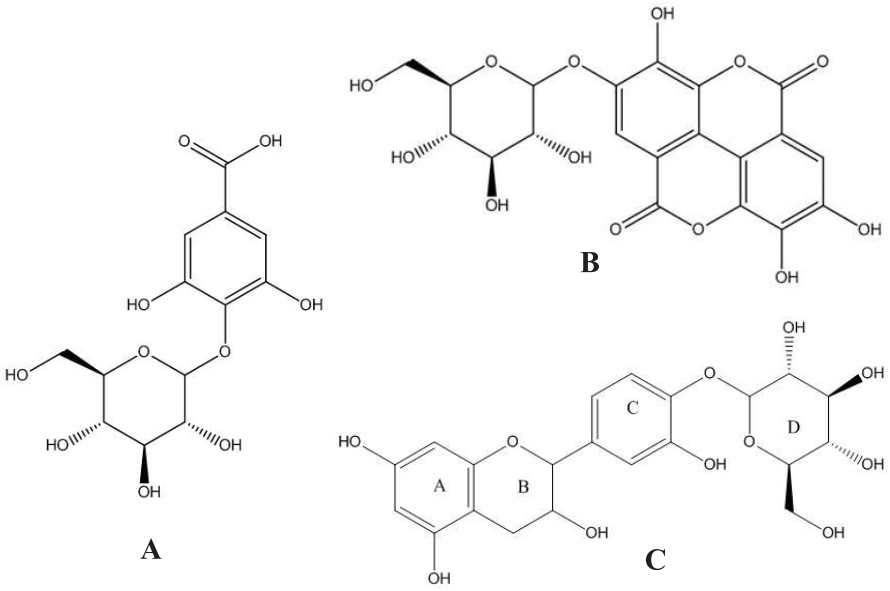

Figure 1. Chemical structure of GAGP (A); EAGP (B); and catechin-4'-O-β-D-glucopyranoside (C).

A_0 is the absorbance of the control and A_1 is the absorbance of the sample extract.

Assessment of lipid peroxidation (LPO)

LPO was determined colorimetrically by thiobarbituric acid (TBA) reactive substances. Briefly, 0.1 ml of pancreas homogenate (10% w/v) was treated with 2 ml of (1:1:1, v/v/v) TBA-trichloroacetic acid (TCA)-HCl reagent (0.37% TBA, 15% TCA, and 0.25 N HCl). All the tubes were kept in a water bath at 100°C for half an hour and cooled. The amount of malondialdehyde (MDA) produced in all samples was calculated by evaluating the absorbance of the supernatant at 535 nm against blank (Oyedemi *et al*., 2010). Percent inhibition was computed using the following equation; % Inhibition = $(A_0 - A_1)/A_0 \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the sample extract.

Determination of biochemical parameters

Biochemical parameters including total cholesterol, triglycerides, low-density lipoprotein, high-density lipoprotein, creatinine, urea, and alkaline phosphatase levels in blood plasma were measured using special kits (Abbott Laboratories, USA) which utilized the colorimetric method in an auto-analyzer (Cherbal *et al*., 2017). Twenty-five rats were randomly divided into five groups (five rats in each group); Group-1, normal untreated rats were given distilled water; Group-2, diabetic untreated rats were given distilled water; Group-3, diabetic rats were given GAGP at a dose of 100 mg/kg, BW; Group-4, diabetic rats were given GAGP at a dose of 200 mg/kg, BW; and Group-5, diabetic rats were given GAGP at a dose of 400 mg/kg, BW. Distilled water and drug preparations were served orally by gastric intubation to the rats of respective groups using force feeding needle, once daily for 4 weeks. All five groups of rats were sacrificed on the last day of treatment after overnight fasting and the blood sample of each animal was collected from cardiac punctures after cervical dislocation before their heartbeats stop. The blood glucose levels were determined at different day intervals by using glucometer and blood was collected from the tail vein. The blood samples were collected in separated BD Vacutainer® Blood Collection Tubes. The blood samples were centrifuged at 1,375 × g for 20 minutes and the serum was separated.

RESULTS AND DISCUSSION

Purification and structural identification of transglycosylation products

Enzyme-catalyzed synthesis of polyphenolic glycosides, as transglycosylation products of crude *M. oleifera* leaves extract, were purified using octa dodecyl sulphate column chromatography followed by structural identification identified on the basis of spectroscopic techniques. According to the analysis using ^{13}C-NMR and ^{1}H-NMR spectra, the isolated compounds were identified as GAGP, ellagic acid-4-O-β-glucopyranoside (EAGP), and catechin-4'-O-glucopyranoside (CGP), respectively (Fig. 1). These chemical structures corresponded to the composition of *M. oleifera* leaves extract which contains approximately 10%–11% gallic acid, 5%–6% ellagic acid, and 2%–3% catechin (Nazir *et al*., 2018). The ^{1} hydrogen (proton) NMR (HNMR) and ^{13} Carbon NMR (CNMR) spectrum of compound GAGP revealed the presence of glycosidic bond in gallic acid glucopyranoside. The broad singlet at δ_H 7.03 corresponds to the two protons of the aromatic ring. The ^{13}CNMR showed one signal resonating at δ_c 114.96, which could be attributed to C-4. The shifting of this signal to the higher field confirms the glycosidic linkage at C-4. The presence of a signal at δ_c 123.6 corresponds to C-2 and C-6 while the signal at 138.9 corresponds to C-3 and C-5 reveals that the aromatic ring of GAGP has a plane of symmetry. More signals are provided in Table 1. GAGP: infrared in K (potassium) bromide powder (ν_{max}/cm^{-1}): ^{1}H-NMR (500 MHz; CD_3OD-d_4/ppm) δ: 7.03 (1H, bs, H-3), 7.03 (1H, bs, H-7), 4.35 (1H, d, J 7.8 Hz, H-1′), 4.26 (1H, dd, *J* 8.3, 7.8 Hz, H-2′), 3.26 (1H, dd, *J* 9.3, 8.3 Hz, H-3′), 4.33 (1H, dd, *J*

Table 1. ^{1}H-NMR and ^{13}C-NMR chemical shift of synthesized transglycosylation products.

	TP-1		TP-2		TP-3	
C	δ^{1}H, *J* (Hz)	δ^{13}C	δ^{1}H, *J* (Hz)	δ^{13}C	δ^{1}H, *J* (Hz)	δ^{13}C
1				126.4	6.84 (d, *J* = 1.93)	130.2
2	7.03 (bs)	123.6		140.6		108.6
3		138.9		132.7		144.8
4		144.9	7.80 (s)	146.2	7.24 (d, *J* = 8.43)	147.96
5		138.9		101.6		116.9
6	7.03 (bs)	123.6		120.2	7.01 (dd, *J* = 2.54, 8.69)	119.9
7				161.2		
1’	4.35 (d, 7.8)	101.9		111.4	4.60 (d, *J* = 1.93)	82.4
2’	4.26 (dd, 8.3, 7.8)	73.8		158.7	3.99 (m)	67.7
3’	3.26 (dd, 9.3, 8.3)	78.4		160.9	2.52–2.94 (m)	29.6
4’	4.33 (dd, 9.3, 9.3)	71.1	6.39 (s)	140.4		156.4
5’	3.37 (m)	79.9		120.5	5.84 (d, *J* = 2.06)	96.5
6’				108.5		157.5
7’				161.2	5.93 (d, *J* = 2.06)	95.9
1”			4.99 (d, 7.1)	101.7	4.05 (m)	100.7
2”			4.08 (m)	74.9	3.48 (m)	73.9
3”			4.30 (m)	77.4	3.38 (m)	80.4
4”			4.19 (m)	70.1	4.13 (m)	71.3
5”			4.06 (s)	78.2	3.40 (s)	78.9
6”	4.06 (dd, 11.3, 5.6)	63.9	4.18 (m)	63.2	3.68 (m)	64.2
6”	4.37 (bd, 11.3)		4.33 (m)		3.89 (m)	

9.3, 9.3 Hz, H-4’), 3.37 (1H, m, H-5′), 4.06 (1H, dd, *J* 11.3, 5.6 Hz, H-6′α), 4.37 (1H, bd, *J* 11.3 Hz, H-6′β). ^{13}C-NMR spectrum (125 MHz; DMSO-d_6/ppm): 166.2 (C-1), 123.6 (C-2), 108.8 (C-3), 144.96 (C-4), 138.9 (C-5), 144.9 (C-6), 108.8 (C-7), 101.9 (C-1′), 73.8 (C-2′), 78.4 (C-3′), 71.1 (C-4′), 79.9 (C-5′), 63.9 (C-6′) (Fig. 1A). Functional properties of GAGP were determined through *in vivo* and *in vitro* experiments, i.e. antioxidative, hypolipidemic and antidiabetic agents.

The ^{1}HNMR and ^{13}CNMR spectrum of EAGP revealed the presence of glycosidic bond in EAGP. The broad singlet at δ_H 7.03 corresponds to the two protons of the aromatic ring. The ^{13}CNMR showed one signal resonating at δ_c 114.96, which could be attributed to C-4. The shifting of this signal to the higher field confirms the glycosidic linkage at C-4. The presence of a signal at *δc* 123.6 corresponds to C-2 and C-6 while the signal at 138.9 corresponds to C-3 and C-5 reveals that aromatic ring of EAGP has a plane of symmetry. More signals are provided in Table 1. EAGP: ^{1}H NMR spectrum (500 MHz; DMSO-d_6/ppm): *δ* 7.80 (1H, *s*, H-5), *δ* 6.39 (1H, *s*, H-5′), *δ* 6.05 (1H, *m*, H-1″), *δ* 4.08 (1H, *m*, H-2″), *δ* 4.30 (1H, *m*, H-3″), *δ* 4.19 (1H, *m*, H-4″), *δ* 4.06 (1H, *m*, H-5″), *δ* 4.18 (1H, *m* H-6″$_a$), *δ* 4.33 (1H, *m* H-6″$_b$). ^{13}C NMR spectrum (125 MHz; DMSO-d_6/ppm): *δ* 126.4 (C-1), 140.6 (C-2),132.7 (C-3), 140.2 (C-4), 101.6 (C-5), 120.2 (C-6), 161.2 (C-7), 111.4 (C-1′), 158.7 (C-2′), 160.9 (C-3′), 146.4 (C-4′),120.5 (C-5′), 108.5 (C-6′), 165.0 (C-7′), 101.7 (C-1″), 74.9 (C-2″), 77.4 (C-3″), 70.1 (C-4″), 78.2 (C-5″), 63.2 (C-6″) (Fig. 1B).

The ^{1}H NMR spectrum of CGP revealed the presence of double doublet of H-6 at δ_H 7.01 of aromatic ring C whereas two doublets at δ_H 6.84 and 7.24 having a coupling constant of 1.93 and 8.43 could be attributed to H-2 and H-5, respectively. The ^{13}CNMR spectrum showed expected signals, which corresponds to all carbon present in the structure. The signal at δ_c 147.9 was at higher filed assigned to a C-4 carbon atom of the aromatic ring, confirming the glycosidic linkage. Another signal at δ_c 144.8 was attributed to C-3 as this carbon of aromatic ring C is attached to a hydroxyl group. The ^{1}H NMR spectrum of compound CGP revealed the presence of two characteristic singlets of H-5 at δ_H 7.80 and H-5’ at δ_H 6.39. The doublet at δ_H 4.99 having the coupling constant (*J* = 7.1 Hz) was assigned to H-1″ which confirms the presence of sugar moiety as β–D-glucose. The ^{13}CNMR spectrum showed expected signals, which corresponds to all carbon present in the structure. The signal at *δ*c 146.2 could be assigned to C-4. This higher field confirms the position of glycosidic linkage to the aglycone moiety. Catechin-4’-O-β-glucopyranoside: ^{1}H-NMR (500 MHz; CD_3OD-d_4/ppm) *δ*: 7.24 (1H, *d*, H-2), 6.84 (1H, *d*, H-5), 7.01 (1H, *d*, H-6), 4.60 (1H, *d*, H-1′), 3.99 (1H, dd, H-2′), 2.52–2.94 (2H, *m*, H-3′), 5.84 (1H, *d*, H-5′), 5.93 (1H, *d*, H-7′). *δ* 4.05 (1H, *m*, H-1″), *δ* 3.48 (1H, *m*, H-2″), *δ* 3.38 (1H, *m*, H-3″), *δ* 4.13 (1H, *m*, H-4″), *δ* 3.40 (1H, *m*, H-5″), *δ* 3.68 (1H, *m* H-6″$_a$), *δ* 3.89 (1H, *m* H-6″$_b$). ^{13}C NMR spectrum (125 MHz; DMSO-d_6/ppm): 130.2 (C-1), 108.6 (C-2), 144.8 (C-3), 147.96 (C-4), 116.9 (C-5), 119.9 (C-6), 82.4 (C-1′), 67.7 (C-2′), 29.6 (C-3′), 156.4 (C-4′), 96.5 (C-5′), 157.5 (C-6′), 95.9.0 (C-7′), 93.3 (C-8′), 100.7 (C-1″), 73.9 (C-2″), 80.4 (C-3″), 71.3 (C-4″), 78.9 (C-5″), 64.2 (C-6″) (Fig. 1C).

Body weight

As shown in Figure 2, this study demonstrated that all diabetes-induced groups had significantly lower BW as compared with the normal control group after the alloxan injection on day-4. The decrease in BW is reported as a marker for the development of diabetes since the BW in diabetic animals’ decreases due to the impairment in insulin action in transforming glucose into glycogen and catabolism of fats. Beta cell destruction also causes unavailability of lipolysis.

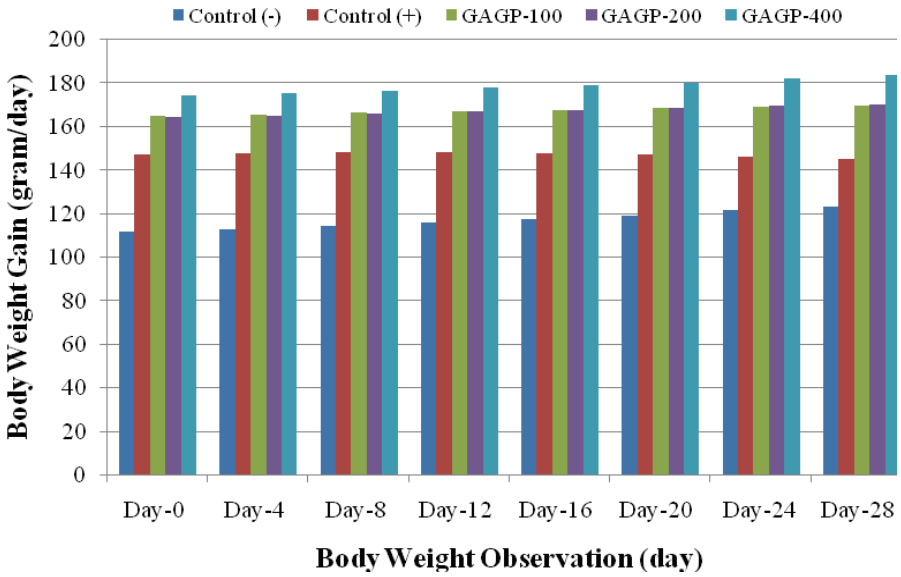

Figure 2. Effect of administration of GAGP on BW gain.

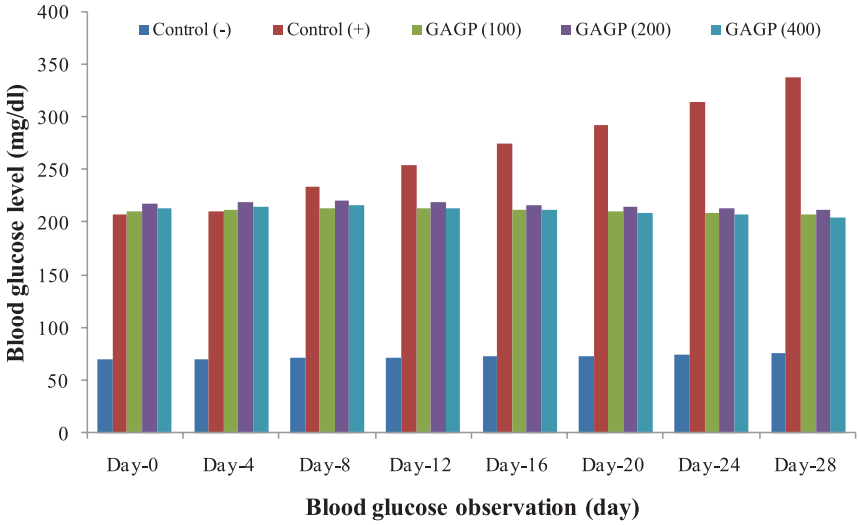

Figure 3. Effect of administration of GAGP on blood glucose level.

Table 2. Effect of administration of GAGP on blood serum profile.

Group	Triglyceride (mmol/l)	Cholesterol (mmol/l)	HDL (mmol/l)	LDL (mmol/l)	Creatinine (µmol/l)	Urea (mmol/l)	Alkaline phosphate (µ/l)
Control (−) rats	1.23 ± 0.06^{a}	1.33 ± 0.07^{a}	0.79 ± 0.03^{b}	0.07 ± 0.01^{a}	21.8 ± 1.66^{a}	4.78 ± 0.17^{a}	341.6 ± 21.20^{a}
Control (+) rats	1.68 ± 0.20^{b}	1.48 ± 0.06^{b}	0.58 ± 0.04^{a}	0.16 ± 0.05^{b}	30.0 ± 1.14^{b}	7.44 ± 0.24^{c}	473.8 ± 58.30^{b}
GAGP (100 mg)	1.57 ± 0.03^{a}	1.47 ± 0.08^{a}	0.69 ± 0.01^{ab}	0.14 ± 0.08^{a}	28.26 ± 1.09^{ab}	6.92 ± 0.15^{bc}	441.8 ± 24.41^{a}
GAGP (200 mg)	1.47 ± 0.08^{a}	1.42 ± 0.05^{a}	0.70 ± 0.04^{ab}	0.14 ± 0.03^{a}	27.36 ± 2.33^{ab}	6.78 ± 0.44^{bc}	439.4 ± 37.26^{a}
GAGP (400 mg)	1.34 ± 0.17^{a}	1.39 ± 0.04^{a}	0.72 ± 0.05^{ab}	0.12 ± 0.02^{a}	26.66 ± 1.47^{ab}	6.32 ± 0.14^{b}	405.7 ± 21.17^{a}

Each value is expressed as mean SD. The values in the column with different letter are significantly different ($p < 0.05$).

However, there was a significant difference ($p < 0.05$) recorded in BW of treated diabetic groups at day-4 as compared with the diabetic control group. The diabetic groups treated with different concentrations of GAGP showed slight increment on BW throughout the experiment. The increase in BW of treated diabetic groups could be due to the preventive action of GAGP on the damage of cells due to severe breakdown of fats and metabolism of protein in the diabetic state.

Blood glucose levels

As shown in Figure 3, diabetic control group rats which were induced with alloxan showed significant difference ($p <$

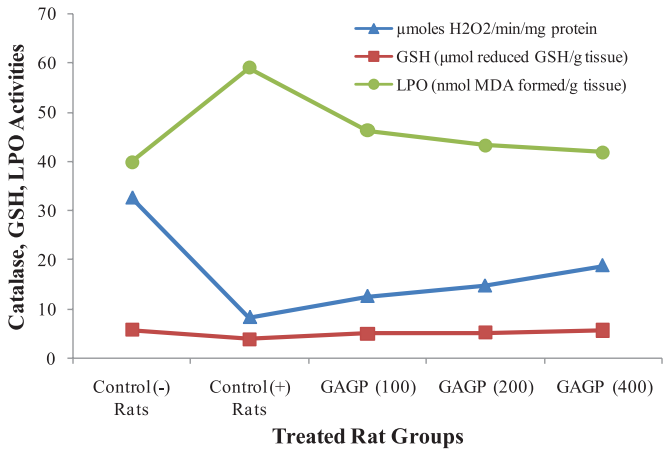

Figure 4. Effect of administration of GAGP on catalase activity, GSH, and LPO.

0.05) in blood glucose levels throughout the study as compared with the normal control group rats probably due to the rapid action of alloxan since it results in rapid depletion of beta cells by DNA alkylation and accumulation of cytotoxic free radicals. In the present study, three different dosages of GAGP at a concentration of 100, 200, and 400 mg/kg of BW showed a significant difference ($p < 0.05$) at day-8 of treatment in blood glucose levels in alloxan-induced diabetic rats compared with diabetic control group. This may be due to the functional properties of GAGP as an antidiabetic agent. However, there is also a significant difference ($p < 0.05$) between the blood glucose levels of the GAGP treated diabetic rats as compared with the diabetic control group from day-12 to end of the study. In contrast, blood glucose levels of untreated diabetic rats remained elevated throughout the experiment.

Blood serum profile

As shown in Table 2, alloxan treatment will increase the chemical composition levels of serum such as alkaline phosphatase, urea, creatinine, low-density lipoprotein (LDL), cholesterol and decrease the high-density lipoprotein (HDL) level. But, treatment with the GAGP reversed the alloxan-induced changes. Serum of triglyceride, cholesterol, alkaline phosphatase, urea, HDL, LDL, and creatinine levels were significantly decreased ($p < 0.05$) as compared with the diabetic control group. Treatment with the GAGP improved the diabetes mellitus conditions as indicated by the parameters of serum profile.

Catalase activity

Catalase is one of an important enzyme in an antioxidant system that plays crucial scavenger enzyme which can be found in peroxisomes. Furthermore, catalase is a type of hemoprotein, which breaks down the harmful H_2O_2 into O_2 and H_2O, which guards the tissue againt highly reactive hydroxyl radicals. In this investigation, catalase levels were found to decline as compared with the diabetic control group, this might be due to inhibition of the enzyme. In contrast, treatment with GAGP demonstrated improved enzymatic activity towards the normal in various dosages. Therefore, the result showed that GAGP has the ability to counter back the oxidative damage in alloxan-induced hyperglycemic rats with an increase in enzymatic activity; additionally, there is a significant difference ($p < 0.05$) present among all the groups as compared with normal control as shown in Figure 4.

Reduced GSH

Reduced GSH or commonly known as GSH is the smallest intracellular molecule with low molecular thiol antioxidant that is ubiquitous in a living environment. GSH occurs naturally in all type of cells and plays an important role in cell protection and acts as a crucial defense against free radicals. In this research work, the diabetic control group showed significantly reduced GSH level in pancreatic tissue compared with normal control group. On the other hand, administration of GAGP at various dosages to alloxan-induced diabetic rats showed significant improvement in GSH levels near to normal as compared with normal control and diabetic control; although there is a significant difference ($p < 0.05$) among the groups as presented in Figure 4.

Lipid peroxidation (LPO)

LPO can be defined as the oxidative deterioration of unsaturated fatty acids. LPO produces numerous products, which some of them are electrophiles (Oyedemi *et al*., 2010). One of the LPO substances which is commonly used as a biomarker for tissue damage is the reactive aldehyde, MDA. MDA is a by-product that generates naturally during the process of LPO. As shown in Figure 4, there was a significant difference ($p < 0.05$) between the diabetic groups as compared with the normal control group. Tissue LPO in diabetic groups was increased as compared with control group because alloxan induces a high level of MDA in diabetic animals which might be due to high glucose stress in diabetes which promoted the production of reactive oxygen species that reacts with polyunsaturated fatty acids inside the cell membrane leading to increase in MDA level. In this study, significantly reduced LPO was recorded in the groups administered with the different dosages of GAGP that almost attained to normal level as compared with the untreated diabetic group.

CONCLUSION

Fungal culture *T. viride* has shown transglycosylation reaction through the activity of CGTase on *M. oleifera* polyphenol constituents to synthesize polyphenol glycosides. Analysis of ^{1}H and ^{13}C NMR of the isolated polyphenol glycosides established the chemical structures of GAGP, EAGP, and CGP. The GAGP was furthermore assayed for its anti-diabetic and antioxidant potential activities. The results show that *in vivo* treatment with GAGP reversed alloxan-induced diabetes. Serum triglyceride, cholesterol, alkaline phosphatase, urea, HDL, LDL, and creatinine levels were significantly decreased ($p < 0.05$) as compared with the diabetic control group. Additionally, GAGP showed significant improvement in GSH levels near to normal and significantly reduced LPO that is almost attained to normal level as compared with the untreated diabetic group.

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CONFLICT OF INTEREST

Nil.

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