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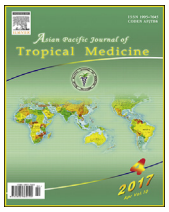

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# *In vitro*, *in vivo* and *in silico* anti-hyperglycemic inhibition by sinigrin

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ABSTRACT

**Objective:** To evaluate the anti-hyperglycemic potential of sinigrin using *in vitro*, *in silico* and *in vivo* streptozotocin (STZ) induced hyperglycemic zebrafish model.

**Methods:** The *in vitro* enzyme inhibition assay was carried out to determine the $IC_{50}$ value against α-glucosidase and α-amylase, *in silico* molecular docking was performed against both enzymes with PyRx tool and simulations were performed using GROMACS tool. Hyperglycemia was induced in zebrafishes using three intraperitoneal injections on alternating days for 1 week at 350 mg/kg of STZ. Hyperglycemic fishes were treated intraperitoneally with 50, 100 and 150 mg of sinigrin/kg of body weight for 24 h and glucose levels were measured.

**Results:** The sinigrin showed very strong inhibition against α-glucosidase and α-amylase with 0.248 and 0.001 24 μM while reference drug acarbose showed $IC_{50}$ value of 73.070 0 and 0.001 7 μM against α-glucosidase and α-amylase, respectively. Kinetic analysis revealed that sinigrin has the mixed type mode of inhibition against α-glucosidase. Molecular docking results revealed its strong binding affinity with α-glucosidase (−10.00 kcal/mol) and α-amylase (−8.10 kcal/mol). Simulations graphs confirmed its stability against both enzymes. Furthermore, in hyperglycemic zebrafishes most significant ($P < 0.001$) reduction of glucose was occurred at 150 mg/kg, moderate significant reduction of glucose was observed at 100 mg/kg and no any significant reduction of glucose was measured at 50 mg/kg.

**Conclusions:** It can be evident from the present results that sinigrin has potent anti-hyperglycemic activity and it may prove to be effective treatment for the hyperglycemia.

## 1. Introduction

Diabetes mellitus is a chronic metabolic disease in which regulation of carbohydrates, protein and lipid metabolism is incorrectly regulated by the pancreatic hormone (insulin) causing increase in blood glucose level *i.e.* hyperglycemia. The hyperglycemia is related with the occurrence of microvascular disorders like neuropathy, loss of vision and diabetic retinopathy and macrovascular disorders such as cardiovascular and amputation that are challenging to manage [1,2]. Globally, an estimated 422 million adults were living with diabetes in 2014, compared to 108 million in 1980. The global prevalence (age-standardized) of diabetes has nearly doubled since 1980, rising from 4.7% to 8.5% in the adult population [3]. This growing tendency of diabetes has become a great challenge for pharmacist worldwide that encourage every possible effort in formulation of new therapeutics to stop its progression.

α-glucosidase (EC#: 3.2.1.106) is a membrane bound enzyme that catalyzes the final step in the carbohydrate digestion, and hence α-glucosidase inhibitors could retard the use of nutritional carbohydrates to inhibit the postprandial hyperglycemia [4]. α-glucosidase catalyzes the catabolism of polysaccharides into monosaccharides which are absorbed in small intestine and cause the diabetes mellitus [5]. Inhibition of α-glucosidase activity is one of the best and easy ways to cope with hyperglycemia by reducing the absorption process of glucose in intestine [6]. Most frequently used α-glucosidase inhibitors are voglibose, miglitol and acarbose and these inhibitors also used as anticancer, anti-HIV and anti-diabetic agents. Unfortunately, the activity of these inhibitors is less than 50% of other anti-

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diabetic drugs such as metformin and sulfonylurea. Latter drugs are linked with some side effects like flatulence, abdominal discomfort and diarrhea [7]. So these side effects are limiting factor to use these medications alone and often to use in combination with other anti-diabetic drugs to enhance its potency.

α-amylase (EC#: 3.2.1.1) is a calcium metalloenzyme that catalyzes the hydrolysis of internal α-1,4-glycosidic linkages in starch to get products like glucose and maltose [8]. Carbohydrates are the important component of daily diet and play a vital role in the energy supply. The composite constituents of dietary carbohydrates should be cleaved into monosaccharides by the combined action of α-amylase and α-glucosidase. The resulting monosaccharides can be absorbed from the lumen of intestine and circulated into the blood [9]. The inhibition of α-glucosidase and α-amylase activity slows down the carbohydrates digestion and leads to decrease in blood glucose level and therefore it could be considered as a potential therapeutic approach for the treatment of diabetes. α-amylase inhibitors therefore have a therapeutic importance and one class of drugs introduced in the treatment of diabetes is the inhibitors of α-amylase [10].

The molecular docking and molecular dynamic simulation has recently established as a powerful technique for high through put screenings. Molecular docking study defines the 'best-fit' positioning of a compound that interacts with the target protein. The chemo-informatics and computational chemistry plays vital role in initial drug examination [11].

Recently zebrafish arise as good human disease animal model such as human pathologies, acquired diseases, genetic disorders, and many physiological processes are extremely conserved throughout the vertebrate evolution [12–17]. Due to the several benefits, zebrafish model has additional importance than earlier models of animal and human diseases. The advantages of the zebrafish comprise its production of visually clear embryos, fecundity and its small size. Furthermore, due to genome structure very similar to that of human it can be used for the studies of human genomic function [18,19]. Many research groups have performed diabetes experiments using zebrafish. In these experimentations streptozotocin (STZ) and glucose were used to induce the hyperglycemia in zebrafish [19–21]; administration of glucose was used for long term diabetes while STZ was used to induce short term acute diabetes.

Sinigrin is a plant-originated aliphatic compound and mostly found in the Brassicaceae family. Sinigrin can be breakdown by myrosinase to allyl isothiocyanate, which applies several biotic effects and also has an important role in the inhibition of cancer and DNA damage caused by carcinogens [22]. Mazumder *et al* [23] has reviewed the therapeutic activities of sinigrin as anticancer, anti-inflammatory, antibacterial, antifungal, antioxidant wound healing and biofumigation. To the best of our knowledge there is no report on anti-hyperglycemic activity, so we have explored the anti-hyperglycemic potential of sinigrin with the aim to develop a safe and effective drug for anti-hyperglycemia.

## 2. Materials and methods

### 2.1. In vitro assay

#### 2.1.1. α-glucosidase inhibition assay

The α-glycosidase inhibition activity of the sinigrin (85440, Sigma–Aldrich) was performed by the following method as described by Saleem *et al* [24]. Briefly, 100 mM phosphate buffer (pH 7.0) containing 0.2 g/L of $NaN_3$, 2 g/L of bovine serum albumin was used for preparation of 0.78 mM substrate *p*-nitrophenyl-α-D-glucopyranoside (Sigma, USA) and α-glucosidase (Sigma, USA) enzyme solutions. The assay mixture comprises of the sinigrin, enzyme solution and substrate. Initially, 10 μL of the test sample and 50 μL of the enzyme solutions were added in the 96 microplate wells and the mixtures were incubated for 5 min at room temperature. Then, 50 μL of the substrate was added and incubated for 10 min at 37 °C. Finally, the reaction was terminated by adding 100 μL of sodium carbonate solution (100 mM). While blank was prepared by adding all the components except enzyme. Absorbance of the reaction mixture was recorded at 405 nm using microplate reader ($OPTI_{Max}$, Tunable Micro Plate Reader). Acarbose was used as a reference inhibitor. All reactions were performed in triplicate and repeated three times. Percentage inhibition was calculated using the following equation.

$$\text{Inhibition } (\%) = \left[\frac{B-S}{B}\right] \times 100$$

Here, the B and S are the absorbances for the blank and samples.

$IC_{50}$ values were calculated by the program Prism 5.0 (GraphPad, San Diego, CA, USA).

#### 2.1.2. α-amylase inhibition assay

The α-amylase inhibitory activity of sinigrin was performed according to a previously reported method [24] with slight modification. Briefly, 40 μL of test compound and 40 μL of α-amylase (α-amylase from porcine pancreas, Sigma A3176-500KU, 089K1661) solution (prepared in buffer of 0.02 M sodium phosphate, pH 6.9 and 0.006 M sodium chloride) were added to 1.5 mL eppendorf tube and incubated for 10 min at room temperature. Then, 40 μL of starch solution (1% in DMSO) was added in the pre incubated tubes and subject to incubate for 10 min at 25 °C. After that, 100 μL of DNSA coloring reagent (10 g of sodium potassium tartrate, 1 g of 3,5-dinitrosalicylic acid and 20 mL of 2 N NaOH to a final volume of 100 mL in distilled water) Next, the test tubes were incubated in a boiling water bath for 5 min and cooled to room temperature. Then, the reaction mixture was diluted up to 400 μL using distilled water and absorbance was recorded at 540 nm. For non-enzymatic reactions, the assays were performed with a blank containing all of the components except the enzyme. The assay measurements % inhibition and $IC_{50}$ calculations were performed using the same procedure as that for the α-glucosidase inhibition assay.

#### 2.1.3. Kinetic study of α-glucosidase

A series of experiments were performed to determine the inhibition kinetics of sinigrin. The concentrations of sinigrin were for 0.00, 0.062 5, 0.125, 0.25, 0.5 and 1.0. Substrate *p*-nitrophenyl-α-D glucopyranoside concentration was between 0.125 and 2.0 mM in all kinetic studies. Pre-incubation and measurement time was the same as discussed in α-glucosidase inhibition assay method. Maximal initial velocity was determined from initial linear portion of absorbance up to 5 min after addition of enzyme at a 30 s interval. The inhibition type on the enzyme was assayed by Lineweaver–Burk plots of inverse of velocities (1/V) versus inverse of substrate

concentration 1/[S] $mM^{-1}$. The EI dissociation constant Ki was determined by secondary plot of 1/V versus inhibitors concentrations while ESI dissociation constant Ki′ was determined by intercept versus inhibitors concentrations.

## 2.2. In vivo

### 2.2.1. Zebrafish husbandry

Adult wild type zebrafish (*Danio rerio*) was purchased from commercial dealer and were acclimated for one month in standard laboratory conditions using a photoperiod of 14 h light and 10 h dark at a temperature of 28.5 °C. Fishes were fed two times daily with dry food and live brine shrimp larvae. Fishes were kept in thermostated tanks under constant chemical, biological and mechanical water filtration and aeration. All fishes used in this experiment were randomly chosen with equal ration of male and female with same age groups (4–6) month. All procedures were performed as described in the 'Principles of Laboratory Animal care' (NIH publication No. 85-23, revised 1985) and study was approved by the Institutional Review Board of Kongju National University (IRB No. 2011-2).

### 2.2.2. Induction of hyperglycemia

Hyperglycemia was induced following the already reported method of Oslen *et al* [19]. Diabetogenic drug STZ was used for hyperglycemia induction. Fishes were anesthetized by placing in the 0.04% Tricain MS-222 (ethyl 3-aminobenzoate methanesulfonate salt, Sigma–Aldrich USA) for (1–2) min. For intraperitoneal injection, an insulin syringe with a 28.5 G needle was used to deliver 0.3% STZ (Sigma–Aldrich USA, STZ was prepared in normal cold saline to a dose of 350 mg/kg) and (60–130) μL dependent on weight of fish. The fishes were administered three intraperitoneal injections of STZ on alternating days for 1 week. After injection, the fishes were maintained in lower temperature (21–23) °C for prolonged and effective induction.

### 2.2.3. Experimental design

Fishes were divided into five groups, fifteen fishes each group, first group receive only normal saline and served as normal control group, second group were administered with only STZ and served as negative control group and remaining third, fourth and fifth groups after induction of hyperglycemia were treated with acute doses (50, 100 or 150 mg/kg body weight, respectively) of sinigrin.

### 2.2.4. Determination of blood glucose level

Glucose was determined following the reported method of Capiotti *et al* [25]. Before the determination of glucose level, fishes were fasted for 12 h. Fishes were anaesthetized as mentioned in induction of hyperglycemic section. Immediately, the tails were cut and blood glucose readings were taken by using glucose meter (ACCU-CHEK, Performa, Korea).

## 2.3. Statistical analysis

Data were analyzed through one-way analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS version 16.0 Inc. Chicago, Illinois, USA). Post-hoc Tukey–Kramer test was applied where normality test failed ANOVA on Ranks test. $P < 0.05$ was considered statistically significant difference. Data are presented as line or bar diagrams constructed using the GraphPad Prism 5 (Version 5.01 GraphPad Software Inc. USA).

## 2.4. In silico

### 2.4.1. Selection of target proteins from PDB

The crystal structures of α-glucosidase and α-amylase with PDBIDs 4J5T and 1DHK, respectively, were accessed from Protein Data Bank (PDB) (www.rcsb.org). The selected target proteins were minimized with Amber force field by employing conjugate gradient algorithm in UCSF Chimera 1.10.1 [26]. The overall proteins architecture and statistical percentage values of helices, beta-sheets, coils and turns were retrieved from online server VADAR 1.8. The Discovery Studio 4.1 Client [27], a visualizing tool, was used to generate the hydrophobicity graph and graphical depiction of target proteins.

### 2.4.2. Designing of ligand structures

The ligand molecule was sketched with ACD/ChemSketch software and minimized by UCSF Chimera 1.10.1. The basic biological properties and hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD) were predicted by employing online tools like Molinspiration (http://www.molinspiration.com/) and Molsoft (http://www.molsoft.com/). Moreover, Lipinski's rule of five was also analyzed to examine the efficacy of ligand structure.

### 2.4.3. Molecular docking analysis and visualization

Molecular docking experiments were performed against α-glucosidase and α-amylase by using PyRx docking tool [28]. The grid box parameters values for α-glucosidase were adjusted as center_x = −18.44, center_y = −20.91, center_z = 8.22 while size_x = 77.93, size_y = 68.98, and size_z = 103.65, respectively. Similarly, for α-amylase the grid values were adjusted as center_x = 102.22, center_y = 40.10, center_z = 18.02 while size_x = 57.60, size_y = 59.83 and size_z = 72.29, respectively. The default exhaustiveness value = 8 was adjusted in both docking to maximize the binding conformational analysis. The docking poses (100 numbers of run) for each docking were adjusted to obtain the best docking results. The graphical visualization of all the docking complexes was carried out using Discovery Studio (2.1.0).

### 2.4.4. Molecular dynamics (MD) simulations assay

The best docked energy (Kcal/mol) complex was selected for MD simulations to observe the residual backbone flexibility. All the MD simulations were performed by Groningen Machine for Chemicals Simulations (GROMACS) 4.5.4 package [29] with GROMOS GROMOS 96 force field [30]. The receptor and ligand topology files were generated by using GROMOS 53A6 forcefield and online PRODRG Server [31], respectively. Furthermore, all the receptor–ligand complexes were solvated and placed in the middle of cubic box with an adjusted 0.8 Å distance. The overall system charge was neutralized by adding ions. The energy minimization (nsteps = 50000) was done by steepest descent approach (1000 ps). Particle Mesh Ewald (PME) method was used for energy calculation [32]. For electrostatic and Van der Waals interactions, cut-off distance

for the short-range VdW (rvdw) was set to 14 Å, whereas Coulomb cut-off (r coulomb) and neighbor list (rlist) were fixed at 9 Å. Finally, a 10 ns molecular dynamics simulation was carried out for complex with nsteps 50000 and analyzed using Xmgrace software (http://plasma-gate.weizmann.ac.il/Grace/).

## 3. Results

### 3.1. In vitro

#### 3.1.1. α-glucosidase and α-amylase inhibitory activities

In present study sinigrin inhibited the α-glucosidase and α-amylase in dose dependent manner. The $IC_{50}$ values for α-glucosidase and α-amylase was found to be 0.2480 and 0.0017 μM, respectively while reference drug acarbose showed the $IC_{50}$ 0.001 24 and 0.001 70 μM against α-glucosidase and α-amylase, respectively.

#### 3.1.2. Inhibition kinetics of α-glucosidase

To understand the inhibition mode of sinigrin against α-glucosidase, inhibition kinetics was performed. The Lineweaver–Burk plot of 1/V versus 1/[S] in the presence of different sinigrin concentrations gave a series of straight lines intersecting within the second quadrant. This behavior indicated that sinigrin inhibits the α-glucosidase by two different pathways, competitively forming enzyme inhibitor (EI) complex and disturbing enzyme-substrate-inhibitor (ESI) complex in noncompetitive manner. EI dissociation constants Ki was determined using the secondary plots of slope versus concentration of sinigrin (Figure 1b) while ESI dissociation constants $Ki'$ was calculated using secondary plots of intercept versus concentration of sinigrin (Figure 1c). A lower value of $Ki$ than $Ki'$ pointed out stronger binding between enzyme and compound, suggesting preferred competitive over noncompetitive manners (Table 1 and Figure 1).

### 3.2. In vivo

#### 3.2.1. Anti-hyperglycemic activity in zebrafish

The hyperglycemic fishes were created by destroying the β-cells of pancreas using the diabetogenic drug STZ after three injections of STZ on alternating days. The hyperglycemic fishes were treated with 50, 100 and 150 mg/kg of sinigrin. After 24 h of administration of sinigrin, the glucose levels were measured as mentioned in experimental section. Our results demonstrated that treatment with 150 mg/kg of sinigrin proved to be very effective for significantly lowering the fasting blood glucose levels ($P < 0.001$; Table 2) in zebrafishes. Treatment with 100 mg/kg suppressed the blood glucose levels significantly ($P < 0.05$; Table 2) in comparison with the negative control while treatment with 50 mg/kg showed the glucose lowering effect but not at significant levels.

**Table 1**

Kinetic analyses of α-glucosidase in the presence or absence of sinigrin. $1/V_{max}$ is the reaction velocity.

| Sinigrin (μM) | $1/V_{max}$ (μM) | $K_m$ (ΔA/Sec) | Inhibition type | *Ki* (μM) | *Ki′* (μM) |
|---|---|---|---|---|---|
| 0.00 | 2592.592 | 0.142 | | | |
| 0.0625 | 4197.530 | 0.256 | | | |
| 0.125 | 4938.271 | 0.312 | Mixed | 0.31 | 0.6 |
| 0.25 | 6296.296 | 0.317 | | | |
| 0.5 | 7407.407 | 0.347 | | | |
| 1.0 | 9629.629 | 0.363 | | | |

Km is the Michaelis–Menten constant, Ki is the EI dissociation constant.

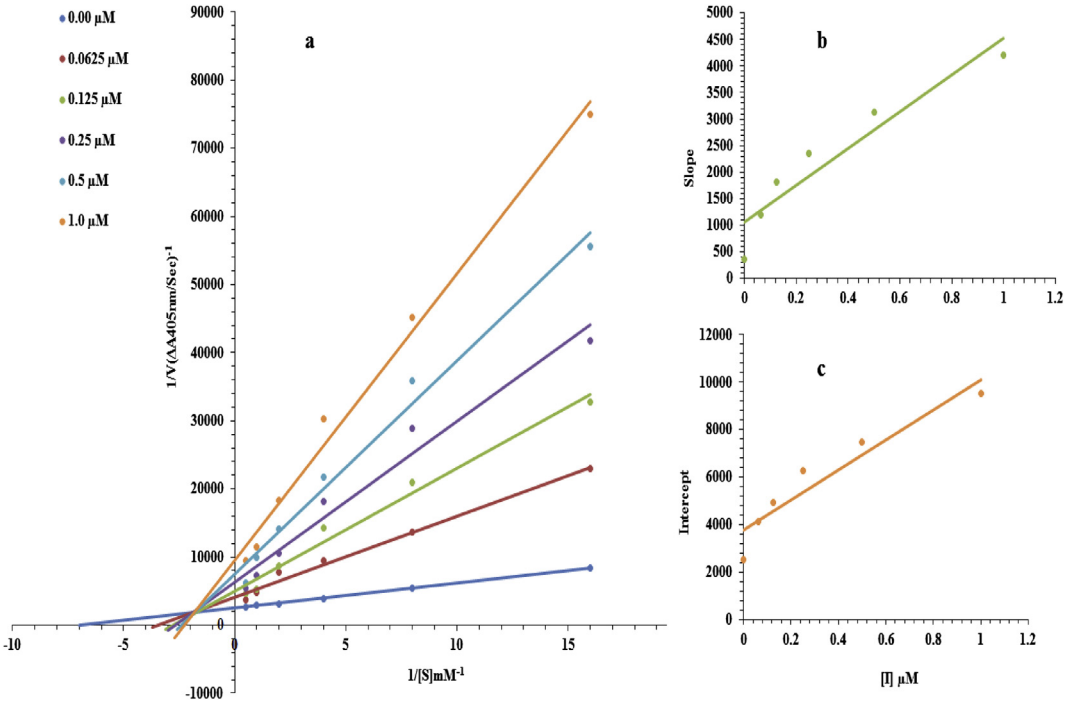


**Figure 1.** Lineweaver–Burk plot for inhibition of α-glucosidase in presence of various concentration of sinigrin.
Substrate *p*-nitrophenyl-α-D-glucopyranoside concentrations were 0.125–2.000 mM. (b) The inserts represent the plot of the slopes (c) or vertical intercepts versus compound concentration to determine inhibition constant.

**Table 2**
Blood glucose levels in zebrafish treated with sinigrin at concentrations of 50, 100 or 150 mg/kg of body weight.

| Groups | Glucose level (mg/dL) | $P^a$ |
|---|---|---|
| Normal control | 60.400 ± 4.672 | >0.05 |
| Negative control | 314.660 ± 55.317 | >0.05 |
| 50 mg/kg | 212.400 ± 29.164 | >0.05 |
| 100 mg/kg | 116.266 ± 38.158 | <0.05 |
| 150 mg/kg | 65.600 ± 10.432 | <0.001 |

Negative control was treated with STZ only and normal control was injected the same volume of normal saline. Values were presented as mean ± standard error of mean.
[a] Compared with negative control group.

### 3.3. In silico

#### 3.3.1. Structural assessment of α-glucosidase and α-amylase

The α-glucosidase and α-amylase are class of hydrolase proteins and actively involved in digestion of food carbohydrates. The α-glucosidase comprises 811 amino acids with residual architecture of 35% helices, 25% β sheets and 38% coils. The porcine pancreatic α-amylase contains 496 residues along with 22% helices, 30% β sheets and 47% coils residual architecture values. The X-ray diffraction studies of α-glucosidase and α-amylase confirmed its resolutions 2.04 Å and 1.85 Å, respectively. The Ramachandran plots of α-glucosidase and α-amylase indicated that 97.6% and 97.1% of residues were present in favored regions. Selected Ramachandran graphs values showed the good accuracy of phi (φ) and psi (ψ) angles among the coordinates of target proteins.

#### 3.3.2. Lipinski rule of five (RO5) evaluations of selected sinigrin

RO5 analysis also justified the therapeutic potential of sinigrin. It was justified that exceeded values of HBA (>10) and HBD (>5) in ligands results in poor permeation in the body. Present chemo-informatics analyses showed that sinigrin possesses ≤10 HBA and <5 HBD with molecular formula of $C_{10}H_{16}$ K $N_2O_9S_2$ and molecular weight of 397.463 4 g/moL confirming their good penetration within the body. Moreover, their log*P* value (−2.10), were also comparable with standard values <5. Sinigrin showed the Polar surface area was 130.88 $Å^2$ and molecular volume 286.94 $Å^2$. However, there are plenty of examples available for RO5 violation amongst the existing drugs [33,34].

#### 3.3.3. Molecular docking analysis

The ligand-protein docked complexes were analyzed on the basis of minimum energy values. Results indicated that sinigrin showed good binding energy values (−10.00 and −8.10 kcal/moL) against α-glucosidase and α-amylase, respectively. The binding energy values justified the binding potential against the target protein. Binding pocket analysis of α-glucosidase and α-amylase showed that sinigrin binds within the active binding region of targeted proteins. The current α-glucosidase docking

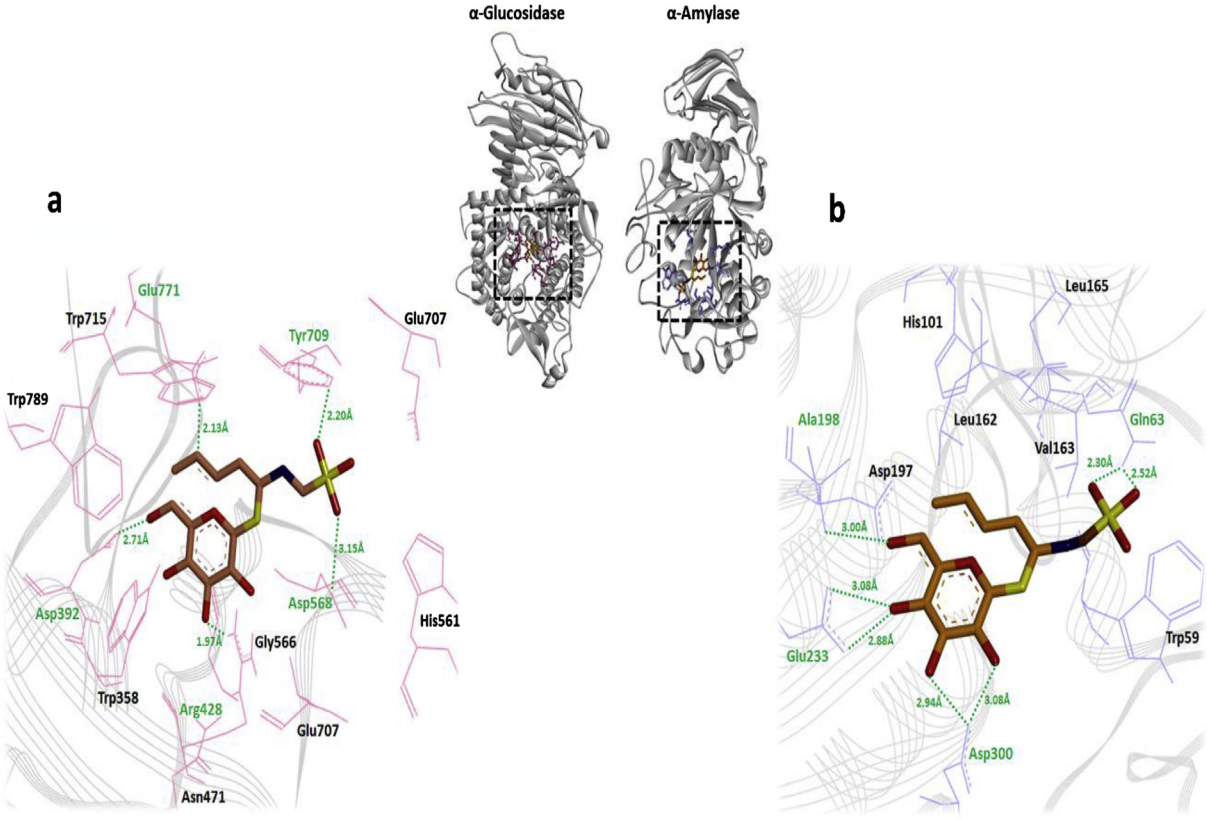


**Figure 2.** Docking complexes of sinigrin against and α-amylase. Two rectangular dotted lines boxes were highlighted the binding residues of both target proteins.
(a) In sinigrin-α-glucosidase binding complex and (b) sinigrin-α- amylase binding complexes shown here, sinigrin are shown in brown color while the functional groups like oxygen, sulfur and nitrogen are labeled with red, yellow and dark blue colors, respectively. The pink (a) or dark blue (b) colors are used to highlight the interacting residues while target proteins are shown in line format. The green and purple color residues are actively participated in hydrogen bonding and their distances (Å) are shown in green dotted lines.

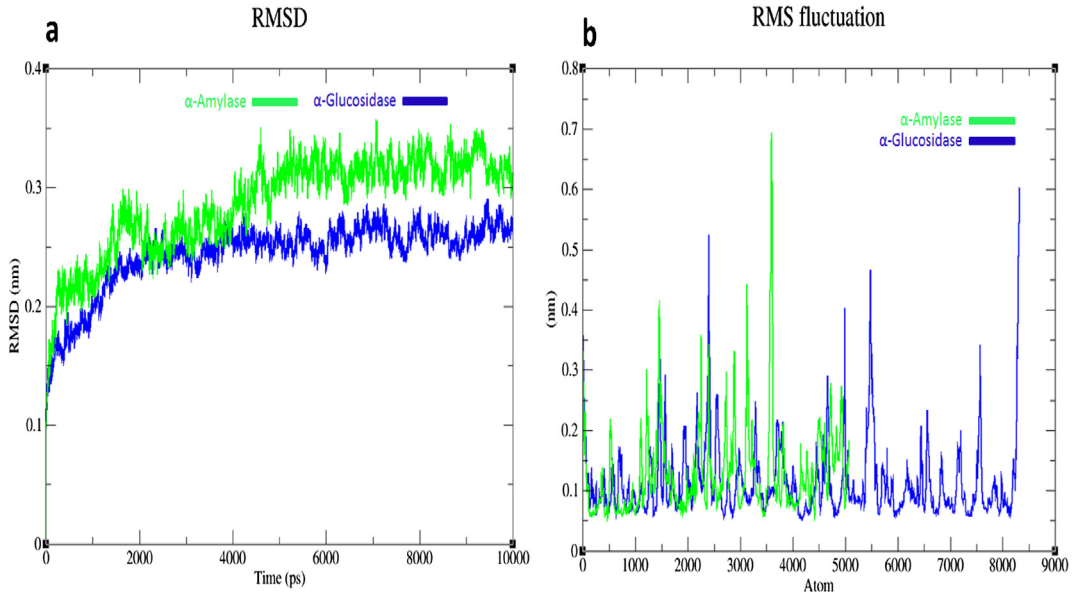


**Figure 3.** RMSD graphs of α-glucosidase and α-amylase. RMSD graphs of α-glucosidase and α-amylase are shown in blue and green colors at time 10 ns, respectively.

results showed the sinigrin binds with the active binding pocket of target protein. Results depicted that sinigrin forms five hydrogen bonds at Asp568, Tyr709, Glu771, Asp392 and Arg428 residues at distances of 3.15 Å, 2.20 Å, 2.13 Å, 2.71 Å and 1.97 Å, respectively (Figure 2a). The α-amylase docking results showed that sinigrin binds with the active binding region of α-amylase and makes four hydrogen bonds. The observed interacting residues of α-amylase active site are Gln63, Asp300, Glu233 and Ala198 which actively participate in interactions. The sinigrin forms double hydrogen bonds with Gln63, Asp300 and Glu233 at different functional groups while Ala198 interacts by single hydrogen bond. The observed binding distances between sinigrin and interacting residues Gln63, Asp300 and Glu233 are 2.30/2.52 Å, 2.94/3.08 Å, 2.88/3.08 Å, and 3.00 Å, respectively (Figure 2b). Based on docking and kinetic results it can be hypothesized that sinigrin binds competitively in the active region of α-amylase and may have strong potential to cure the hyperglycemia.

### *3.3.4. MD simulation analysis*

The residual flexibility of receptor with sinigrin was confirmed by MD simulation study. The Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuations (RMSF) graphs were predicted to observe the receptor fluctuations at different time scale. Figure 3a showed the RMSD graphs of both α-glucosidase and α-amylase. Initially, both graph lines were showed an increasing trend with RMSD values range from 0.1 to 0.2 (nm) at time (0–2000) ps. After that minor steady fluctuations were observed from 2000 ps to 4000 ps in both complexes. From 4000 ps 1000 ps the α-glucosidase is still remaining steady and showed little fluctuations throughout the simulation time. While the α-amylase showed higher fluctuations in the simulation time frame. The comparative analysis clarified that α-glucosidase simulation graph is more stable throughout the simulation time period as compared to α-amylase. Figure 3b showed the RMSF results which reflect that both C and N-terminal lobes of α-glucosidase and α-amylase are fluctuating throughout the simulation period. In α-amylase loop conformation is more fluctuated and showed higher peaks as compared to α-glucosidase.

## 4. Discussion

Diabetes mellitus is a metabolic disorder characterized by disrupted insulin production leading to hyperglycemia. To control this disease, the management of high blood glucose levels is the main element of this disease. The present study was undertaken to evaluate the anti-hyperglycemic potential of sinigrin. Three different approaches were used in current study: *in vitro* α-glucosidase and α-amylase inhibition assay, *in vivo* Streptozotocin (STZ) induced hyperglycemic zebrafish model and *in silico* molecular docking and dynamic simulation. Pharmaceuticals that suppress the carbohydrate-metabolizing enzymes have been known to decrease postprandial hyperglycemia [35]. The results of enzyme inhibition showed that sinigrin has the potency to inhibit the both enzymes α-glucosidase and α-amylase, sinigrin showed more strong inhibition potency against α-glucosidase as compared to α-amylase. This is well agreed with the report of Kwon *et al* [36] that compounds originated from plants had been shown to have strong inhibition potencies against α-glucosidase. The inhibition activity of these enzymes was confirmed and might be responsible for decreasing the rate of hyperglycemia. This effect would delay the catabolism of starch and oligosaccharides, resulting decreased absorption of glucose and therefore delayed increase in hyperglycemia [37].

When sinigrin were administered to STZ induced zebrafishes the maximum reduction of glucose levels was recorded at 150 mg/kg. Metabolic breakdown of sinigrin may lead to the formation of isothiocyanate which is responsible for many biological activities [23]. Our results were in line with the Washida *et al* [38] where sinigrin reduced the plasma triglycerides levels. Unfortunately, we were unable to measure the triglycerides and cholesterol levels because of very low blood volume in zebrafish. Current results revealed that sinigrin have the potency to be good hyperglycemic inhibitor.

Molecular docking of sinigrin against α-glucosidase confirmed its binding affinity by using hydrogen-bonding. Literature study showed that hydrogen-bonding affinity has been considered as a significant parameter for evaluating the drug permeability [39]. The active binding residues of α-glucosidase which are functionally participate in the inhibition and signaling pathways were verified from literature study. It has been observed that Glu771 and Asp568 were considered as key functional player in the inhibition kinetics and downstream signaling pathways [40]. Moreover, another study also proposed the binding pocket residues such as Asp568, Trp381, Trp710, Trp715 and Trp789 are also present in the same binding domain of α-glucosidase [41]. Furthermore, in α-amylase docking the active binding residues were justified from literature study [42].

The present study demonstrated that sinigrin possess remarkable anti-hyperglycemic potential against STZ induced hyperglycemic adult zebrafishes. Its α-glucosidase and α-amylase inhibitory activity may also be translated into the potential to reduce the hyperglycemia. The docking and simulations results revealed its strong and stable binding affinity with both targeted proteins. Based on our results, sinigrin may be used for the development of anti-hyperglycemic drugs. However, further study is needed to investigate the molecular mechanism pathway which is involved in the inhibition of hyperglycemia.

## Conflict of interest statement

The authors declare no conflict of interest.

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