

Characterization of Flavonoids and Saponins from *Gleditsia triacanthos* by LC-ESI/MS/MS Analysis: Pharmacological Assessment of the Anti-hyperglycemic and Anti-ulcerogenic Activities of *G. triacanthos* methanolic Fruit Extract and its *n*-Butanol Fraction

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ABSTRACT

Introduction: *Gleditsia triacanthos* is known to possess various pharmacological activities.

Objective: The composition of *n*-butanol fraction of *Gleditsia triacanthos* methanolic seedless fruit extract was identified from the LC-ESI/MS/MS spectra. Total methanolic extract of the seedless *G. triacanthos* fruits (MEGT) and its *n*-butanol fraction (BFGT) at three dose levels 70, 140 and 280 mg/kg were studied for their anti-diabetic and anti-ulcerogenic effects. **Materials and Methods:** The anti-diabetics properties of MEGT and BFGT were orally assessed in streptozotocin (STZ; 55 mg/kg; i.p.)-induced hyperglycemic rats. Their anti-ulcerogenic activities were also evaluated in ethanol-induced peptic ulcer in rats. **Results:** Two phenolic acids, five flavonoids as well as four saponins were identified from BFGT. Both MEGT and BFGT showed high potential in decreasing the elevated serum glucose, total triglycerides and total cholesterol levels in rats, dose dependently, comparable with the anti-diabetic reference drug; gliclazide (Glz; 10 mg/kg; p.o.). They also showed an elevation insulin and α -amylase serum levels. On the other hand, MEGT and BFGT showed significant ulceroprotective activities through decreasing both number and severity of ethanol-induced gastric lesions in rats, dose dependently, comparable with the anti-ulcer reference drug; ranitidine (20 mg/kg; p.o.) with MEGT at 280 mg/kg showing highest activity. **Conclusion:** From all the previous results, it can be concluded that the observed pharmacological properties are attributed to the augmented activities of the saponin and flavonoidal content of *G. triacanthos* fruits.

Key words: Anti-diabetic activity, *Gleditsia triacanthos*, Hypoglycemic activity, Ulceroprotective effect.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic condition with chronic hyperglycemic symptoms and metabolic disorders. It is probably due to either insulin deficiency, insulin resistance or failure of β -cells of the pancreas to generate insulin, carbohydrates, fats and proteins¹. Moreover, diabetic patients reportedly have a higher incidence of peptic ulcer disease. Peptic ulcer disease and DM are two serious chronic diseases with many medical and socioeconomic consequences². Researchers found that DM was independently associated with increased risk of peptic ulcer bleeding³. Herbal medicines with antihyperglycemic activity have been used as alternative treatments of DM due to their reduced side effects and lower cost compared to synthetic hypoglycemic drugs⁴. Following the World Health Organization recommendations for DM (WHO 1980), the search for safer and more effective hypoglycemic pharmaceuticals from medicinal plants has continued to be an important area of active research⁵.

Gleditsia triacanthos L. (Leguminosae), a 45 m long tree, is cultivated in Egypt public parks and gardens for ornamental purposes⁶. Extracts and isolates from different parts of *G. triacanthos* have been reported to possess different pharmacological potentials such as analgesic⁶, anti-oxidant⁷, anti-diabetic⁸, oncostatic and cytotoxic activities^{7,9}.

Saponins are known to be the main constituents of fruits of different *Gleditsia* species¹⁰⁻²¹. Also, several reports discussed the occurrence of flavonoids in different organs of *Gleditsia* species^{7,22-27}.

The goal of the present study was to investigate the constituents of the *n*-butanol fraction of the methanolic fruit extract of *G. triacanthos* (BFGT) and to assess its anti-hyperglycemic and anti-ulcerogenic activities in comparison to those of the total methanolic fruit extract *G. triacanthos* (MEGT) in male rats. To fulfill this goal, BFGT was analyzed using UPLC-ESI/MS/MS technique in the negative ion mode.

DM was induced in rats by streptozotocin (STZ); a well-known compound used to destruct the β cells

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of the pancreas of rats thus causing an increment in the serum glucose level as well as serum levels of triglycerides and cholesterol which were dramatically increased²⁸⁻³¹. On the other hand, ulcers were induced in male rats by oral injection of ethyl alcohol³²⁻³⁴.

MATERIAL AND METHODS

Plant material

G. triacanthos fruits were obtained from The Zoological Garden, Giza, Egypt, in October 2018 and identification was confirmed by Mrs. Therese Labib senior specialist for plant identification. A voucher specimen was deposited in the Herbarium of NRC (CAIRC), Voucher Number 230.

Preparation of MEGT and BFGT

The air-dried fruits of the *G. triacanthos* (3 kg) were extracted with CH₂Cl₃ (3X6 L) followed by MeOH (3X6 L). The combined MeOH extract was evaporated under vacuum to yield 90 g of MEGT. Part of MEGT (40 g) was kept in the refrigerator until use in the pharmacological studies. The remaining part (50 g) of MEGT was suspended in water and extracted with water saturated *n*-butanol (5X500 ml) to yield 43 g of *n*-butanol fraction (BFGT). Part of the *n*-butanol fraction (30 g) was kept in the refrigerator until use in the pharmacological studies. The other part (13 g) was dissolved in distilled water (0.2%) and the aqueous solution was passed through a column packed with the polymer gel Diaion HP-20 (200 g). The adsorbed material was eluted with H₂O, 25, 50, 75, and 100% methanol. The 50 and 75% eluted fractions containing flavonoids were combined and freeze-dried (7g). The 100% fractions containing saponins were combined and freeze dried (5 g). The combined flavonoidal and saponin fractions were separately subjected to LC-ESI/MS/MS analysis.

UPLC-ESI/MS/MS analysis

Ultra-performance liquid chromatographic (UPLC) analysis coupled with an ESI mass spectrometer detector allowed simultaneous isolation of the compounds and determination of the molecular weights of isolated peaks. Samples were dissolved in HPLC analytical grade methanol, filtered using a membrane disc filter (0.2 µm), and subjected to UPLC-ESI-MS/MS analysis. Samples (5 µl) were injected into the UPLC instrument, Waters® equipped with a reversed-phase C-18 column (ACQUITY UPLC - BEH C18 1.7 µm particle size 2.7 mm). The mobile phase was filtered using a 0.2 µm membrane disc filter and sonicated before injection. The Elution flow rate was adjusted to 0.2 ml/min; the gradient was composed of two eluents: water acidified with 0.1% formic acid and acetonitrile acidified with 0.1% formic acid. Negative ion modes were used on a triquadrupole (XEVO) mass spectrometry, Waters® Corporation, Milford, MA01757 U.S.A. 30 eV cone voltage, 3 kV capillary voltage at 150 °C, 440 °C desolvation temperature were applied. Detection of mass spectra was in the ESI range *m/z* 100–1000 for flavonoids and 800-1800 for saponins using the software Maslynx 4.1 and tentatively identified by comparing the retention time (Rt) peaks in the mass spectrum and their fragmentation pattern with reported data.

Laboratory animals

Adult male Wistar albino rats (150-200 g) were used for the current experiment. Animals were obtained from the National Research Centre animal house colony. They were housed in plastic cages under standard conditions of temperature (25 ± 1°C), relative humidity (55 ± 10%), 12h/ 12h light/dark cycles and fed on standard pellet diet and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee of the National research Centre (Approval number: 13/165).

Chemicals

Streptozotocin (STZ) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Gliclazide (Glz); standard drug; was obtained from Servier Canada (Laval, QC, Canada). All chemicals used were purchased from ADWIC (CAI, Egypt). All other chemicals used were of highest available analytical grade.

Induction of diabetes mellitus

Diabetes Mellitus was induced by a single i.p. injection of STZ (55 mg/kg). Rats were weighed and injected with STZ dissolved in a citrate buffer (0.1 M, pH 4.5)³⁵. After 48 h blood samples were withdrawn from the retro-orbital venous plexus under light ether anaesthesia and the serum was separated by centrifugation for the determination of glucose level. Only rats with serum glucose levels more than 200 mg/dl were selected and considered diabetic animals that have been subjected to further experimentation.

Experimental design

Diabetic rats were randomly divided into eight groups each containing 6-10 animals. Group I, the untreated diabetic group, served as the negative control group while group II served as the standard group being treated with gliclazide (Glz; 10 mg/kg; p.o.) for 14 consecutive days³⁶. Groups II, IV and V were orally treated with total methanolic extract *Gleditsia triacanthos*, (MGT) at the three dose levels 70, 140 and 280 mg/kg, respectively. Groups VI, VII and VIII were orally given *n*-butanol fraction of *Gleditsia triacanthos* (BFGT) 70, 140 and 280 mg/kg; respectively. Administration of different doses of MEGT, BFGT and Glz once daily was continued for 14 days. The used doses of MEGT and BFGT were based on the acute toxicity and LD₅₀ studies previously reported⁶. Moreover, a universal control group receiving only saline was observed throughout the experiment.

Estimation of biochemical parameters

At the end of the treatment, blood samples were collected from overnight fasting rats. Sera were separated for determination of serum levels of glucose, insulin, triglycerides, total cholesterol and α-amylase. The rats were then sacrificed by cervical dislocation and the liver tissues were isolated. Liver tissues were homogenized.

Serum glucose, triglycerides, and cholesterol were estimated according to the methods of Trinder³⁷, Fosati and prencipe³⁸, as well as Alain et al.³⁹, respectively. Serum insulin was estimated by a radioimmunoassay technique using the ALPCO Insulin (Rat) ELISA kit according to the method of Judzewitsch et al.⁴⁰. The activity of α amylase was determined by the method of Gella et al.⁴¹. Estimation of liver reduced glutathione (GSH) was adopted according to Beutler et al.⁴²

Gastric ulcer model induced by ethanol (Anti-ulcerogenic assessment)

Forty-eight male Wistar rats were randomly divided into eight groups (6 rats per group). Group I received distilled water orally and served as negative served as control while group II served as the standard control group by receiving rantidine (20 mg/kg.wt.). Groups III- V received orally 70,140 and 280 mg/kg b.wt. of MEGT, respectively. BFGT at 70,140 and 280 mg/kg b.wt. was orally administered to groups VI-VIII. Acute erosion of the gastric mucosa in fasting rats was induced by intragastric administration of absolute ethanol (1 ml v/v)⁴³. Animals were sacrificed after 1 h of ethyl alcohol administration by cervical dislocation. The stomachs were then excised along the greater curvature and examined for mucosal necrotic lesions, red streaks, and red erosions macroscopically. The total lesion number was counted and lesion severity was measured⁴⁴.

Statistical analysis

All the results were expressed as Mean ± S. E. Data was statistically evaluated with Graphpad prism software. Hypothesis testing methods included one-way analysis of variance (ANOVA) followed by Tukey post hoc test, whereas the ulcer number and severity were estimated using one Kruskal-Wallis non-parametric ANOVA followed by Dunn's multiple comparisons test, P < 0.05 was considered to indicate statistical significance.

RESULTS

UPLC-ESI/MS/MS analysis

The analysis of molecular masses and formulae in addition to LC-ESI/MS/MS spectra of the *n*-butanol fraction of *G. triacanthos* methanolic fruit extract (BFGT) led to identification of 11 compounds (Table 1) including phenolic acids 1-2, flavonoids 3-7, triterpenoidal saponins (8-11). 4-*O*- and 5-*O*- caffeoylquinic acids (1 and 2) were identified based on the presence of a major [M - H]⁻ ion peak at m/z 353 with a fragment ion m/z 191 corresponding to a quinic acid moiety and a fragment ion at m/z 179 corresponding to a caffeic acid moiety with respect to their retention times⁴⁵. This was also confirmed by the presence of a base fragment peak m/z 173 corresponding to the loss of caffeic acid and a water molecule which is a characteristic fragmentation pattern for 4-caffeoylquinic acid and can differentiate it from other isomers. The occurrence of Caffeoylquinic acid in its adduct form was revealed from the presence of a fragment [2M-H]⁻ ion peak at m/z 707⁴⁶.

Compounds 3 and 4 displayed principal deprotonated ion peaks at m/z 287 with typical distinctive fragmentation patterns of fustin and

aromadendrin (dihydrokaempferol), respectively. Compound 5 was identified as isoquercitrin showing [M-H]⁻ ion peak at 463. It showed a typical fragmentation pattern with fragments corresponding to a deprotonated radical aglycone at m/z 300 and an aglycone at m/z 301. Compound 7 was identified as quercetin showing [M-H]⁻ ion peak at 301 and a typical fragmentation pattern²⁷. The detailed fragmentation pattern of compound 6 allowed the identification of luteolin-7-*O*-glucoside with a major base peak at m/z 285 demonstrating the loss of a hexose sugar moiety⁴⁷.

The structures of the triperpenoidal saponins were established based on the [M-H]⁻ and their fragment ions corresponding to the loss of 132 (pentoses) and 162 (hexoses) mu and after considering the characteristic sugar pattern and aglycones of *Gleditsia* saponins^{48,49}.

Anti-hyperglycemic assessment

Effect of MEGT and BFGT on serum glucose level in diabetic rats

Streptozotocin-induced diabetic rats exhibited significant blood glucose level increase when compared to negative control group. The standard drug Glz showed significant decrease in elevated serum glucose levels by 45% when compared to the STZ-induced diabetic group. Test groups treated with total methanol extract of MEGT at dose levels 70,140, and 280 mg/kg b.wt. showed significant decrease in elevated serum glucose levels of rats by 24%, 31%, and 39%, respectively. Similarly, treatment with *n*-butanol fraction of *G. triacanthos* (BFGT) at the same dose levels decreased elevated serum glucose levels by 34%, 36% and 38%, respectively in comparison to the control diabetic group (Table 2).

Table 1: LC-ESI/MS-MS analysis of BFGT.

Compound number	Identified Compound	Retention time (min)	[M - H] ⁻	Molecular formula	MS/MS fragment ions
Flavonoids and Phenolics					
1	5- <i>O</i> -Caffeoylquinic acid	1.68	353	C ₁₆ H ₁₈ O ₉	191, 179, 161,135
2	4- <i>O</i> -Caffeoylquinic acids	1.82	353	C ₁₆ H ₁₈ O ₉	191, 179, 173, 135
3	Fustin	4.94	287	C ₁₅ H ₁₂ O ₆	269, 259, 225, 163, 149, 135, 109
4	Dihydrokaempferol (Aromadendrin)	4.94	287	C ₁₅ H ₁₂ O ₆	259, 243, 201, 151, 125
5	Isoquercitrin	5.91	463	C ₂₁ H ₂₀ O ₁₂	301, 300, 271, 255, 179, 175, 151
6	Luteolin 7- <i>O</i> - glucoside	6.79	447	C ₂₁ H ₂₀ O ₁₁	285, 240.9, 266.9, 256.9, 242.9, 216.9, 199, 175, 151, 133
7	Quercetin	7.63	301	C ₁₅ H ₁₀ O ₇	179, 151, 121, 107
Saponins					
8	Caspicaoside F	3.91	1777	C ₈₀ H ₁₃₀ O ₄₃	1645, 1615
9	Gleditsia saponin C'	6.69	1615	C ₇₄ H ₁₂₀ O ₃₈	1483, 1349, 1217, 897
10	Gleditsioside J	6.93	1631	C ₇₄ H ₁₂₀ O ₃₉	1499, 1469, 897
11	Gleditsia saponin G'	9.50	1351	C ₆₄ H ₁₀₄ O ₃₀	1333, 1085, 965, 469

Table 2: Effect of MEGT and BFGT on the serum levels of glucose, triglycerides and cholesterol in STZ-treated diabetic rats

	Glucose	Triglycerides	Cholesterol
Normal	86.9 ± 4.2	96.4 ± 2.5	95.5 ± 5.0
STZ (55 mg/kg)	221.8 ± 10.6*	387.8 ± 12.9*	153.9 ± 12.4*
Glz (10 mg/kg)	120.4 ± 11.7 [®]	124.1 ± 11.3 [®]	89.6 ± 6.6 [®]
MEGT (70mg/kg)	167.3 ± 13.4 [®]	102.3 ± 6.3 [®]	94.6 ± 3.7 [®]
MEGT (140mg/kg)	151.8 ± 12.3 [®]	122.2 ± 8.8 [®]	92.7 ± 4.7 [®]
MEGT (280mg/kg)	134.15 ± 8.4 [®]	148.2 ± 7.9 [®]	87.5 ± 7.6 [®]
BFGT (70mg/kg)	144.6 ± 6.8 [®]	151.9 ± 11.7 [®]	79.9 ± 6.2 [®]
BFGT (140mg/kg)	141.4 ± 3.8 [®]	153.5 ± 13.8 [®]	78.2 ± 6.7 [®]
BFGT (280mg/kg)	137.2 ± 5.6 [@]	148.8 ± 11.8 [@]	73.88 ± 6.8 [@]

Results are expressed as means ± SEM. Statistical analysis was carried out by ANOVA followed by Tukey's multiple comparisons test. * Significant difference from normal group at P < 0.05. @ Significant difference from STZ-treated group at P < 0.05.

Effect of MEGT and BFGT on serum insulin level in diabetic rats

Test group receiving STZ showed a 54 % decrease in serum insulin level. Glz produced a significant increase of 131 % in reduced serum insulin in comparison to the STZ-induced diabetic rats. Rats treated with MEGT at 70, 140 and 280 mg/kg significantly increased the levels of reduced serum insulin by 63 %, 126 % and 146 %, respectively while rats treated with BFGT at 70, 140, 280 mg/kg b.wt. exhibited an increase of 122%, 108% and 168% when compared to the control group (Figure 1a).

Effect of MEGT and BFGT on serum α -amylase activity in diabetic rats

Diabetic rats exhibited a 68 % significant decrease in the activity of serum α -amylase activity when compared to normal control group. Group treated with standard drug Glz showed a 156 % significant increase in the serum α -amylase activity when compared to group of diabetic rats. MEGT and BFGT at 70, 140 and 280 mg/kg b.wt.

dose levels showed significant increase of 172 %, 167 %, 152% and 130 %,125%, 112%, respectively in the serum amylase activity, in comparison to the control diabetic group (Figure 1b).

Effect of MEGT and BFGT on serum levels of MDA and GSH in diabetic rats

A significant 2.9-fold increment of serum MDA was shown in the diabetic rats in comparison with the normal control group. Glz exhibited significant decrease in the serum MDA of STZ-induced diabetic rats by 56 % as compared to the diabetic control group. Treatment with MEGT (70, 140 and 280 mg/kg) exerted significant decrease in the serum MDA by 43%, 57% and 59%, respectively, while treatment with SFGT (70, 140 and 280 mg/kg) showed significant decrease in the serum MDA level by 24%, 32% and 40% respectively as compared to the control diabetic group.

Moreover, STZ-induced diabetic rats showed 71 % decrease in serum GSH in comparison to normal control group. Group of diabetic rats treated with Glz showed significant increase in the serum GSH level 175

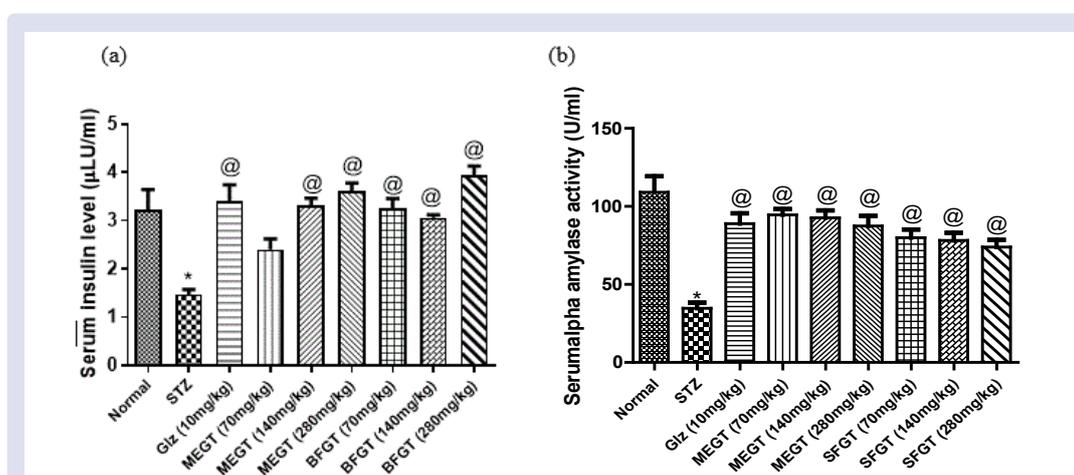


Figure 1: Effect of MEGT and BFGT on the serum levels of insulin (a) and amylase (b) in STZ-treated diabetic rats. Results are expressed as means \pm SEM. Statistical analysis was carried out by ANOVA followed by Tukey's multiple comparisons test. * Significant difference from normal group at $P < 0.05$. @ Significant difference from STZ-treated group at $P < 0.05$.

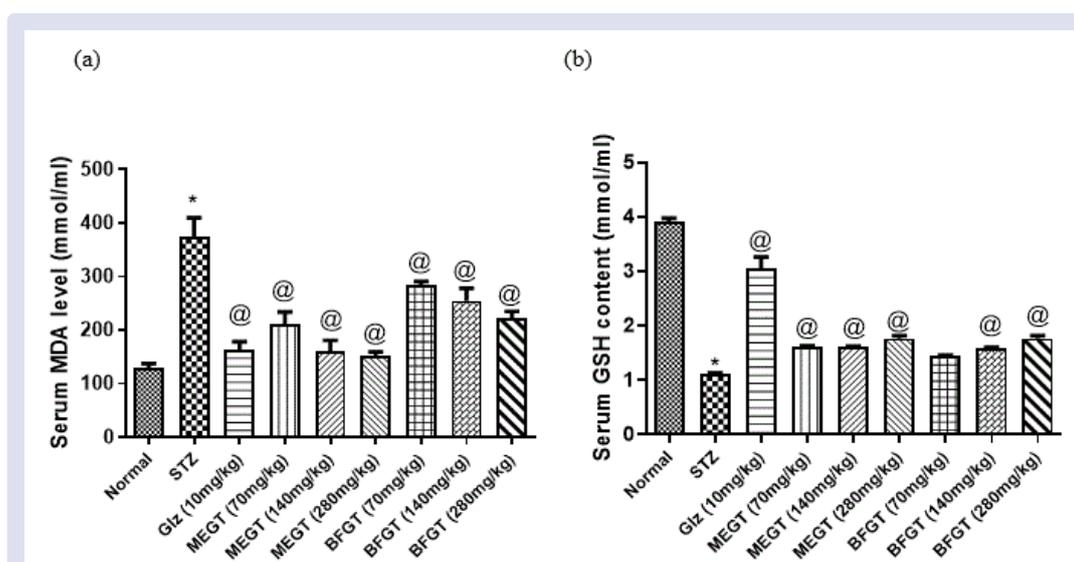


Figure 2: Effect of MEGT and SFGT on the serum levels of lipid peroxides (a), reduced GSH (b) in STZ-treated diabetic rats. Results are expressed as means \pm SEM. Statistical analysis was carried out by ANOVA followed by Tukey's multiple comparisons test. * Significant difference from normal group at $P < 0.05$. @ Significant difference from STZ-treated group at $P < 0.05$.

% when compared to the control diabetic group. Treatment with MEGT (70, 140 and 280 mg/kg) showed significant increase in the serum GSH level by 45 %, 45% and 59%, respectively, whereas treatment with BFGT (70, 140 and 280 mg/kg) exerted a serum GSH level increment by 30%, 42% and 59%, respectively as compared to the control diabetic group (Figure 2a,b).

Effect of MEGT and BFGT on serum levels of triglycerides and total cholesterol in diabetic rats

A significant increase of elevated serum triglycerides and total cholesterol levels reaching 5 folds and 1.6 folds, respectively was shown in STZ-induced diabetic rats in comparison to normal control ones. Group treated with Glz produced significant decrease in elevated serum triglycerides and total cholesterol levels by 68 % and 41 % when compared to the STZ-induced diabetic group. Groups treated with MGT and SGT at 70, 140 and 280 mg/kg b.wt. showed significant decrease in elevated serum triglyceride level by 73%, 68%, 61% and 60 %, 60%, 61%, respectively in comparison to the control diabetic group (Table 2).

Similarly, test groups treated with MGT and SGT at 70,140 and 280 mg/kg b.wt. exhibited significant decrease in elevated serum total cholesterol level by 38 %, 39 %,42%, and 47%, 49%, 51 %, respectively, in comparison to the diabetic group.

Anti-ulcerogenic assessment

Effect of MEGF and BFGF on serum ulcer number and severity in ethanol-induced ulcer rat model

MEGT and BFGT showed significant ulceroprotective activity where they showed a significant decrease in both number and severity of ethanol induced gastric lesions in rats when compared to control group. receiving standard drug ranitidine (20mg/kg b.wt.). MEGT at its highest dose level (280 mg/kg b.wt.) showed inhibition of ulcer number and severity by 70 and 82%, respectively.

BFGT at 140 and 280 mg/kg b.wt. decreased the number and severity of gastric lesions in rats by 80%, 83% and 85%, 96%, respectively when compared to the control group. Group IV receiving SGT at 70 mg/kg b.wt. showed only a significant inhibition in ulcer number with a 73% protection. These effects were comparable with standard reference drug ranitidine (20 mg/kg) (Table 3).

DISCUSSION

The composition of BFGT was identified from the LC-ESI/MS/MS spectra. One phenolic acid and its dimer, five flavonoids and four saponins were characterized. The five identified flavonoids were previously reported from various parts of different *Gleditsia* species. Fustin and dihydrokaempferol were previously detected in the spines of *G. sinensis*^{26,27}, Isoquercitrin previously detected from spines of *G.*

sinensis was isolated from the leaves of *G. sinensis*²³, *G. japonica*²² and *G. triacanthos*^{7,24}. Luteolin-7-O-glucoside was previously isolated from leaves of *G. japonica*²², *G. triacanthos*⁷ and *G. sinensis*²³. Quercetin was previously reported from spines of *G. sinensis*²⁶, *G. japonica* leaves²² and *G. triacanthos* leaves²⁴. The two Caffeoylquinic acid isomers were previously isolated from leaves of *G. japonica*²² whereas 5-O-caffeoylquinic acid was detected in *G. triacanthos* leaves⁵⁰.

The saponin structures were established based on their MS data⁴⁸ and guided by their characteristic sugar pattern^{10-20,51}. The identified saponins reported here for the first time were caspicaoside F previously isolated from *G. caspica* fruits¹⁰, Gleditsia saponin C' was previously isolated from *G. sinensis*^{12,20,21} and also obtained after partial alkaline hydrolysis of Gleditsia saponin C from *G. japonica*⁵², Gledtsioside J was isolated before from fruits of *G. sinensis*²⁰ and gleditsia saponin G' was previously obtained from *G. japonica* after alkaline hydrolysis of Gleditsia saponin G⁵¹.

The impact of MEGT and BFGT on the STZ-induced hyperglycemia and their role in lowering the serum lipids in diabetic rats was also investigated in the current study. The results showed that MEGT and BFGT at the three dose levels (70, 140 and 280 mg/kg b.wt.) markedly decreased the elevated serum glucose levels of diabetic rats' dose dependently in comparison to the control diabetic group. On the other hand, oral treatment of diabetic rats with MEGT and BFGT also dramatically elevated the serum levels of insulin and α -amylase in a dose dependent manner. It has been previously proven that DM is closely associated to hyperlipidemia. Lack of insulin secretion in diabetic patients develops hydrolysis of triglycerides into diglycerides, unesterified fatty acids and free glycerol⁵³. The fatty acids may be re-esterified into triglycerides may leading to hypertriglyceridemia that activates the mechanism by which DM causes hyperlipidemia⁵⁴. The current study showed that treatment with MEGT and BFGT exhibited significant decrease in elevated serum levels of triglycerides and total cholesterol in comparison to the control diabetic group.

Oxidative stress is the hallmark of the pathogenesis of DM. On the other hand, the ability of anti-oxidants to protect against the deleterious effects of hyperglycemia and also to enhance glucose metabolism and uptake, should be considered as a lead alternative in DM treatment. In the present study, STZ-induced diabetes was associated with a marked elevation in the oxidative stress biomarkers evidenced by significant elevation of serum MDA as well as decrease in serum GSH in comparison to normal control group. Oral treatment with MEGT and BFGT exerted prominent decline in the serum MDA and exerted a serum GSH level increment as compared to the control diabetic group.

Furthermore,DM has been previously reported to increase vulnerability of the gastric mucosa to various ulcerogens. The remarkable alterations observed in patients with chronic DM decrease gastric secretion and motility⁵⁵. In the current research the impact of MEGT and BFGT on ethanol induced ethanol-induced ulcer in rats at three dose levels 70,

Table 3: Effect of MEGT and BFGT on ethanol-induced ulcer number and severity.

	Ulcer number	%Protection	Ulcer severity	%Protection
Saline	12.64 ± 0.741		43.09 ± 3.212	
Ranitidine (20mg/kg)	0.5 ± 0.25*	96.04	0.72 ± 0.83*	98.32907867
MEGT (70mg/kg)	4.83 ± 0.792	61.78797468	16 ± 1.929	62.86841495
MEGT (140mg/kg)	4.33 ± 0.802	65.74367089	11.16 ± 1.706	74.10071942
MEGT (280mg/kg)	3.67 ± 0.210*	70.96518987	7.5 ± 0.807*	82.59456951
BFGT (70mg/kg)	3.33 ± 0.333*	73.65506329	14.16 ± 0.435	67.13854723
BFGT (140mg/kg)	2.50 ± 0.428*	80.22151899	7 ± 0.849*	83.75493154
BFGT(280mg/kg)	1.83 ± 0.703*	85.5221519	1.66 ± 0.732*	96.14759805

Results were expressed as mean ± SEM. Statistical analysis was carried out by one Kruskal-Wallis non-parametric ANOVA followed by Dunn's multiple comparisons test. * Significant difference from saline control group P<0.05

140 and 280 mg/kg b.wt., was also assessed. MEGT and BFGT showed a substantial decrease in the amount and intensity of ethanol-induced gastric lesions in rats, relative to the rats receiving the standard drug ranitidine (20 mg/kg b.wt.), indicating prominent ulceroprotective efficacy. At its highest dose level, MEGT (280 mg/kg b.wt.) demonstrated significant inhibition of the number and severity of ulcers. Moreover, flavonoid and saponin rich BFGT decreased the number and severity of gastric lesions in rats when compared to the ranitidine treated group in a dose dependent manner.

The anti-diabetic and anti-ulcerogenic effect of MEGT and BFGT might be attributed to its high content of flavonoids and saponins where both classes were reported to possess both activities. Flavonoids were reported to show anti-diabetic and anti-oxidant activities⁵⁶⁻⁵⁸. Flavonoids described in the current study were previously reported to possess anti-hyperglycemic activity. Isoquercitrin showed protective activity against DM⁵⁹ and improved insulin sensitivity⁶⁰ and quercetin caused significant decrease in blood glucose level and attenuated oxidative stress associated with diabetes in rats^{61,62}. Moreover, luteolin-7-*O*-glucoside was reported to ameliorated DM⁶³ and inhibited alpha amylase activity⁶⁴. Also, caffeoylquinic acid-rich extract from *Pandanus tectorius* fruit decreased insulin resistance and modulates hepatic glucose and lipid metabolism in diabetic mice⁶⁵.

Flavonoids were also reported in literature to exhibit anti-ulcerogenic activities by protecting the gastrointestinal mucosa from lesions through the contribution of various mechanisms⁶⁶. Flavonoidal fractions from different plants were reported to possess anti-ulcer activities⁶⁷. Moreover, flavonoidal fraction from *G. triacanthos* leaves exhibited anti-oxidant activity with luteolin 7-*O*-glucoside showing highest free radical scavenging activity⁷. Luteolin 7-*O*-glucoside also was reported to provide protective effects in the rat gastric mucosa against ethanol-induced gastric injury⁶⁸. Quercetin possesses beneficial effects on ulcer formation and gastric secretions in rat experimental ulcer model. However, the anti-ulcer activity of quercetin is attributed to its anti-oxidant property, which involves free radical scavenging and reduction of lipid peroxidation⁶⁹. Moreover, caffeoylquinic acid-rich extract from *Ligularia stenocephala*⁷⁰ and isolated dicaffeoylquinic acid isomer from *Arctium lappa*⁷¹ exhibited significant anti-ulcer effect.

The anti-diabetic activities of MEGT and SFGT might also be attributed to its triterpenoidal saponin component. Triterpenoidal saponin rich fraction from *Stauntonia chinensis* was reported to exhibit hypoglycemic and hypolipidemic effects in db/db mice⁷². Similarly, Singh et al. (2014) has reported the anti-diabetic potential of triterpenoid saponin isolated from *Primula denticulate* where it declined blood glucose via elevating insulin secretion from pancreatic β -cells⁷³. Furthermore, the hypoglycemic property of triterpenoid saponin isolated from *Polyscias fruticosa* leaves⁷⁴ inhibited porcine pancreas α -amylase and prominently reduced the postprandial blood glucose level in a high-sucrose diet fed mice.

Previous reports also showed potent protective effect of the saponin fractions from different origins on ethanol- and indomethacin-induced gastric mucosal lesions in rats. The saponin fraction from the *Camellia sinensis* (tea plant) seeds and its triterpenoidal saponin isolates were previously reported to possess a marked ulceroprotective activity against ethanol-induced gastric lesions⁷⁵. *Conyza blinii* extract rich with triterpenoidal saponins was reported to exhibit an intense protection activity against ethanol-induced gastric ulcers through scavenging of free radicals, suppressing the inflammatory responses thus protecting the gastric mucosa⁷⁶.

CONCLUSION

The present study showed prominent evidence on the anti-diabetic and anti-ulcerogenic activities of MEGT and BFGT evaluated by STZ-induced diabetes and ethanol-induced gastric ulcers in rats,

respectively. The anti-diabetic and the anti-ulcerogenic properties of MEGT and BFGT may be attributed to the combined activities of their saponins and flavonoidal constituents.

CONFLICTS OF INTEREST

None.

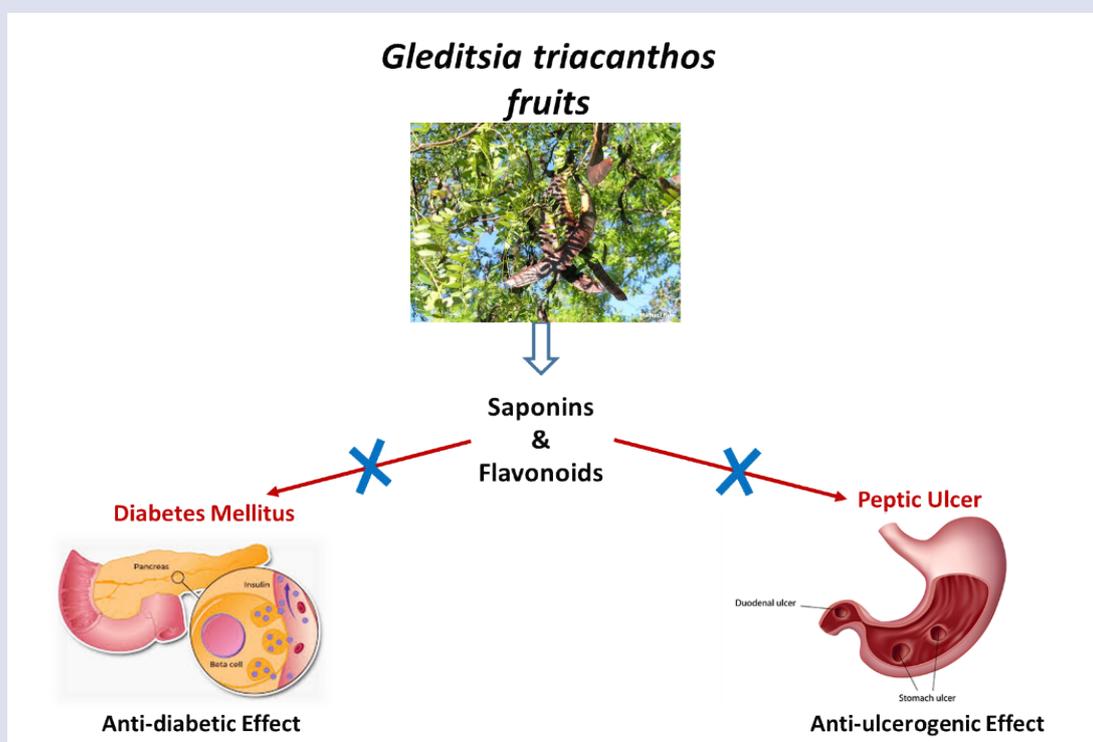
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GRAPHICAL ABSTRACT



Eleven compounds were identified from *Gleditsia triacanthos* fruits where the methanolic fruit extract and its *n*-butanol fraction exhibited anti-diabetic and anti-ulcerogenic activities in rats.

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