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Investigation of Fenugreek (*Trigonella foenum-graecum)* Seed Extract in Combating Glucotoxicity via Modulation of the Canonical Wnt Signaling Pathway: *In Vivo* and *In Silico* Approach

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Abstract

Diabetes mellitus is a chronic metabolic disorder characterized by prolonged hyperglycemia, in which glucose reacts covalently with plasma proteins through a non-enzymatic process known as glycation. This leads to the formation of advanced glycation end-products (AGEs), which play a central role in the development of diabetic complications, including osteoporosis. These accumulated AGEs disrupt the canonical Wnt signaling pathway, thereby increasing bone resorption and reducing bone formation, ultimately leading to osteoporosis. This present study has been designed to explore the antidiabetic potential of Fenugreek (*Trigonella foenum-graecum)* seed extract (TSE) in inhibiting AGEs formation. Based on the study results, TSE at two different doses (200 and 700 mg/kg/day) significantly lowered the blood glucose levels in methylglyoxal (MGO)-induced diabetic mice model. Since MGO acts as a key precursor in the glycation process, these results suggest that TSE may help to slow glycation and AGEs accumulation. Furthermore, molecular docking analysis also revealed that two compounds of TSE such as, Yuccagenin and Diosgenin act as key bioactive components to exhibit the strong binding affinities (−9.8 kcal/mol) with Sclerostin, indicating its potential role in reducing the bone degradation and preventing diabetes-associated osteoporosis.

Key words: Feenugreek (*Trigonella foenum-graecum*) seed, glucotoxicity, AGEs (Advanced glycation end-products), MGO (Methylglyoxal), Wnt-signaling pathway, sclerostin.

Introduction

Diabetes mellitus (DM) is known as a complex metabolic disorder characterized by chronic high blood sugar, impaired metabolism due to insufficient insulin secretion, action, or both. Glucotoxicity is supposed to be the major contributing factor to induce DM, which affects nerves, kidneys, eyes, and increases the risk for cardiovascular diseases, including microvascular disorder (Yadav *et al.*, 2023). An increasing rate in the prevalence of DM among the Bangladeshi population is also observed over the time due to unhealthy diets and sedentary lifestyles, leading to increased body mass index (BMI) and fasting plasma glucose levels (Alam *et al.*, 2022). One of the key mechanisms underlying diabetic complications, including nephropathy, retinopathy, and neuropathy, is the formation of advanced glycation end products (AGEs). AGEs are generated through the Maillard reaction, a non-enzymatic process in which reducing sugars react with protein amino groups of protein to form Schiff bases, which subsequently rearrange into Amadori products and then degrade into reactive dicarbonyls, such as glyoxal, methylglyoxal (MGO), and deoxyglucosones. These intermediates undergo oxidation, dehydration, and cyclization to form

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irreversible AGEs (Yu et al., 2024). AGEs are yellow-brown, fluorescent, and insoluble adducts that accumulate on long-lived proteins, impairing their function (Vistoli et al., 2013). Hyperglycemia accelerates glycation of plasma proteins (e.g., albumin, fibrinogen, globulins)(Abate and Delbarba, 2015a; Okano *et al*., 2002) and collagen, leading to disrupted drug binding, platelet activation, oxidative stress, impaired fibrinolysis, immune dysregulation, and defects in bone remodeling and skeletal integrity(Banerjee, 2017; Guillon *et al*., 2021). Particularly, collagen glycation affects osteoblast differentiation, leading to bone remodeling defects and skeletal fragility (Byun *et al*., 2017; Ott *et al*., 2014). In the late stages of glycation, irreversible AGEs accumulate, driving the progression of diabetic complications, including osteoporosis in elderly patients (Pinto-Junior *et al*., 2018). The canonical Wnt signaling pathway is critical for the regulation of bone metabolic homeostasis and skeletal development (Ma and Hottiger, 2016). However, accumulation of AGEs down-regulates the Wnt canonical signaling pathway, which negatively affects bone strength to progress osteoporosis in diabetic patients. Several studies have confirmed that disruption of the Wnt signaling pathway is involved in hyperglycemia-induced abnormalities of bone metabolism in osteoporosis (Peng *et al*., 2019). Based on these research gaps, this study has been designed to investigate the roles of AGEs in diabetes, focusing on the Wnt signaling pathway of osteoporosis, which will provide further insight into its pathogenesis and provide new strategies for the treatment of this disease.

Trigonella foenum-graecum (fenugreek), commonly known as “meethi seeds,” has long been used in traditional medicine for its diverse therapeutic properties, particularly in managing diabetic complications (Ocvirk *et al*., 2013). Fenugreek is well recognized for its ability to regulate blood glucose levels and improve lipid profiles in diabetic patients when consumed in powdered form (Goyal *et al*., 2016). Several studies have also shown its antidiabetic effect in streptozotocin-induced diabetic models, where fenugreek has been reported to reduce oxidative stress, support early diabetes management, and improve short-term memory (Alam *et al*., 2022; Moradi and Zadeh, 2015). Despite these potentialities in diabetic complication management, no study has yet explored the antidiabetic potential of *T. foenum-graecum* (TFE) seed extract in AGEs-induced diabetes, specifically targeting the Wnt signaling pathway (Hina *et al*., 2025). Therefore, the present study aims to evaluate the efficacy of fenugreek seeds in inhibiting the formation of advanced glycation end-products (AGEs) *in vivo* by using methylglyoxal (MGO)-induced diabetic mice model and to investigate its preventive role in osteoporosis through modulation of the canonical Wnt signaling pathway via *in-silico* approaches.

Methods and Materials

Collection of plant material and extraction: The seeds of Fenugreek (*Trigonella foenum-graecum)* were collected from old Dhaka, Bangladesh, during the month of March 2023. The authentication of the plant sample was confirmed by consulting with the National Herbarium's taxonomist (DACB Accession Number is 100718), and a voucher specimen of this plant was deposited at the Bangladesh National Herbarium, Dhaka, Bangladesh. Firstly, the seeds of Fenugreek were properly washed and dried in sunlight for several days. Afterward, the dried seeds were crushed into fine powder using a high-capacity grinding machine. Then, fifty gram (50g) of powdered materials were soaked in 500 ml of ethanol for 10 days and the filtration was done three times to get a clearer extract, followed by evaporation through a rotary evaporator to get the concentrated extract.

Chemicals and reagents: Ethanol, n-hexane, chloroform, acid and all other reagents used in phytochemical screening were collected from local market (Hathkhola market, Tikatuli, Bangladesh). Methylglyoxal (MGO) was purchased from Sigma Aldrich (India); Metformin was a generous gift from Incepta Pharmaceuticals Ltd., Dhaka Bangladesh. All the chemicals used in the current study were of the highest analytical grade. Glucometer of BestCheck basic, Taiwan was taken from Shahbag, Dhaka,

Bangladesh. All the analysis kits related to mice study were purchased from Lazz- Pharma Ltd., Dhaka, Bangladesh.

Fractionation and partitioning: Fractionation of the crude ethanolic extract was done by using modified Kupchan partitioning method by using liquid-liquid extraction to separate compounds based on solubility in different solvents. Solvents used in this process are n-Hexane, ethyl acetate, and chloroform. At first, 50ml of concentrated extract was taken for fractionation, and the remaining ethanol from the extract was evaporated by using a water bath at 78ºC and 2g of solid extract were obtained.

n-Hexane partition: TFE concentrated extract was mixed with 100ml of water and 100ml of n-hexane. Then the solution was poured into a separating funnel with shaking to mix well. The lid was opened in between shaking to release the gas created by shaking. After 30 minutes, two different layers were obtained. The upper layer was the n-Hexane layer, which contained nonpolar compounds of the extract, and the lower layer was the aqueous layer, which contained polar compounds of the extract. The layers were collected in different beakers. The n-Hexane was evaporated to obtain the isolated compounds.

Ethyl-acetate partition: The aqueous layer was obtained from n-Hexane partition, when the TFE concentrated extract was mixed with 100ml of water and 100 mL of ethyl acetate. The same procedure was done as the n-Hexane partition. Two layers were seen, where the upper layer was the ethyl-acetate layer, which contained polar compounds of the extract, and the lower layer was the aqueous layer. The layers were collected in different beakers. The ethyl acetate was evaporated to obtain the isolated compounds.

Chloroform partition: To get the chloroform fraction, the aqueous layer obtained from the ethyl-acetate partition was added with 100ml of water and 100 mL of chloroform. The same procedure was done as n Hexane partition, and here are also two layers seen. As chloroform was denser than water, the chloroform layer was seen at the bottom, which contained organic components of the extract. The layers were collected. Chloroform was evaporated to obtain the isolated compounds, and the aqueous layer was stored at a cool temperature.

Phytochemical screening: The confirmatory qualitative phytochemical screening of *TFE* was performed to identify the main classes of compounds (tannins, saponins, flavonoids, alkaloids, phenols, glycosides, steroids, and terpenoids). Phytochemical screening was conducted for ethanol, n-hexane, ethyl-acetate, and chloroform extracts of the seeds of *TFE*, following the standard protocols of previously reported research (Shah *et al*., 2020; Ganjewala and Gupta, 2013). Wagner's test was conducted by adding dil. HCl to plant extract and adding Wagner's reagent, resulting in a reddish-brown precipitate indicating alkaloids presence. By adding 10% NaOH to the plant extract, the Flavonoids Alkaline Reagent Test finds flavonoids by noting a brilliant yellow tint that becomes colorless when diluted HCl is added. For the detection of phenolic compounds, a type of plant extract were performed through a ferric chloride test, resulting in a greenish black color formation. The ferric chloride test detected tannins by forming a dark green color when a mixture of plant extract and 5% ferric chloride solution was mixed. Salkowski's test was used to detect terpenoids by mixing 0.5 mL of crude extract with 2 mL of chloroform and 3 mL of H_2SO_4. The presence of coumarins was confirmed by adding 1.5 ml of 10% NaOH to 2 ml of plant extract, resulting in a bright yellow color. Salkowski's test detects steroids by mixing the crude extract with chloroform and H2SO4, resulting in a red interface.

Antidiabetic study (In vivo diabetic mice model):

Experimental animals: Swiss albino mice (around 18-25 g) at the age of eight weeks were collected from ICDDR,B, Dhaka, Bangladesh. The *in vivo* anti-diabetic experiments were conducted following the previously described protocol (Sivashanmugam and Chatterjee, 2020). All animal procedures were reviewed and approved by the Ethical Committee of Jagannath University in accordance with the Institutional Animal Care and

Use Committee (IACUC) guidelines (Ref. No. 18/2023, JnU, ERC). They were kept in animal cages under standard environmental conditions (22-25°C, humidity 60-70%, 12 h light: 12 h dark cycle) and allowed to have access to food and unrestricted water. The mice were provided with a standard pellet diet obtained from ICDDR,B, Dhaka. Throughout the anti-diabetic study, the animals had continuous access to this diet and water ad libitum. The laboratory environment was maintained in a clean and hygienic condition using antibacterial cleaning procedures. The bedding (husk) used for the animals was autoclaved and disinfected, while the cages were sterilized and equipped with feeding and water bottles before housing the animals. Strict hygienic measures were followed to prevent infections in diabetic mice.

Induction of diabetes: In the present study, methylglyoxal (MGO) was used as *in vitro* precursor of AGEs to induce DM in mice, following the previously reported protocol (Nigro *et al*., 2014; Di Lorote *et al*., 2004). Metformin served as the standard drug (positive control) due to its well-documented MGO-derived AGE-inhibitory properties (Chaturvedi *et al*., 2018; Shah and Dikshit, 2018; Widjaja *et al*., 2019). MGO was administered intraperitoneally once daily at a dose of 60 mg/kg for three consecutive weeks to induce oxidative stress, inflammation, and vascular complications characteristic of DM (Sankaralingam *et al*., 2018). Blood samples were collected from the tail vein, and plasma glucose levels were determined using the BestCheck glucometer (Taiwan). Mice exhibiting fasting blood glucose levels between 8.5 and 11.5 mmol/L were considered diabetic. Healthy mice were categorized by age group and used to evaluate the effects of plant extracts on normal physiological conditions. The seed extracts of TFE, were administered orally at two different doses of 200 mg/kg and 700 mg/kg/day, respectively. Metformin (MET) was given as a positive control group at a dose of 300 mg/kg/day. All the treatments were administered via oral gavaging using a 16-gauge gastric tube once daily for the final two weeks of the study. This method simulated the traditional oral use of ethnomedicines for treating various ailments, followed by previously reported methodologies (Shah *et al*., 2020). Throughout the experiment, mice were closely monitored through daily cage-side observations. Parameters such as body weight, food intake, and water intake were recorded daily, while blood glucose levels were measured weekly. Additionally, physical and behavioral parameters were observed for any abnormalities, including changes in skin, eyes, mucous membranes, and respiratory patterns, as well as alterations in circulatory (heart rate, blood pressure), autonomic (salivation, lacrimation, perspiration, piloerection, urination, defecation), and central nervous system functions (drowsiness, gait, tremors, convulsions).

Acute toxicity study: The acute toxicity evaluation of TFE was conducted in accordance following the previously described method (Ragasa *et al*., 2015). In brief, six swiss albino mice (8 weeks old, weighing 18–25 g) were used for the study. The animals were fasted for three hours prior to the experiment and one hour following oral administration of the plant extracts, with access to water ad libitum. Initially, the first mouse in each experimental group received a single oral dose of 1 g/kg of TFE, and subsequent mice received the same doses sequentially after observing the effects on the preceding animal. The mice were housed separately and closely observed for signs of behavioral and physical toxicity. Observations were made continuously for the first 30 minutes and intermittently over a period of 4 hours, followed by continuous monitoring for 24 hours (Shah *et al*., 2020). During this period, cage-side observations were conducted daily to monitor changes in skin, eyes, and mucous membranes (nasal), as well as alterations in respiratory rate, circulatory system (heart rate and blood pressure), autonomic responses (salivation, lacrimation, perspiration, piloerection, urination, and defecation), and central nervous system activity (drowsiness, gait, tremors, and convulsions). Based on the results of the acute toxicity assessment, no mortality or significant toxic symptoms were observed at the tested doses. Therefore, 200 mg/kg and 700 mg/kg doses of TFE

were selected for subsequent evaluation of their anti-diabetic activities.

Hypoglycemic assay: For conducting the anti-diabetic study, all the mice were randomly divided into four groups, containing four mice (n = 4) of each group, following the previously described method (Shah *et al*., 2020). Induction of diabetes was performed by intraperitoneal administration of methylglyoxal (MGO) at a dose of 60 mg/kg body weight once daily for two consecutive weeks (Wasana *et al*., 2021). This administration of MGO has been reported to cause oxidative stress, inflammation, and vascular complications associated with DM. After proper induction of diabetes, a total of twenty (20) mice were allocated into seven experimental groups as follows: i) Negative control group: Only distilled water ii) Diabetic control group: MGO (60 mg/kg body weight) iii) Positive control group: Metformin (300 mg/kg/day body weight) iv) Low dose: *T. foenum graecum* seed extracts (TFE) at 200 mg/kg/day body weight v) High dose: *T. foenum graecum* seed extracts (TFE) at 700 mg/kg/day body weight. All the treatments were continued orally once daily by oral gavage using a 16-gauge gavage needle for 15 consecutive days in the morning.

Molecular docking study : Molecular docking studies were executed following a previously described method by using AutoDock Vina version 1.1.2 software. Previously reported twenty-five (25) compounds from fenugreek seed, having potential anti-diabetic effect, were selected as ligands, and Sclerostin was used as a target protein. The compounds are Riboflavin, Quercetin, Disogenin, Myristicin, Kaempferol, 2-Dodecenal, Luteolin, Isovitexin, Thymol, Yuccagenin, beta Tocopherol, Apiole, Carvone, Vitexin, Linolenic Acid, Limonene, Irilone, Deoxyrhapontin, Arachidonic Acid, Isoquercetin, Rutin, Levomenol, Biochanin A, Formononetin, and Gitogenin.

Preparation of protein: The three-dimensional structure of Sclerostin (PDB id: 6L6R) was downloaded in PDB format from the Protein Data Bank (www.rcsb.org). The retrieved PDB structure contains water molecules, heavy atoms, cofactors, metal ions, etc., and this structure does not have information about topologies, bond orders, and formal atomic charges. Hence, the downloaded PDB structure was prepared using the 'prepare protein' protocol of Discovery Studio 4.0. The target protein was prepared by removing all water molecules, ligands, and other hetero atoms from the structure. Hydrogen atoms were added to the atoms to satisfy the valence. The structure was then energy minimized using Swiss PDB Viewer in order to get a stable conformation.

Preparation of ligand molecule: Twenty-five (25) ligand molecules were selected, and compounds were downloaded in 3D SDF format from PubChem. The structures of the ligand molecules were geometrically optimized and energy minimized using Open Babel. Finally, docking simulation was performed using PyRx (0.8) software considering the protein as a macromolecule and compounds as ligand by maintaining grid box size 135.1420, 134.3354, and 94.3387 Å along X, Y, and Z directions, respectively, where the whole protein was covered by the grid box. Both the protein and docked structure were saved in PDB format to calculate the non-bonding interactions. Discovery Studio Visualizer was utilized to predict, analyze, and visualize the interactions between ligand and amino acid residues of the receptor protein.

Statistical analysis: Statistical analysis was performed by using GraphPad Prism software. All the experiments were performed in triplicate, and the results are presented as mean ± SD (standard deviation). One-way ANOVA and the Turkey test were used for *in vivo* analysis. The obtained results of three replicates are represented as the mean ± SEM of triplicate experiments. (*) $p < 0.05$ and (***) $p < 0.001$ vs. negative control group; (#) $p < 0.05$ vs. diabetic control group.

Results

Phytochemical constituents: Phytochemical screening assays confirmed the presence of alkaloids, phenolics, terpenoids, steroids, flavonoids, tannins, phenols, and glycosides in the crude ethanolic extract

and its different fractions of *T. foenum-graecum*, as shown in Table 1 and Figure 1. From the results, the n-hexane and aqueous fractions were confirmed with almost all of the phytoconstituents except alkaloids and saponins, respectively. On the other hand, chloroform fractions were found with the presence of glycosides and saponins, whereas ethyl acetate fractions were confirmed with terpenoids, steroids, and flavonoids, etc. Mentionably, the crude ethanolic extract fraction (TFE) has been seen to confirm the presence of almost all of the phytoconstituents than the other fractions of aqueous, chloroform, ethyl acetate, and n-hexane fractions (Figure 1); thus, the rest of the experiment was continued with this crude ethanolic extract, TFE.

Table 1. Qualitative phytochemical analysis conducted through ethanolic, hexane, chloroform, ethyl acetate, and aqueous extract of *TFE*.

Sl. No	Chemical constituents	Phytochemical test	Crude Ethanolic Extract (TFE)	n-Hexane Extract	Chloroform Extract	Ethyl Acetate Extract	Aqueous Extract
1	Alkaloids	Wagner reagent test	++++	-	-	-	++
2	Phenolic compund	Ferric Chloride test	++++	+	-	-	+
3	Terpenoid	Salkowski test	++++	+	-	+	+++
4	Steroid	Salkowski test	++++	+	-	++	++
5	Flavonids	Alkaline reagent test	++++	+	-	++	+++
6	Glycoside	Keller Killiani test	++++	++	++	-	+++
7	Tannin	Ferric Chloride test	++++	+	-	-	+
8	Saponins	Foam test	++++	+++	++	+	-

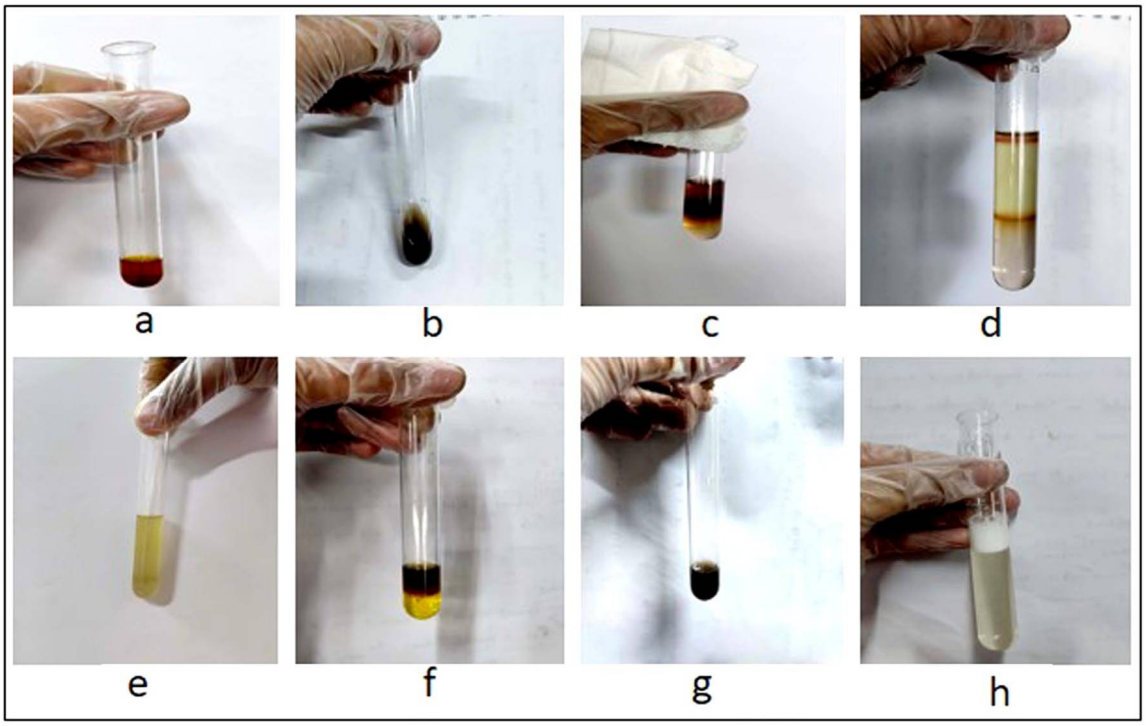

Figure 1. Phytochemical analysis of TFE crude ethanolic extract a) Alkaloids, b) Phenolic compounds, c) Terpenoids, d) Steroids, e) Flavonoids, f) Glycosides, g) Tannins, h) Saponins.

Evaluation of the effect of TFE crude ethanolic extract as a novel anti-diabetic agent in an in vivo diabetic mice model

Acute toxicity test: From the acute toxicity study, it is obvious that the single oral administration of TFE at 2 g/ kg in four diabetic mice does not show any symptoms of mortality, even any signs of acute toxicity during the physical and behavioral observations during this study. Thus, it is assumable that the approximate LD50 value of TFE extract is more than 2 g/kg. Therefore, TFE at two different concentrations (200 and 700 mg /kg/day), denoting low and high doses were used for the evaluation of an *in vivo* antidiabetic mice model.

Effect on blood glucose level: The treatment of TFE at two different concentrations (200 and 700 mg /kg/day) was seen to decrease the blood glucose level significantly, as shown in Figure 2. From the study results, the hypoglycemic activity of TFE was observed in a dose-dependent manner during the study periods, also in coordination with the previous study results (Goyal *et al*., 2016; Hina *et al*., 2025). Mentionable that MGO-induced diabetic mice were noticed with increased blood glucose compared to the normal control group.

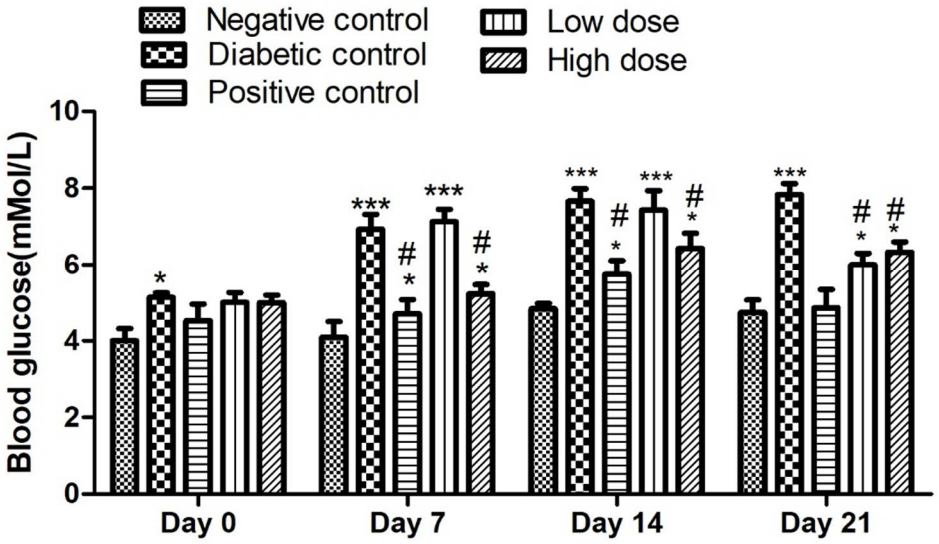

Figure 2. Effect of TFE on blood glucose level (mMol/l) in MGO induced diabetic mice model.

Effect on body weight: In case of body weight, there were no significant differences in the average weights across all the groups initially at the start of the study. By Day 7, the diabetic control (DC) group showed an increase in weight compared to the normal control group, indicating hyperglycemia-induced weight gain. This trend continued until Day 21, when the diabetic control group recorded the highest weight (30.1 g), compared to the normal control group (23.3g). By Day 21, the high-dose group maintained an average weight of 28.4 g, while the low-dose group showed a similar trend with 27.4 g. This suggests a dose-dependent normalization of weight in TFE-treated groups, indicating the anti-diabetic effect of TFE (Figure 3).

Molecular docking study results: For performing the molecular docking analysis, firstly, Sclerostin(6L6R) was used to interact with previously discovered twenty-five (25) molecules of fenugreek. Then, top ten (n = 10) compounds for Sclerostin (6L6R) were selected based on the Autodock vina docking program's best docking scores. Then, molecular docking analysis was performed between sclerostin (6L6R) and the major active phytoconstituents of fenugreek to evaluate and investigate the potential targets of fenugreek. From

the obtained docking scores, Yuccagenin and Diosgenin had the best docking score of -9.8 kcal/mol as shown in (Table 2 , Figure 4 & 5) with H-bond formation at the position of LEU575, TRP550, and TRP550, ARG553 respectively. Inversely, the lowest binding score was noticed with Luteolin (-7.6 kcal/mol), having H-bond formation at the position of GLU115, THR116, and ARG118.

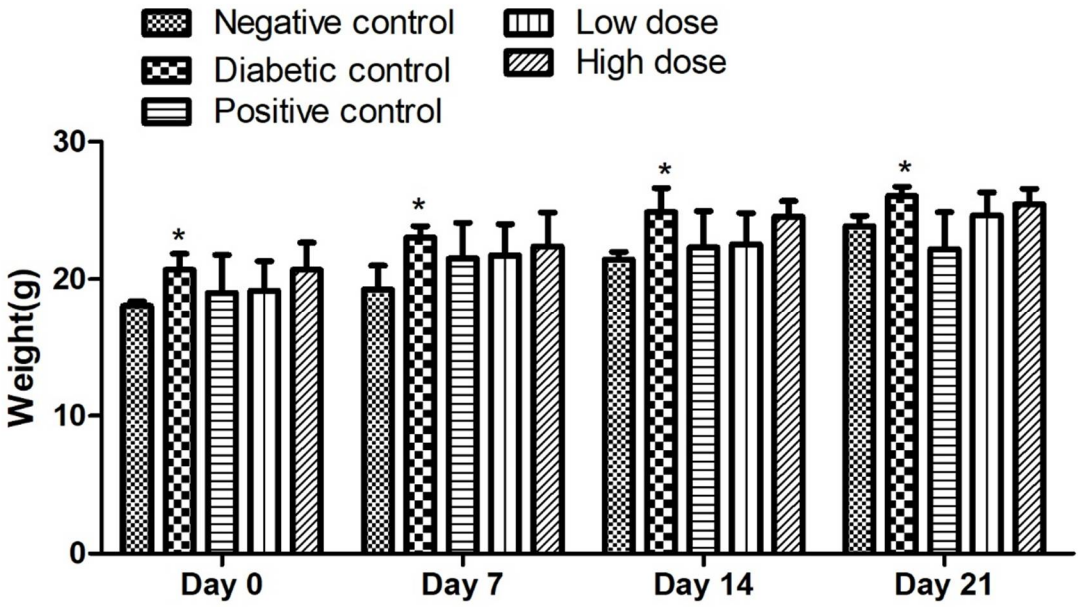

Figure 3. Effect of TFE on body weight (g) in MGO induced diabetic mice model.

Table 2. Docking scores of top ten (10) compounds for Sclerostin (6L6R) , major target protein of Wnt signaling pathway.

Protein with PDB ID	Ligand with CID	Molecuklar Docking score (Kcal/mol)	Interacting residues
Sclerostin (6L6R)	Yuccagenin (CID:3083608)	-9.8	LEU575, TRP550
	Diosgenin (CID:99474)	-9.8	TRP550, ARG553
	Gitogenin (CID:441887)	-9.7	GLN551, LEU575, TRP550
	Rutin (CID:5280805)	-8.6	VAL567, ASP570, ARG118, ASP348, ASN117, TRP134, ILE569
	Isovitexin (CID:162350)	-8.3	PHE133, GLN135, LYS130, VAL314, PRO311, GLY313, ARG173
	Isoquercetin (CID:5280804)	-8.1	PHE174, ILE175, ASP212, THR214, ARG216, SER179, ILE177, ASN178
	Desoxyrhaponticin (CID:5316606)	-7.9	ARG118, ASP348, GLU115, ILE568, ASP570, ARG340, LEU95, LEU572
	Irilone (CID:5281779)	-7.8	ARG341, ALA327, VAL314, ILE389, PHE388, HIS584
	Vitexin (CID:5280441)	-7.7	ASP351, ASP570, THR116, ARG118, ASN117
	Luteolin (CID:5280445)	-7.6	GLU115, THR116, ARG118

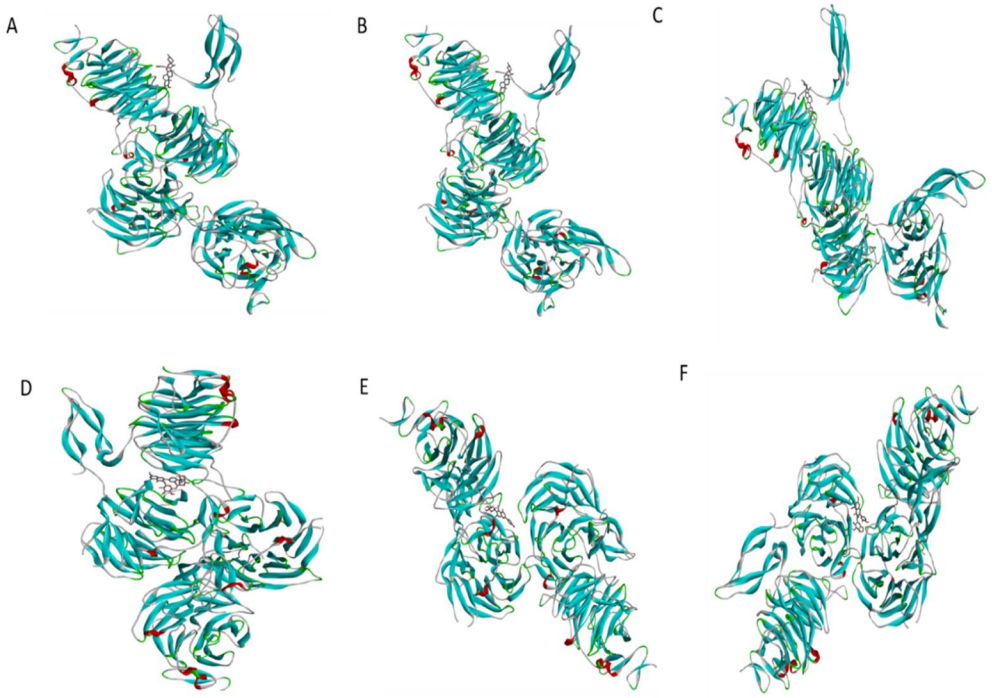

Figure 4. Molecular docking study of various active phytoconstituents against Sclerostin (6L6R) were performed by auto dock vina. The complex structure of Sclerostin (6L6R) with A) Yuccagenin B) Diosgenin C) Gitogenin D) Rutin E) Isovitexin, and F) Isoquercetin.

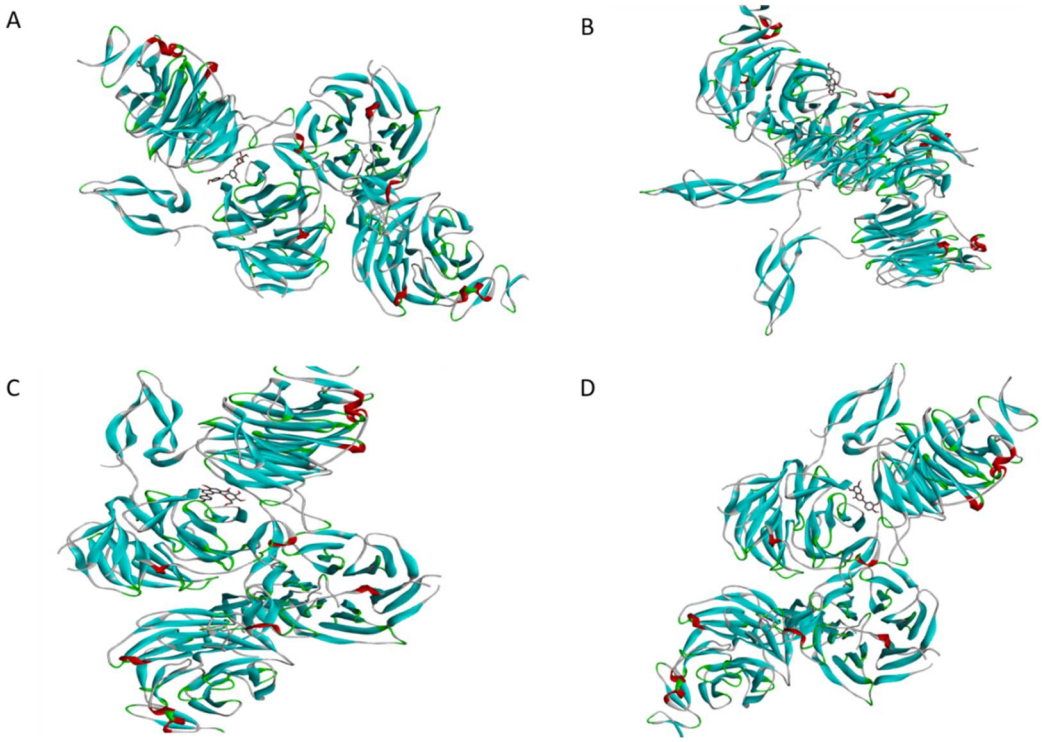

Figure 5. Molecular docking study of various active phytoconstituents against Sclerostin (6L6R) were performed by auto dock vina. The complex structure of Sclerostin (6L6R) with A) Desoxyrhaponticin B) Irilone C) Vitexin, and D) Luteolin .

Discussion

Protein glycation is considered as one of the major contributing factors for many chronic disorders, including DM reported through several research studies (Lee *et al*., 2020; Vistoli *et al*., 2013). When reducing sugars (such as galactose, mannose, glucose etc.) interact with the free amino groups of proteins, protein glycation occurs via schiff reactions with the irreversible formation of stable intermediatory products, namely advanced glycation end-products (AGEs) (Abate and Delbarba, 2015b; Lee *et al*., 2016). Several highly reactive compounds (MGO, GO, GA etc.) act as precursors of AGEs formation, which over time lead to the accumulation and act crucially in the development of diabetic complications (Hatfield, 2005; Nigro *et al*., 2014).

Fenugreek (*T. foenum-graecum*) seeds are native to various regions of the world, such as Asia, Europe, Australia, Africa, and the Americas. The seed is rich in essential phytochemicals and is widely used in the preparation of medicinal extracts and powders for its high dietary fiber and essential nutrients that support healthy growth and development. Numerous studies have documented its diverse pharmacological and therapeutic properties, including antimicrobial, anticholesterolemic, emollient, febrifuge, carminative, restorative, laxative, galactagogue, uterine tonic, expectorant, antioxidant, anticarcinogenic, anti-inflammatory, antiviral, demulcent, and hypotensive activities (Goyal *et al*., 2016; Hina *et al*., 2025). These medicinal attributes have promoted the widespread use of fenugreek extracts and powders in both the food and pharmaceutical industries (Rahman *et al*., 2021). Based on the identified research gaps, this study was designed to investigate the role of advanced glycation end-products (AGEs) in diabetes, with a specific focus on the Wnt signaling pathway involved in osteoporosis. This approach aims to enhance the understanding of disease pathogenesis and contribute to the development of new therapeutic strategies.

Preliminary qualitative identification of the different important phytochemical groups (alkaloids, phenolics, terpenoids, steroids, flavonoids, tannins, phenols, and glycosides) in *T. foenum graecum* seed extracts (TFE) provided the base for continuation with this crude ethanolic extract for further study. Based on our study results, ethanolic seed extracts of *T. foenum-graecum* (TFE) exhibited significant anti-diabetic activity in diabetic mice model in MGO-induced model. For evaluating the antidiabetic activity of TFE, two different doses (200 and 700 mg/kg/day) were chosen based on an the acute toxicity study to continue the *in vivo* study in the MGO-AGEs-induced diabetic mice model. Throughout the antidiabetic evaluation study period, a decreasing trend of body weight was observed in all of the experimental mice, although the changes were not statistically significant to mention. However, the diabetic control group (MGO-AGEs induced) showed noticeable weight loss of approximately in comparison with their initial body weight. Interestingly, the crude ethanolic extracts (TFE), at both doses, were noted to mitigate the weight loss. These findings suggest that TFE might have anti-glucotoxic effects, potentially by mitigating weight changes associated with diabetes. It is also evident that TFE was able to lower the blood glucose level of MGO-administered mice in a dose-dependent manner, which is also in agreement of previous study reports (Moradi and Zadeh, 2015). It is also presumed that the TFE could reduce the formation of AGEs and thus it might lower the level of Sclerostin, which might reduce the bone resorption in diabetic patients and could be effectively addressed as an alternative therapy in the prevention of the formation of AGEs in diabetic patients.

Computational drug discovery plays a crucial role in drug discovery and research, including the discovery and development of new compounds, identification of drug targets, validation of drug–target interactions, lead discovery and optimization, as well as preclinical evaluation (Karakaya *et al*., 2019). Nowadays, structure-based screening techniques are among the most widely used approaches for identifying small molecules with desirable drug-like properties for new drug discovery

beyond the hectic and time-consuming biological experiment-based study (Sultana *et al*., 2024). In this study, we investigated the binding affinity and bound conformations of potential interactions of residual amino acids with intermolecular distances of the small molecules of sclerostin (6L6R). Then, the molecular docking analysis was performed between Sclerostin (6L6R) and the major active phytoconstituents of fenugreek to evaluate and investigate the potential targets of fenugreek. Based on our obtained results, Yuccagenin and Diosgenin have shown the highest docking score towards Sclerostin around – 9.8 kcal/mol with the formation of different types of chemical bonds at multiple positions. Besides that, the second-highest docking score was noticed during interactions with Gitogenin(−9.7 kcal/mol) and Rutin (−8.6 kcal/mol), having H-bond formation at GLN551, LEU575, TRP550 and VAL567, ASP570, ARG118, ASP348, ASN117, TRP134, ILE569, respectively. However, the least binding affinities, having the lowest docking score, were noticed against Luteolin (−7.6 kcal/mol). Moreover, several intermolecular interactions, including hydrogen bonds, were involved, which strongly defend the potential role of these ligands towards Sclerostin. In particular, the binding affinities and different bonding of Sclerostin towards its target proteins were noted to activate its associated canonical Wnt-Signaling Pathway in osteoporosis (Table 2 and Figure 4 & 5). Therefore, these active phytoconstituents of fenugreek can be a source of potential anti-diabetic agents targeting Canonical Wnt-Signaling Pathway in diabetes induced chronic osteoporosis condition. Based on these findings, a further study including other target proteins of the Canonical Wnt-Signaling Pathway involved in diabetes induced osteoporosis will be executed in the future by using these major active phytoconstituents of fenugreek to evaluate and investigate the potential targets of fenugreek. In summary, *T. foenum-graecum* (fenugreek) seed extract demonstrated significant antidiabetic potential in this study of MGO-induced diabetic mice model, which highlighting its potential as a source of novel bioactive phytoconstituents for the development of new therapeutic antidiabetic agents.

Conclusion

The findings of this study concluded that the crude ethanolic seed extracts of *T. foenum-graecum* has prominent antidiabetic effect by significantly reducing body weight as well as blood glucose levels in MGO-induced *in vivo* diabetic mice model. It is also evident from molecular docking study results that different phytoconstituents of TFE has the promising ability to inhibit MGO –derived AGEs accumulation. The overall results provide scientific proof for the traditional use of *T. foenum-graecum* seed in folk medicine to manage diabetes and its associated complications. Further research related to the mechanisms behind its antidiabetic effects, and bioactivity-guided investigations as well as clinical studies is needed to isolate the key compound responsible for the claimed antidiabetic activity of *T. foenum-graecum* seed to explore novel antidiabetic agents.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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