

Exploration of Controlled Hypoglycemic Effect in Streptozotocin-Induced Type 1 Diabetes and Antibacterial Activity of Combination of Ethanolic Extract of Garlic (*Allium Sativum*) and Ginger (*Zingiber Officinale*) Stem; *In Vivo* Studies Supported by *In Silico* Study

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ABSTRACT

Ginger and Garlic have high pharmacological properties. Garlic and ginger stem extract (1:1) with absolute ethanol (GGESE) has been studied for their potential health benefits, including antidiabetic, antibacterial effects as well as their potentiality to aid in body weight reduction. Normal food along with GGESE extract was given to two groups of mice, and a weight variation test was performed. Along with normal foods, after 13 weeks of GGESE administration a moderately weight loss was observed in male mice group, and the difference was statistically significant. Two hours before blood collection, a single dose of 400, 800 mg/kg of the GGESE extract was given orally to control/experimental group of mice to determine the effects on blood glucose (BG) and oral glucose tolerance test (OGTT). Streptozotocin-induced type 1 diabetic mice, the test group, was treated at same dosage level to examine its antidiabetic effects in terms of BG control, body weight. The dose of 800 mg/kg showed the highest BG reduction that was from 19.7±0.51 mg/dl to 11.78±0.57 mg/dl in diabetic mice ($p < 0.01$). By identifying the zone of inhibition against different gram positive and gram-negative bacteria, antibacterial activity was evaluated at 1000, 800 and 600 µg/disc mg/ml concentration of the extract. 1000 µg/disc showed highest zone of inhibition for *S. aureus* (18±0.97 mm), *B. cereus* (16±0.83 mm), *S. typhi* (15±0.81 mm), *P. aeruginosa* (16±0.59 mm). Furthermore, during our docking study, it was observed that ginger and garlic exhibited the highest fitness scores of -5.71 and -6.04, respectively, when interacting with the peroxisome proliferator-activated receptor gamma (PPARγ) enzyme, out of the ten major isolated compounds. The study evidenced scientifically the beneficial use of GGESE as an alternative medicine in management of diabetes and bacterial infection.

Keywords: Diabetes Mellitus; GGESE; Sitagliptin; Streptozotocin; Zone of Inhibition; *In Silico* Studies; Receptor-Ligand Binding

Introduction

Exploring the potential therapeutic benefits of a combined extract of garlic and ginger stem in controlling blood glucose levels and preventing bacterial infections in individuals with type 1 diabetes. The study is based on the hypothesis that the bioactive compounds found in garlic and ginger stem may have synergistic effects in reducing hyperglycemia and inhibiting bacterial growth [1,2]. Millions of people worldwide suffer from diabetes, a chronic metabolic disorder. In all cases, type 1 diabetes has been reported for nearly 5-10%, in the last twenty years. It is characterized by the incapability of the million pancreases to produce ample quantities of insulin, which can lead to increased blood glucose levels and several complications. One of the common complications of diabetes is bacterial infections, which are more prevalent in individuals with poorly controlled blood glucose levels [3,4]. Garlic may enhance insulin sensitivity in peripheral tissues, such as the liver, muscles, and adipose tissue. Insulin resistance, which is a common feature of type 2 diabetes, occurs when peripheral tissues become less responsive to the actions of insulin, leading to elevated blood glucose levels. By enhancing insulin sensitivity, garlic may improve glucose uptake and utilization in peripheral tissues, thereby reducing blood glucose levels [5]. Ginger has been shown to enhance insulin sensitivity in peripheral tissues, which can lead to improved glucose uptake and utilization by these tissues, thereby reducing blood glucose levels. Ginger may help to inhibit glucose production in the liver, which can also help to reduce blood glucose levels.

It may increase glucose uptake in muscle cells, which can improve glucose utilization and reduce blood glucose levels [6]. Garlic and ginger are widely recognized for their medicinal properties and have been traditionally used for various therapeutic purposes. Several studies have suggested that these two herbs have hypoglycemic and antibacterial effects, respectively [6,7]. However, the potential synergistic effects of a combined extract of garlic and ginger in treating diabetes and preventing bacterial infections have not been extensively studied. *In silico* molecular docking is a powerful computational technique used in drug discovery and molecular biology research. It enables scientists to investigate and predict the interactions between small molecules (ligands) and target proteins, providing valuable insights into their binding affinity and potential therapeutic applications. *In silico* docking has a significant impact on the rational design and optimization of pharmacological concepts, assisting in the selection of potential substances for additional experimental validation [8]. Consequently, the goal of this study is to explore the potential antibacterial activity on nine different pathogens and hypoglycemic effects on type 1 diabetes caused by streptozotocin by using combinedly prepared ethanolic extracts of ginger and garlic stem extract. The findings of this research could provide insightful information about the development of novel therapeutic agents for diabetes management and related complications. Furthermore, by using simulation technique, ligand-protein binding affinity will be calculated here.

Materials and Methods

Identification and Collection of Plant material

The stem of *Allium Sativum* (Garlic) and *Zingiber officinale* (Ginger), were collected from distinctive parts of the northern locale of Bangladesh. Within the month of April, we collected garlic and ginger plant stems. The plants were distinguished by Dr. Shayla Sharmin Shetu, Taxonomist and Assistant Professor, Department of Botany, Jahangirnagar University.

Preparation of Extract

Each plant material was dried, ground into a powder (1500 g), and doused in 3 L of ethanol at room temperature (23.0 ± 2) °C.) for 15 days with 3–5 days of interim. The filtrate was obtained through cheesecloth and No. 1 Whatman channel paper. Rotary evaporator (RE 200, Sterling, UK) used under reduced weight at temperatures below 50°C. The extract was set in a sealed shut glass tube. Approximately 25 gm of the extract was suspended in 20 ml of DW, and the suspension was shaken enthusiastically on a vortex blender. For hypoglycemic study, the concentration 35.33 mg/mL of the extract was prepared, for the antibacterial study as well [1,9,10].

Chemicals and Machineries

All chemicals and reagents were of explanatory grade. Carbon tetrachloride was obtained from Merck (Germany). Accu-Chek Aviva Plus Blood Glucose Observing System, Roche Diabetes Care, USA; with strips were acquired from Dhaka, Bangladesh. Sitagliptin (phosphate salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro [1,2,4] triazolo[4,3-a] pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl) butan-2-amine) used in this study was supplied by Incepta Pharmaceuticals Limited, Bangladesh. Agar supplement was obtained from Merck, India. Kanamycin (30 µg/disc, Oxoid, Britain) was utilized as a standard anti-microbial circle.

Animals and Experimental Set-Up

Swiss albino mice weighing from 25 to 30 grams were obtained from Jahangirnagar University in Savar, Bangladesh. The animals were kept on a natural day-night cycle with adequate ventilation in the chamber and were given normal laboratory food and refined water freely. All the investigations were carried out in a quiet, secluded environment. The Planning & Development Committee, Department of Pharmacy, Jagannath University, and BCSIR, Bangladesh, gave their approval to the study protocol. Prior to the trial, the mice were given a seven-day acclimatization period to laboratory conditions. The Department of Pharmacy, Jagannath University and BCSIR gave their approval to the study protocols, and all animal studies were conducted in accordance with their criteria.

Phytochemical Screening

Using freshly made GGESE extract solutions and the techniques outlined by Nishan et al., a qualitative analysis of phytoconstituents

including reducing sugars, combined reducing sugars, phenolic compounds, flavonoids, tannins, saponins, gums, steroids, terpenoids, alkaloids, glycosides, acidic compounds, amino acids and proteins were carried out [11,12].

Acute Toxicity Study

Following OECD guidelines, an acute oral toxicity study of GGESE was conducted. There were no fatalities during the 14 days of treatment with a GGESE dose with 4000 mg/kg body weight (Even 6000 mg/kg passed the acute toxicity test). All treated animals were able to tolerate the GGESE doses, and there was no statistically significant difference in body weight between the treated and untreated groups. There were no unusual behaviors or significant behavioral alterations in the animals. In a test of acute oral toxicity, the hair, face, eyes, and nose showed no adverse effects. For example, there were no tremors, visions, salivations, or diarrheal symptoms. The regressive actions and posture, as well as emotional states, were also consistent. Both the control and treatment groups weighed the same. Each animal got feed and water [13,14].

Weight Variation Test

Based on sex, 48 male and female mice were divided into four different groups. The animal was 7 days old overall. Normal food was given to both the male and female mouse groups. Two further groups received regular meals infused with ginger and garlic stem paste. Twelve mice were present in each group. Every week, their body weight was recorded and calculated as a mean weight after being measured [13].

Anti-Diabetic Effect

A well-known normoglycemic model was used to test the extract's hypoglycemic effect [11,15]. The animals in Group I (the normal control group) received saline, Group II (the positive control group) received Sitagliptin (100 mg/ 70 kg body weight) [16,17], and Groups III and IV received GGESE extracts in different strengths (400 mg/kg and 800 mg/kg, respectively). The glucose oxidase method was used to estimate blood glucose levels at 0 minute, 30 minutes, 60 minutes, and 120 minutes [14]. The Roche Diabetes Care Accu-Chek Aviva Plus Blood Glucose Monitoring System was used to measure glucose levels.

In Vitro Antibacterial Activity

Microorganisms: To reveal the antibacterial effect of GGESE nine bacterial species are used. The Gram-positive bacteria were *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*), *Bacillus cereus* (*B. cereus*), and *Mycobacterium tuberculosis* (*M. tuberculosis*), and the gram-negative bacteria were *Salmonella typhi* (*S. typhi*), *Salmonella paratyphi* (*S. paratyphi*), *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Proteus mirabilis* (*P. mirabilis*). Zone of inhibition was measured by using these bacterial microorganisms. The pharmacy department of Jagannath University, and BCSIR provided these microorganisms.

Anti-Bacterial Screening: At 37 °C and pH 7.4±0.2, On Nutrient agar (Merck, India) media, all bacterial strains were grown and retained in existence. Overnight, the bacteria were sub cultured. All the extracts were diluted in their respective solvents for the investigation of the antibacterial activity. The extract-specific concentrations were given in terms of µg/disk. The discs, which had a diameter of around 5 mm, were punched out of Whatman No. 1 filter paper using a punching machine. The discs were placed in a Culture plate, autoclaved, and dried at 180°C in the oven. The disc diffusion method was used to test the antibacterial effects, with some slight modifications. The filter paper discs (5 mm in diameter) were individually coated with 24 µL of 800 g/disk and 30 µL of 1000 g/disk of various plant extracts before being placed within 15 minutes on the agar plates that had already been inoculated with the test microorganisms. Prior to incubation at 37 °C for 24 hours, at 4 °C for 3 hours petri dishes were kept. The inhibitory zones' sizes were measured in millimeters. The experiments were all run twice. A blank disc coated with methanol, ethanol and distilled water was used as a negative control. 30 g of kanamycin in a disc was used as a positive control [15,16].

In Silico Molecular Docking Study: The potent active compounds of Ginger and Garlic against the active site of the peroxisome proliferator-activated receptor gamma (PPAR γ) [16] enzyme were predicted using Glide, a molecular docking tool in Schrödinger Maestro (version 10.1, Schrödinger, LLC New York, NY, USA) [17]. The compounds utilized in the study were obtained from a thorough review of the literature [18].

Ligand and Protein Preparation: The five main chemicals that were identified from garlic (*Allium sativum*) and their chemical compositions, including Allicin (PubChem ID: 65036), Diallyl sulfide (DAS) (PubChem ID: 11617), Diallyl disulfide (PubChem ID: 16590), Diallyl trisulfide (PubChem ID: 16315), and S-allyl cysteine (PubChem ID: 9793905), were obtained from the PubChem Project database. These compounds were structurally plotted in 3D using Ligprep 2.5 in Schrödinger Suite 2013. The ionization states of these compounds were generated at pH (7.0 ± 2.0) using Epik 2.2 in Schrödinger Suite. Similarly, five major compounds isolated from Ginger (*Zingiber officinale*), namely Gingerol (PubChem ID: 442793), Shogaol (PubChem ID: 5281794), Zingerone (PubChem ID: 31211), Zingiberene (PubChem ID: 92776), and Curcumin (PubChem CID: 969516), were retrieved from the database of the PubChem Project. Schrödinger Suite 2013's Ligprep 2.5 was used to structurally layout these compounds in three dimensions. Their ionization states were produced in the Schrödinger Suite's Epik 2.2 at pH (7.0 2.0). Peroxisome proliferator-activated receptor gamma (PPAR) enzyme's 3D structure (PDB: 2XYJ) was retrieved from the Protein Data Bank in order to prepare the protein. The obtained structure was prepared and refined using the protein preparation wizard in Schrödinger Maestro (version 10.1). Charges and bond orders were assigned, hydrogens were added to the heavy atoms, and selenomethionines were converted to methionine. All water portions were removed. Reorientation was performed for cer-

tain hydroxyl and thiol groups, and optimization maximum of amide groups of glutamine, asparagine, the imidazole ring of histidines, the protonation states of histidines, glutamic acid, and aspartic acid was carried out at neutral pH. The OPLS_2005 force field was then used to minimize, with a maximum heavy atom RMSD set at 0.30 Å.

Receptor Grid Generation: Grids were created in the Glide software using the OPLS_2005 force field's default parameters of a van der Waals scaling factor of 1.00 and a charge cut-off of 0.25. For the receptor, a cubic box of specific dimensions was created around the centroid of the active site residues, which represents the ligand activation site. This bounding box had dimensions of 16 Å × 16 Å × 16 Å and was utilized for the docking experiments. An important stage in the procedure was determining the target protein's active binding site.

Glide Standard Precision Ligand Docking: Using the Glide module in Schrödinger Maestro (version 10.1), flexible ligand docking with standard precision (SP) was performed. During the docking process, penalties were applied to non-cis/trans amide bonds. The molecules were docked to the peroxisome proliferator-activated receptor gamma (PPAR γ) enzyme using Glide SP docking, and hits with docking scores above 4 kcal/mole were further subjected to redocking in XP mode, keeping the default docking parameters. The docking calculations didn't include any bonding restrictions [19,20]. A Monte Carlo random search technique was used to produce ligand poses for each input molecule, and the Glide docking score was used to predict the binding affinity of these compounds to the PPAR enzyme. An empirical E model scoring function was used to evaluate the potential energies of the docked molecules. The OPLS_2005 force field

was used for post-docking reduction, and one posture per ligand was preserved [21]. Additionally, the strain energies of the ligands' bound and free forms were computed. The docking score was increased by a penalty equal to one-fourth of the strain energy difference for hits that showed an energy difference of more than 4 kcal/mole between the two forms.

Statistical Analysis

Dunnett's test was used to evaluate the data, which were reported as the mean standard deviation for the zone of inhibition and the mean standard error of the mean for the hypoglycemic effect, respectively. The significant level showed at $P < 0.05$, $P < 0.01$ and $P < 0.001$.

Results

Acute Toxicity Result

During the observation period, none of the animals showed any behavioral, neurological, or physical changes at the maximum dose of 6000 mg/kg of garlic and ginger stem extract, as measured by symptoms like restlessness, convulsions, and coma. There was no mortality seen at the test dose. Thus, it was discovered that the overall LD₅₀ of plant extracts was larger than 6000 mg/kg.

Phytochemical Screening

The presence of phenolic and terpene compounds, major polyphenols, coumarins, phenolic groups, alkaloids, and steroids, reducing sugar, combined reducing sugar, tannins, flavonoids, saponins, steroids, terpenoids, alkaloids, glycosides, anthraquinones, protein, and acidic compounds were all revealed by phytochemical analysis of the combinedly prepared garlic and ginger stem extracts.

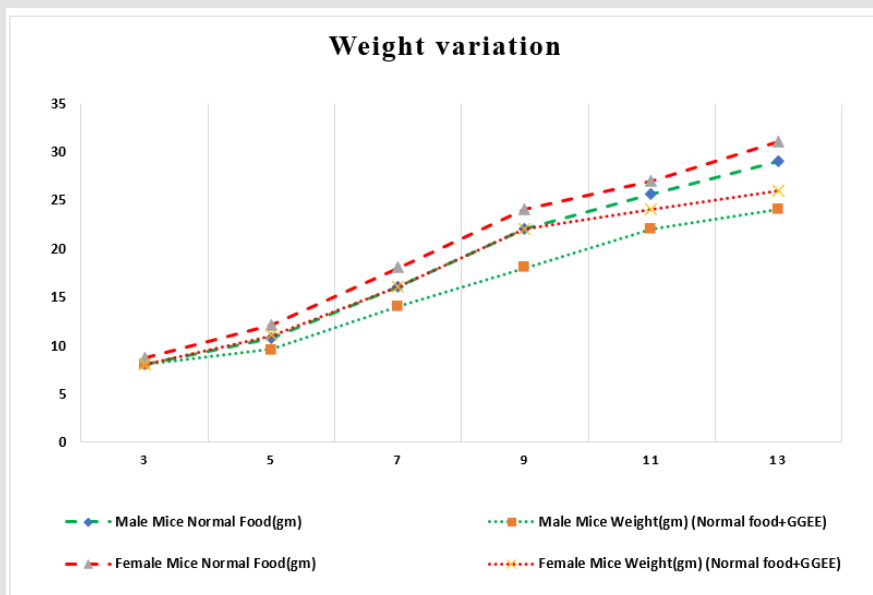


Figure 1.

Weight variation: Normal Feeding vs Ginger and Garlic Stem past with Conventional Feeding

Ginger and garlic stems had little impact on research animals. When given normal food, both male and female mice put on a lot of weight in comparison to other animal groups that were given normal food coupled with ginger and garlic stem. With typical feeding, female mice grow to be about 31 gm in 13 weeks. On the other hand, the group of female mice that consumed ginger and garlic in stem simultaneously gained about 26 gm. The group of male mice dispersed 24 gm in 13 weeks after consuming ginger and garlic stem together with regular diets. Contrarily, some groups show 29 gm at the same time when consuming typical items (Figure 1).

Hypoglycemic Effect in Normal Mice

(Table 1) The table shows the blood glucose levels (in mg/dl) of different groups at various time points throughout the 45-day age mice. The groups include a control group (G1), a diabetic control group (G2, Streptozotocin-induced type 1 Diabetes), a diabetic group treated with GGESE at 400mg/ml (G3), a diabetic group treated with

GGESE at 800mg/ml (G4), a diabetic group treated with Sitagliptin (a standard antidiabetic drug) (G5), and a group treated only with GGESE (G6). The results indicate that the diabetic control group (G2) had higher blood glucose levels at 30 min, 60 min and 120 min time points compared to the control group (G1). However, treatment with GGESE (in groups G3 and G4) or Sitagliptin (in group G5) showed a reduction in blood glucose levels compared to the diabetic control group (G2), with the most significant reduction observed in G5 treated with Sitagliptin at 100 mg/70 kg as a standard treatment of diabetic care. Interestingly, the group treated only with GGESE (G6) had lower blood glucose levels than the control group at all-time points, indicating a potential balanced glycemic effect of GGESE. Among all the extracts, GGESE 400 mg/ml reduced blood sugar level in diabetic induced mice at the time point of 30 minutes, 60 minute and 120 minutes without fluctuation. It reduced blood glucose from 19.5 ± 0.45 mg/dl (0 minute) to 14.42 ± 0.42 mg/dl (120 minutes). This was the significant change compared to control group and moderate change compared to standard group. In between 16.13 ± 0.21 mg/dl stood for 30 minutes and 15.54 ± 0.30 mg/dl for 60 minutes time point.

Table 1.

| Different Time Points of Blood Glucose Levels (mg/dl) in a same day (The Age of Animal: 45 days) | | | | |
|--|-----------------------------|------------------------------|------------------------------|-------------------------------|
| Groups | 0 Min Blood Glucose (mg/dl) | 30 Min Blood Glucose (mg/dl) | 60 Min Blood Glucose (mg/dl) | 120 Min Blood Glucose (mg/dl) |
| G1 (Control) | 11.87 ± 0.16 | 14.28 ± 0.71 | 15.41 ± 0.37 | 12.53 ± 0.19 |
| G2 (Diabetic control) | 13.42 ± 0.32 | 18.7 ± 0.20 | 21.46 ± 0.26 | 20.78 ± 0.43 |
| G3 (Diabetic + GGESE, 400mg/ml) | 19.5 ± 0.45 | $16.13 \pm 0.21^*$ | $15.54 \pm 0.30^*$ | $14.42 \pm 0.42^*$ |
| G4 (Diabetic + GGESE, 800mg/ml) | 19.7 ± 0.51 | $15.0 \pm 0.52^*$ | $13.47 \pm 0.33^{**}$ | $11.78 \pm 0.57^{**}$ |
| G5 (Diabetic + Sitagliptin, 100 mg/70 kg) | 18.5 ± 0.41 | 10.08 ± 0.22 | 10.0 ± 0.21 | 8.50 ± 0.46 |
| G6 (Randomly GGESE feeding) | 8.61 ± 0.55 | 9.24 ± 0.48 | 9.71 ± 0.37 | 10.8 ± 0.50 |

The high concentration of ginger and garlic stem extract showed significant result at 60 minutes and 120 minutes after administration of GGESE. In the case of diabetic control mice, GGESE 800 mg/ml showed around 40 percent blood glucose reduction rate in diabetic control group. The values reported for this group show a decrease in blood glucose levels over time, with the mean values decreasing from 19.7 ± 0.51 mg/dl at 0 minutes to $11.78 \pm 0.57^{**}$ at 120 minutes. Which was significant compared to both control and standard group. Other than 120 minutes after GGESE administration time zone, both 30 minutes and 60 minutes showed significant values those were consecutively 15.0 ± 0.52 mg/dl and 13.47 ± 0.33 mg/dl. According to the findings, diabetic mice treated with GGESE and Sitagliptin had lower blood glucose levels than the diabetic control group. When compared to the diabetic control group and the standard group, the blood glucose levels at 30 minutes, 60 minutes, and 120 minutes in the G3 (Diabetic + GGESE, 400mg/ml) group were moderately reduced. In G4 (Diabetic + GGESE, 800mg/ml), the blood glucose levels were significantly reduced at 60 minutes and 120 minutes compared to the

G2, G3, and G5. However, the reduction in blood glucose levels by Sitagliptin was more significant than GGESE at all-time points. But as a medicinal plant extract GGESE 800 mg/ml showed significant blood glucose lowering activity compared to the standard treatment group G5. At a level of $P < 0.01$, Sitagliptin demonstrated its significance.

When compared to the control, the dose of 800 mg/kg GGESE significantly decreased $P < 0.01$, according to Dunnett's test. These findings revealed that Sitagliptin had a high significance level and that GGESE's hypoglycemic action at a dosage of 800 mg/kg was significant.

In Vitro Antibacterial Activity

The antibacterial activity of GGESE was tested against nine pathogenic bacteria. All gram-positive and gram-negative bacterial samples were compared to the Kanamycin as a standard group. GGESE exhibited significant antibacterial activity against both Gram-positive and Gram-negative bacteria at the concentration of 800 and 1000 μ g/disc, as shown in Table 2. It was determined that GGESE's inhibition zone

against Gram-positive bacteria was moderately significant. In *S. aureus*, the zone of inhibition ranged from 14±0.57 mm to 18±0.97 mm, while in *B. cereus* it ranged from 13±0.77 mm to 16±0.83 mm. Another gram-positive bacterium, *B. subtilis*, showed a mild change in inhibition zone from 10±0.87 mm to 12±0.92 mm. However, the zone of inhibition for *M. tuberculosis* was too low significant compared to the standard drug, ranging from 8±0.94 at 600 µg/disc to 1000 µg/disc.

S. typhi had a zone of inhibition ranging from 13±0.82 mm to 15±0.81 mm at concentrations of 1000 µg/disc when the antibacterial activity of GGESE was tested against Gram-negative bacteria. *S. paratyphi*, on the other hand, was resistant to GGESE. Furthermore, GGESE exhibited a moderately high zone of inhibition against *E. coli* and *P. aeruginosa*, which were 14±0.56 mm and 16±0.59 mm, respectively. However, the zone of inhibition against *P. mirabilis* was relatively low.

Table 2.

| Name of the Bacteria | | GGESE | | Kanamycin | |
|----------------------|------------------------|--------------|-------------|-------------|--------------|
| | | 1000 µg/disc | 800 µg/disc | 600 µg/disc | 30 µg/disc |
| Gram-positive | <i>S. aureus</i> | 18±0.97** | 14±0.57* | 10±0.63 | 25.20 ± 0.66 |
| | <i>B. subtilis</i> | 12±0.92* | 10±0.87 | 7±0.76 | 22.20 ± 0.40 |
| | <i>B. cereus</i> | 16±0.83** | 13±0.77* | 10±0.64 | 27.00 ± 0.62 |
| | <i>M. tuberculosis</i> | 8±0.94 | 6±0.76 | 3±0.68 | 25.00 ± 0.41 |
| Gram-negative | <i>S. typhi</i> | 15±0.81** | 13±0.82* | 9±0.63 | 26.00 ± 0.39 |
| | <i>S. paratyphi</i> | NI | NI | NI | 25.00 ± 0.37 |
| | <i>E. coli</i> | 14±0.56* | 12±0.79 | 9±0.66 | 27.00 ± 0.44 |
| | <i>P. aeruginosa</i> | 16±0.59* | 12±0.83 | 10±0.67 | 25.00 ± 0.38 |
| | <i>P. mirabilis</i> | 9±0.75 | 6±0.88 | 5±0.78 | 23.00 ± 0.51 |

In Silico Study: Molecular Docking Study for Anti-Diabetic Activity

(Figure 2, Tables 3 & 4).

Table 3.

| Compound name | Compound Information | Protein/Receptor Name | Docking score (kcal/mol) |
|--------------------|--|-----------------------|--------------------------|
| Allicin | MF: C ₆ H ₁₀ OS ₂ MW: 162.3 g/mol | PPAR γ | -5.538 |
| Diallyl sulfide | MF: C ₆ H ₁₀ S MW: 114.21 g/mol | PPAR γ | -5.71 |
| Diallyl disulfide | MF: C ₆ H ₁₀ S ₂ MW: 146.3 g/mol | PPAR γ | -5.17 |
| Diallyl trisulfide | MF: C ₆ H ₁₀ S ₃ MW: 178.3 g/mol | PPAR γ | -4.74 |
| S-allyl cysteine | MF: C ₆ H ₁₁ NO ₂ S MW: 161.22 g/mol | PPAR γ | -3.48 |

Note: Simulation result; Receptor- peroxisome proliferator-activated receptor gamma (PPAR γ) with isolated compounds of Garlic (*Allium sativum*):

Table 4.

| Compound name | Compound Information | Protein/ Receptor Name | Docking score (kcal/ mol) |
|---------------|--|------------------------|---------------------------|
| Gingerol | MF: C ₁₇ H ₂₆ O ₄ MW: 294.4 g/mol | PPAR γ | -5.16 |
| Shogaol | MF: C ₁₇ H ₂₄ O ₃ MW: 276.4 g/mol | PPAR γ | -5.55 |
| Zingerone | MF: C ₁₁ H ₁₄ O ₃ MW: 194.23 g/mol | PPAR γ | -5.84 |
| Zingiberene | MF: C ₁₅ H ₂₄ MW: 204.35g/mol | PPAR γ | -5.89 |
| Curcumin | MF: C ₂₁ H ₂₀ O ₆ MW: 368.4g/mol | PPAR γ | -6.04 |

Note: Simulation result; Receptor- peroxisome proliferator-activated receptor gamma (PPAR γ) with isolated compounds of Ginger (*Zingiber officinale*).

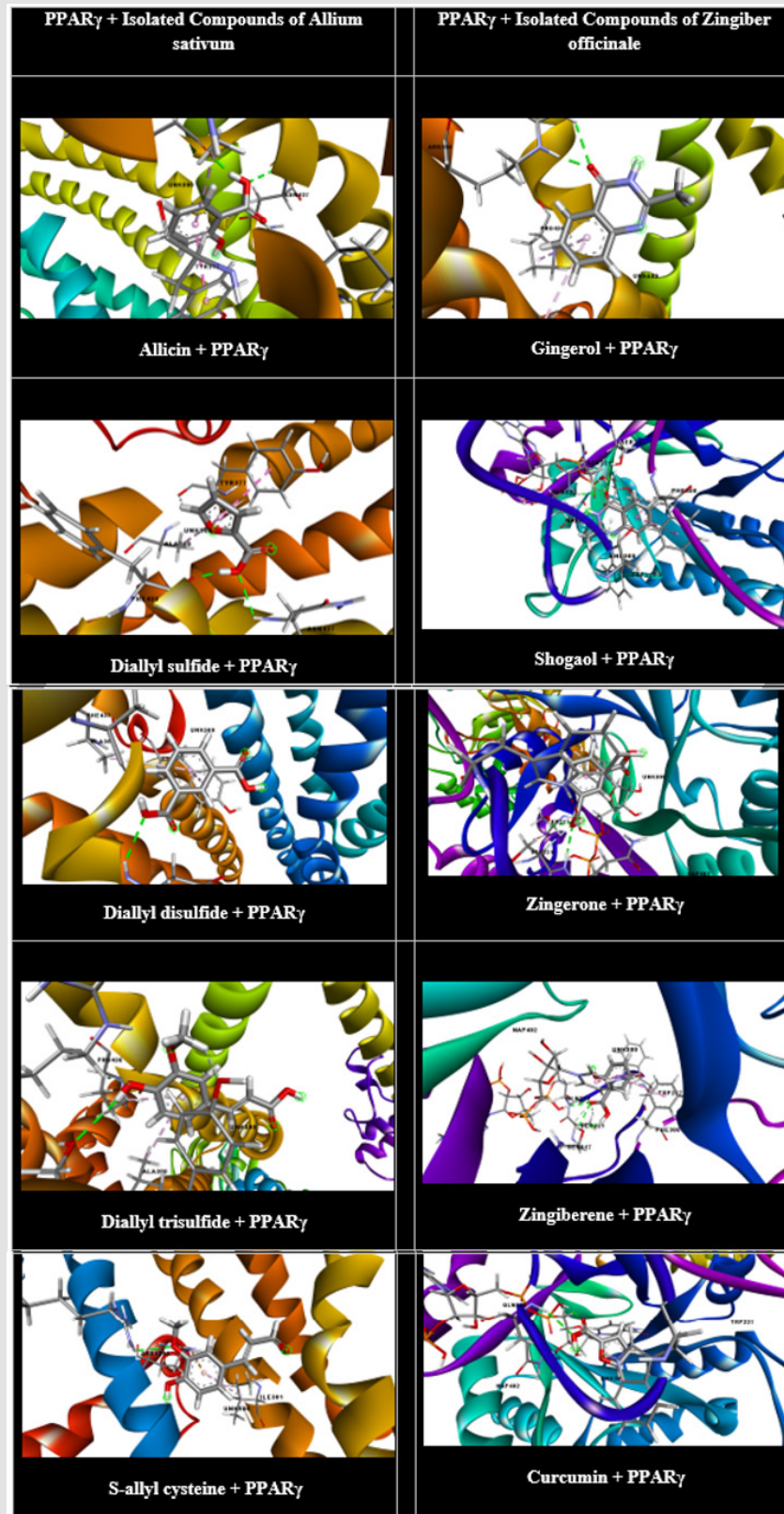


Figure 2: Illustration of the molecular docking contact between isolated *Allium sativum* and *Zingiber officinale* compounds and the PPAR gamma enzyme.

Discussion

The medical care system still faces the task of developing diabetes mellitus treatments with fewer side effects. It has long been presumed that diabetes mellitus is associated with chronically elevated blood glucose levels. And it interferes with glucose uptake as well as glucose metabolism. As a result, research on medicinal plants is expanding to create reasonably safe antidiabetic plant-based products, either alone or in combination with existing medications [1,20]. In this study, a combined ethanolic extract of the stems of Garlic (*Allium sativum*) and Ginger (*Zingiber officinale*) significantly reduced fasting glucose levels in hypoglycemic mice model. After 2 hours of therapy, the blood glucose level had significantly decreased as compared to the control. To the best of our knowledge, this is the initial investigation of GGSE's hypoglycemic and antibacterial properties. Therefore, the precise method of action is yet to be determined. Plants are prominent sources of potentially useful frameworks for the development of new chemotherapeutic agents. The first step towards this goal is the in vitro antibacterial activity assay [19,21]. Therefore, our aim was to identify the antibacterial activity of GGSE.

The results indicate that the weight of male and female mice increased with time, which is expected as they are growing. However, the weight gain was slightly lower in the group that received normal food plus GGSE compared to the group that received only normal food. The difference in weight gain between the two groups was more noticeable male mice than in female mice.

At 13 weeks, male mice that received normal food plus GGSE had a weight of 24 grams, while those that received only normal food had a weight of 29 grams. Similarly, female mice that received normal food plus GGSE had a weight of 26 grams, while those that received only normal food had a weight of 31 grams. These results suggest that GGSE may have a slight inhibitory effect on weight gain in male and female mice. It is particularly important to keep in mind that the analysis did not account for additional elements like physical exercise or genetics that might have affected the mice's weight. Additionally, the sample size is modest which might restrict how broadly the results can be applied.

Conclusion

The given data provides some evidence that GGSE may have a slight inhibitory effect on weight gain in male and female mice, but further studies are needed to confirm this effect and to explore its potential implications for human health. Overall, the results suggest that GGSE has moderate antimicrobial activity compared with Kanamycin against the tested bacteria. Gram-positive bacteria appear to be more susceptible to both GGSE and Kanamycin than Gram-negative bacteria. Among the Gram-positive bacteria, *S. aureus* and *B. cereus* are the most susceptible to GGSE, while *B. subtilis* is the least susceptible. *M. tuberculosis* is the least susceptible among all bacteria tested. Among the Gram-negative bacteria, *S. typhi* and *P. aeruginosa*

are more susceptible to GGSE, while *E. coli* and *P. mirabilis* are less susceptible. *S. paratyphi* was not susceptible to either antimicrobial agent at the tested concentrations. The results showed that the diabetic control group (Group 2) had significantly higher blood glucose levels compared to the control group (Group 1) at all-time points. However, treatment with GGSE (Group 3 and Group 4) resulted in a significant decrease in sugar values at half hour, one hour and two hours compared to Group 2. These results suggested that GGSE may have potential as a therapeutic agent for managing blood glucose levels in diabetic patients. Interestingly, Group 4, which received a higher dose of GGSE, showed a decrease in blood glucose levels even at 0 minutes, although this was not seen in Group 3.

This could indicate a dose-dependent effect of GGSE on blood glucose regulation, with higher doses being more effective. It is also noteworthy that the positive control group, which received Sitagliptin, showed the most significant decrease in blood glucose levels at all-time points compared to other groups. This is consistent with the known glucose-lowering effects of Sitagliptin, which is a commonly used medication for managing blood glucose levels in diabetic patients. Lastly, Group 5, which received only GGSE without being diabetic, had lower blood glucose levels at all-time points compared to the control group. This indicates that GGSE may have some hypoglycemic effects even in non-diabetic individuals, which could have potential implications for the use of GGSE as a health supplement or functional food. Overall, the results of this study suggest that GGSE may have potential as a therapeutic agent for managing blood glucose levels in diabetic patients, and further research is warranted to explore its efficacy and safety in clinical settings. These findings indicated that GGSE may be effective in the management of diabetes and has a hypoglycemic principle. Further research is necessary to identify the isolated compounds from combinedly prepared GGSE extract and identifying the mechanism of actions of GGSE will be a dramatic part of future research. The presented molecular docking study, a computer-aided drug design technique, to investigate the binding interactions between five major compounds from *Zingiber officinale* (ginger) and *Allium sativum* (garlic) with the peroxisome proliferator-activated receptor gamma (PPAR γ) enzyme.

The docking scores, binding energies, and glide energies were calculated to evaluate the affinity and potential interactions between the compounds and the target receptor [22,23]. The results of the docking simulations indicated that macarangin and scopoletin, derived from *Zingiber officinale* and *Allium sativum*, respectively, exhibited the best fitness scores of -5.7 and -6.04 kcal/mol with the PPAR γ enzyme. These scores suggest a strong interaction and binding affinity between these compounds and the receptor. The docking scores of the other compounds, such as gingerol, shogaol, zingerone, zingiberene (from ginger), and allicin, diallyl sulfide, diallyl disulfide, diallyl trisulfide, and S-allyl cysteine (from garlic), also indicated favorable interactions with PPAR γ , although to a slightly lesser extent [24]. It is important to note that docking scores alone do not provide conclusive

evidence of the pharmacological effects or efficacy of the compounds. The pharmacological properties of these isolated compounds and their potential as pharmaceutical candidates or therapeutic agents must be confirmed by additional research. These additional investigations could include *in vitro* and *in vivo* experiments, such as cellular assays, animal models, and clinical trials, to assess the compounds' effects on PPAR γ activity and their potential therapeutic applications. Moreover, it would be valuable to investigate the specific binding modes and interactions between the identified compounds and the PPAR γ enzyme through further analyses, such as molecular dynamics simulations or structural studies. Understanding the precise binding mechanisms and the impact of these compounds on the protein structure could provide insights into their potential as PPAR γ modulators and aid in the design of more potent and selective compounds [8].

In conclusion, the molecular docking study presented here offers a preliminary assessment of the binding affinity and potential interactions between major compounds from ginger and garlic with the PPAR γ enzyme. The findings suggest that macarangenin and scopoletin exhibit strong binding affinity with PPAR γ , warranting further investigations. However, additional research, including experimental validation and structural analyses, is necessary to fully comprehend the pharmacological effects and therapeutic potential of these compounds as PPAR γ modulators [25].

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Conflicts of Interest

The authors declare no conflict of interest. All authors are equally contributed.

Kutub Uddin Ahamed and Nazim Uddin Ahmed did planning performing several test and guided overall project; Nilufa Akter, Jannatul Ferdous, Sharmin Jahan and Md. Asfaqul Islam performed *in vivo* test, literature review, and analysis; Md. Mostafa Moin Uddin and Md. Reaz Uddin performed molecular docking, and extract preparation, Amira Mostafa, Nisarar Nizhum and Fatematuj-Johora did literature review, collected plant materials, animal incubating, performed *in vitro* test and helped to write discussion part.

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