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In silico and in vitro analysis reveal multi-target anti-hyperglycaemic properties of gedunin, a limonoid from neem (*Azadirachta indica*)

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Abstract

Background: Insulin secretion and insulin related pathways have been the prime targets in the treatment of diabetes for a long time. However, recently a lot of attention is being directed towards addressing hyperglycaemia as the main perpetrator of the symptoms in this metabolic disorder. This new treatment approach also involves greater inclination to plant derived therapeutic agents for their safety and probable minimal side effects. The objective of the present study was to scientifically elucidate the potential of gedunin (a limonoid from Neem tree) as an anti-hyperglycaemic agent.

Methods: The effect of gedunin on pancreatic and salivary amylase activity and glucose transport across yeast cell membrane was tested at three different concentrations (5 μM, 10 μM and 20 μM) using known inhibitor acarbose as the standard. Multiple Ligand Simultaneous Docking was used to study the interaction of gedunin with salivary and pancreatic amylase and determine binding affinity and specificity of this interaction.

Results: The in vitro results documented a steady, linear pancreatic alpha amylase (ovine) inhibition in a concentration dependent manner with gedunin showing lower IC_{50} value of 20.25 μM against acarbose (IC_{50} = 31.12 μM**)** a known enzyme inhibitor used as standard in the present study**.** The inhibition of salivary amylase by gedunin was also distinct. Yeast cell glucose uptake studies revealed remarkable inhibition of glucose absorption at 10 μM and 20 μM concentration of gedunin (5.45% and 13.87% respectively with respect to control). Corroborating the in vitro findings even in the docking studies gedunin exhibited higher docking score (− 8.12 Kcal/mol) and higher enzyme inhibition potency (Ki = 1.12 μM) with human pancreatic amylase-substrate complex as compared to acarbose (docking score-5.24 Kcal/mol, Ki = 110.8 μM). The studies further suggested a non-competitive, mixed kind of inhibition by gedunin. As evident from this current in vitro study, gedunin had shown significant inhibition of alpha amylases and glucose uptake at much lower concentration (5, 10 and 20 μM) than previous studies where the concentrations used were (20.7–124.3 μM).

Conclusion: This study lays strong evidence to the rationale of gedunin being an important lead compound to developing a promising hyperglycaemic agent, simultaneously targeting glucose absorption in the intestine and enzymatic digestion of polysaccharides.

Keywords: Diabetes, Gedunin, Alpha amylase, In silico docking, MLSD

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Introduction

Diabetes mellitus is a metabolic disorder that results due to disruption of carbohydrate metabolism and clinically characterised by chronic elevated plasma glucose level. The hyperglycaemic condition arises either due to tissue's inability to respond to insulin secretion or due to insufficient insulin production by pancreatic cells as suggested by Reaven et al. [1]. Multiple enzymes, like alpha glucosidase, human salivary amylase (HSA) and human pancreatic alpha amylase (HPA), protein tyrosine phosphatase (PTP), DipeptidylpeptidaseIV (DPP IV), and sucrase have been documented by researchers to be involved in the pathophysiology of insulin independent (IIDM) or Type2 diabetes mellitus (T2DM) [2]. Hence inhibition of these enzymes could be an attractive therapeutic approach to retard postprandial hyperglycaemia (PPHG). The reduction of glucose absorption through interference in the activity of alpha glucosidase and alpha amylase is considered as a first line defence and effective strategy against elevated post prandial glucose concentration in the management of diabetes [3–6]. Acarbose, miglitol and voglibose are the few widely prescribed alpha glucosidase (EC 3.2.1.20) inhibitors that act mainly by inhibiting carbohydrate digestion and retarding absorption [7, 8]. However, inhibitors of alpha amylase (EC 3.2.1.1) are thought to be better suppressors of PPHG since it resists abnormal accumulation of maltose [9].

Although a few alpha amylase inhibitors have been reported, these present several side effects like diarrhoea, sudden hypoglycaemia, weight gain, anorexia, allergic reaction and unusual bleeding [10, 11]. Inability of these current medicines to efficiently maintain consistent normal glucose level with minimal adverse effects impels exploration of other options especially those of herbal origin. It must be noted that natural products are being increasingly considered as important sources of anti-diabetic agents. Many reviews that extensively describe and substantiate the significance of natural products in anti-diabetic treatment have been published in the last few years which include reports on Chinese medicinal plant originated therapeutic agents, flavonoids and desert plants [12–14]. It has been reported that certain components of medicinal plants like alkaloids, terpenoids, tannins etc. have a role to play in control of hyperglycaemia [13]. It therefore follows that plant derived products are potential candidates for lead compounds to effective therapeutics in treatment of diabetes and must be explored with greater scrutiny.

Gedunin, is a basic limonoid and an active ingredient of the class tetranortriterpenoid, derived from plant *Azadirachta indica*. Though a wide array of research has recently demonstrated the use of gedunin in the treatment of different types of cancers [15] various inflammatory diseases [16, 17] and post diabetic complications [18] its only now also being recognized as a possible therapeutic agent for metabolic disorders like diabetes. Although a few proof-of-concept studies have been conducted [19, 20] there is an insufficient scientific validation of its effect on hyper glycaemia in type 2 diabetes (NIDDM).

The objective of the present investigation was to analyse the therapeutic potential of gedunin as an anti-hyperglycaemic agent. A strategic all-round approach was employed wherein effect of gedunin on starch breakdown enzymes namely salivary and pancreatic amylase as well as on glucose absorption machinery were evaluated through in silico and in vitro means. The *in- silico* approach involved the analysis of the binding affinity and specificity of gedunin with human salivary and pancreatic alpha amylase and analysis of nature of inhibition through docking. The in vitro approach on the other hand included ovine pancreatic alpha amylase and human salivary amylase inhibition assay and yeast cell glucose uptake assay. In this study the in vitro results complimented well with the *in-silico* findings suggesting a multitargeted hyperglycaemic effect of gedunin.

Materials & methods

Chemicals and reagents

Goat pancreas for alpha amylase enzyme extraction was obtained from the butchery and was maintained at 4 °C, while being transported immediately to the laboratory. All the chemicals and reagents used in the study were of analytical grade or ultrapure grade. Soluble starch, acetic acid, sodium chloride, 3, 5-dinitrosalicylic acid (DNS), monosodium and disodium phosphate were purchased from Sisco Research Laboratories Pvt. Ltd. (SRL). gedunin was purchased from Santacruz Biotechnology, USA. Dimethyl sulfoxide (DMSO) was purchased from HI Media Laboratories Pvt. LTD. Acarbose (Glucobay®) was from Bayer AG, Berlin, Germany. Baker's yeast was obtained in-house. Glucose assay kit was purchased from Accurex Biomedical Pvt. Ltd., India. Absorbance was taken on UV visible Spectrophotometer (Biochrom Libra S70 double beam spectrophotometer, Biochrom Pvt. Ltd., Cambridge).

Multiple ligand simultaneous docking (MLSD)

Data retrieval

In the present study, we have used multiple ligand simultaneous docking approach (MLSD) [21] for docking studies. The MLSD program is based on AutoDock Tools 4.2 platform [22] with improved LGA and hybrid PSO algorithm [23, 24].

Molecular interaction studies were performed in the active sites of human pancreatic amylase and human salivary amylase receptors. The X-ray crystal structure of human pancreatic (HPA) (PDB ID: 1HNY) [25] and

human salivary amylase (HSA) (PDB ID:1SMD) [26] were downloaded from RCSB protein data bank (http://www.pdb.org).

The ligand compounds gedunin (test compound) [CID: 12004512], acarbose (standard drug) [CID:4369394] and amylose or alpha-maltotriose (natural substrate of amylase) [CID:192826] were downloaded from PubChem database [27].

Preparation of receptor and ligands

The proteins were subjected to energy minimization using steepest descent algorithm based on GROMOS96 43B1 forcefield parameter set [28]. The target protein was prepared using AutoDock Tools version 4.2 [22] program. After adding polar hydrogen atom to the protein, non-polar hydrogens were merged. Kollman charge was added to the amino acids of the receptor. The 3D grid box was prepared with a size of 50 × 50 × 45 grid points with a spacing of 0.5 Å at the geometric centre of the target enzyme. We kept the grid centre as 61.01, 46.405, 62.204 for (x, y, z) respectively.

The ligands were prepared by setting the appropriate number of rotatable bonds and Gasteiger charge [29] was added prior to grid preparation. A conventional grid generation procedure was applied followed by grid parameter setting for each ligand. Similarly, the generated .dpf files have been merged to a single docking parameter file to initiate MLSD starting configuration. Each ligand was randomly initiated with their own parameter sets to achieve its configurations. We have performed separate docking for each protein (HPA and HSA) to compare the binding affinity and geometric differences between the ligands.

Inhibition studies on pancreatic and salivary α-amylase

For pancreatic amylase assay goat pancreas was washed free of blood with saline, trimmed of other tissues and 200 mg was weighed and homogenized in 10 ml ice cold phosphate buffer (0.2 M, pH 7.4). Homogenate was centrifuged at 5000 rpm for 10 min at 4^0 C. The supernatant collected was used as source of crude alpha amylase [30].

For salivary amylase assay, mouth was rinsed, saliva was collected and diluted 1:20 with distilled water. The diluted saliva was centrifuged and the supernatant was used for assay [31].

The assay was performed according to the protocol described by Miller [32] and the reducing sugar (maltose equivalent) liberated was quantified by the 3, 5-dinitrosalicylic acid (DNSA) method. Gedunin was dissolved in minimum amount of dimethyl sulfoxide (DMSO) to obtain concentrations of 5 μM, 10 μM and 20 μM. Standard alpha glucosidase inhibitor acarbose was used as positive control (5 μM,10 μM, 20 μM).

The percentage inhibition was calculated using the following given formula [33],

$$(\%)inhibition = \left(\frac{Abs(Control) - Abs(Sample)}{Abs(Control)}\right) \times 100$$

The IC_{50} values were calculated and determined from plots of percent inhibition versus inhibitor concentration.

Estimation of glucose uptake by yeast cells

Glucose transport across the yeast cell membrane was monitored according to the protocol by Cirillo [34]. Commercial baker's yeast dissolved in distilled water was subjected to repeated centrifugation (3000×g, 5 min) until clear supernatant fluid was obtained and a 10% (v/v) of the suspension was prepared in distilled water. Various concentrations of gedunin (10–20 μM)) were added to 1 mL of glucose solution (10 mM) and incubated together for 10 min at 37 °C. Reaction was started by adding 100 μL of yeast suspension followed by vortexing and further incubation at 37 °C for 60 min. The tubes were centrifuged (2500×g, 5 min) and amount of glucose in the supernatant was estimated using the kit (GOD-POD).

The percentage glucose uptake by yeast cells was calculated for control (solution of 10 mM glucose with yeast cells), test drug gedunin (solution of 10 mM glucose with yeast cells and test drug gedunin-10 μM to 20 μM) and standard drug metformin (solution of 10 mM glucose with yeast cells and standard drug metformin-10 μM to 20 μM) using the following modified formula by Rehman et al. [35].

$$Glucose\ Uptake\ (\%) = \left(\frac{Abs(0\ \ min) - Abs(10\ \ min)}{Abs(0\ \ min)}\right) \times 100$$

Statistical analysis

Values are Mean ± SD for three independent experiments. The statistical significance of differences observed in the study was confirmed by performing student t-test using Microsoft Excel.

Results

Molecular docking study

A total of 3 compounds, namely, amylose, acarbose and gedunin, were screened in the binding sites of the mentioned receptors in the following manner: a) HPA/HSA + amylose b) HPA/HSA + acarbose c) HPA/HSA + gedunin d) HPA/HSA + amylose + acarbose e) HPA/HSA + amylose + gedunin. Results of the same are presented in Table 1.

For HPA and HSA, the results of single substrate docking revealed that compared to gedunin, the binding energy of acarbose is closer to the binding energy of the substrate molecule, amylose.

Table 1 Single and multiple ligand docking analysis

No.	Complex	H-bond amino acids	Free Energy Of Binding (kcal/mol)	Ki (μM)
1.	HPA + amylose	Gln63, His201, Arg195, Asp197, His299, His305, Ile235	−5.31	23.55
2.	HPA + acarbose	His101, Arg195, His201, Val234, Ile235, His299, His305, Ala307	−5.38	113.73
3.	HPA + gedunin	Gln63, His101, His201	**−7.66**	**2.42**
4.	HPA + amylose+acarbose	Gln63, Ala106, Thr163, Arg195, Lys200, His201, His299, His305	−5.40	110.8
5.	HPA + amylose+gedunin	Gln63, His101, His201	**−8.12**	**1.12**
6.	HSA + amylose	Gln63, Tyr151, Arg195, Lys200, His201	−5.89	47.98
7.	HSA + acarbose	Trp59, Gln63, Gly104, Ala106, Ser163, His201, His299, His305	−5.68	68.84
8.	HSA + gedunin	Gln63, His101, His201	**−6.36**	**21.91**
9.	HSA + amylose+acarbose	Trp58, Trp59, Tyr151, Arg195, Lys200, His201, Leu237, Gly306, Lys352	−5.90	47.19
10	HSA + amylose+gedunin	Glu63, His101, Arg195	**−7.29**	**4.51**

When the inhibitors are compared with each other, gedunin showed much promising binding energy values of –8.12 kcal mol-^{1} and -7.29 kcal mol^{-1} than acarbose (– 5.4 kcal mol^{-1} and -5.9 kcal mol$^{-1)}$ with HPA and HSA respectively. Same pattern of result was seen when inhibition constant Ki values of gedunin (2.42 μM for HPA and 4.52 μM for HSA) and acarbose were compared. In the absence of substrate amylose, the binding affinity of gedunin with HPA is 1.4 times better than acarbose and the inhibition potential of gedunin is almost 47 times more than acarbose.

Multiple ligand simultaneous docking revealed different degrees of inhibitor interactions with the enzymes in the presence and absence of substrate. There is 6% reduction in docking energy and more than 50% decrease in Ki value when gedunin binds with HPA simultaneously in the presence of the substrate amylose. These differences are negligible for acarbose when bound with the enzyme in the presence and absence of substrate. Though the overall docking energy and inhibition constant of HSA with gedunin is less than HPA, here also more than 90% decrease in Ki value and almost 34.23% less docking energy with gedunin in the presence of amylose was seen.

On comparing the multiple ligand docking of the two inhibitors with each other it was observed that, gedunin showed more than 50% decrease in docking energy and 99% less Ki value and 95% fall in inhibition constant with respect to acarbose for HPA.

Comparisons of single docked structures with multiple ligand binding for each inhibitor reveal less binding energy of Ki values for both gedunin and acarbose.

It is observed that all the three ligands, amylose, gedunin and acarbose share some common H-bonding amino acid residues like His101, His305, Gln63, Ile235with HPA (Fig. 1) and Gln63, His201 residues with HSA. Similarly, gedunin and acarbose share some common H-bonding amino acid residues like Glu63, Trp59 and His201 with HPA (Fig. 2 and Fig. 3) and Glu63, His101 and Trp58 with HSA (Fig. 4 and Fig. 5) in presence of amylose.

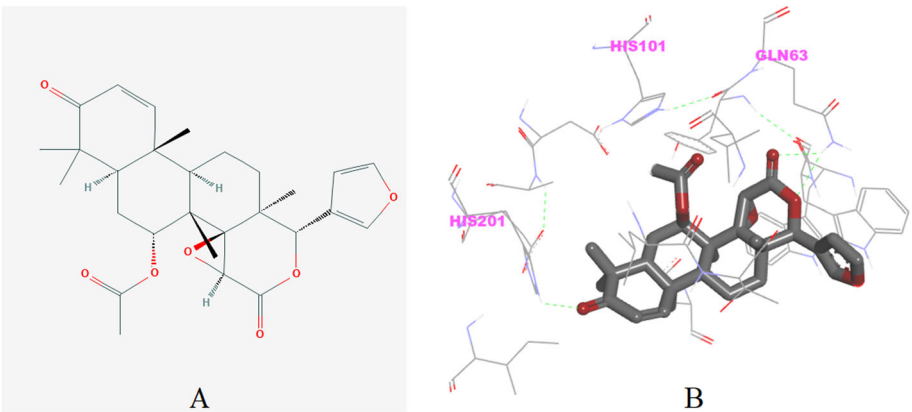

Fig. 1 Molecular docking interaction pattern of gedunin with the active site of HPA (PDB ID: 1HNY) generated using AutoDock Tools 4.2 platform (**a**) 2D structure of gedunin (PubChem CID: 12004512) (**b**) 3D docking representation of gedunin in the binding pocket showing the hydrogen bond interactions

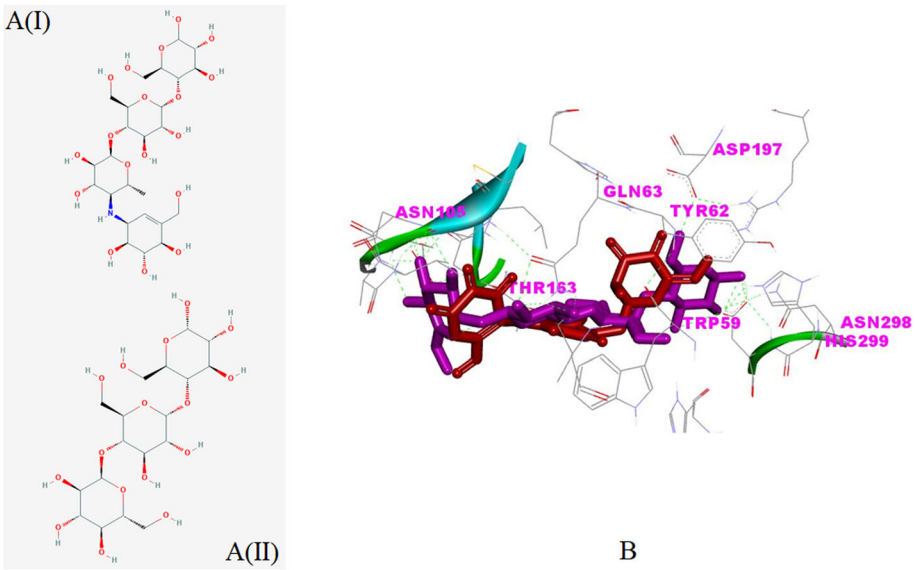

Fig. 2 Molecular docking simultaneous interaction pattern of amylose and acarbose with the active site of HPA (PDB ID: 1HNY) generated using AutoDock Tools 4.2 platform [**A(I)**] 2D structure of Acarbose (PubChem CID: 41774) [**A (II)**] 2D structure of amylose (PubChem CID: 192826) (**B**) 3D docking representation of simultaneously docked compounds (amylose- substrate in red and acarbose- inhibitor in magenta) in the binding pocket showing the hydrogen bond interactions

Pancreatic α- amylase inhibition

Results of pancreatic α- amylase inhibition by acarbose are presented in Fig. 6. From the data obtained, it was seen that gedunin showed noteworthy inhibitory activity when compared with acarbose. The inhibition by gedunin varies from 12.66% to 49.64% in the concentration range of 5 μM to 20 μM. For the same concentration range, there was inhibition of 18.75% to 36.5% of alpha amylase by acarbose. While the standard inhibitor showed only 17.75% increase in enzyme inhibition from lowest concentration to highest concentration, our test drug showed a

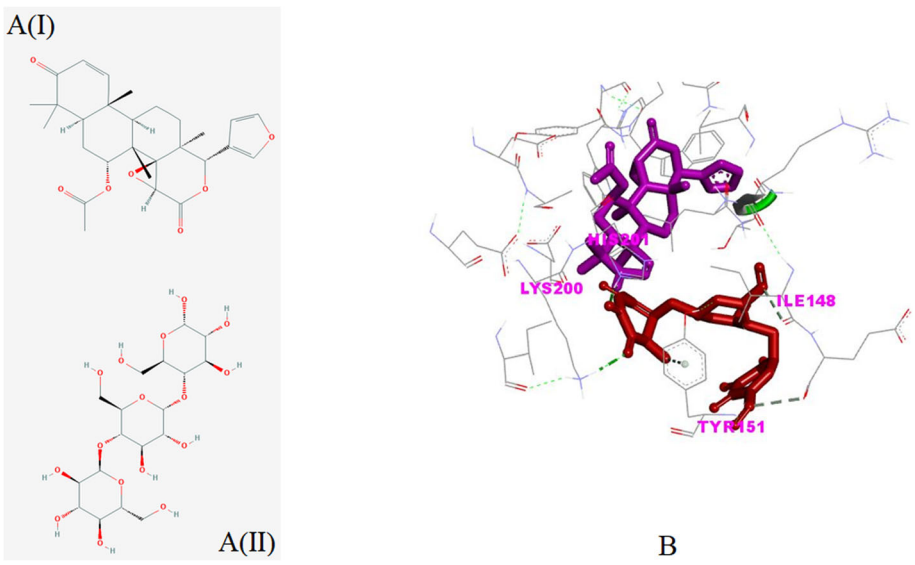

Fig. 3 Molecular docking simultaneous interaction pattern of amylose and gedunin with the active site of HPA (PDB ID: 1HNY) generated using AutoDock Tools 4.2 platform [**A(I)**] 2D structure of gedunin (PubChem CID: 12004512) [**A (II)**] 2D structure of amylose (PubChem CID: 192826) (**B**) 3D docking representation of simultaneously docked compounds (amylose- substrate in red and gedunin- inhibitor in magenta) in the binding pocket showing the hydrogen bond interactions

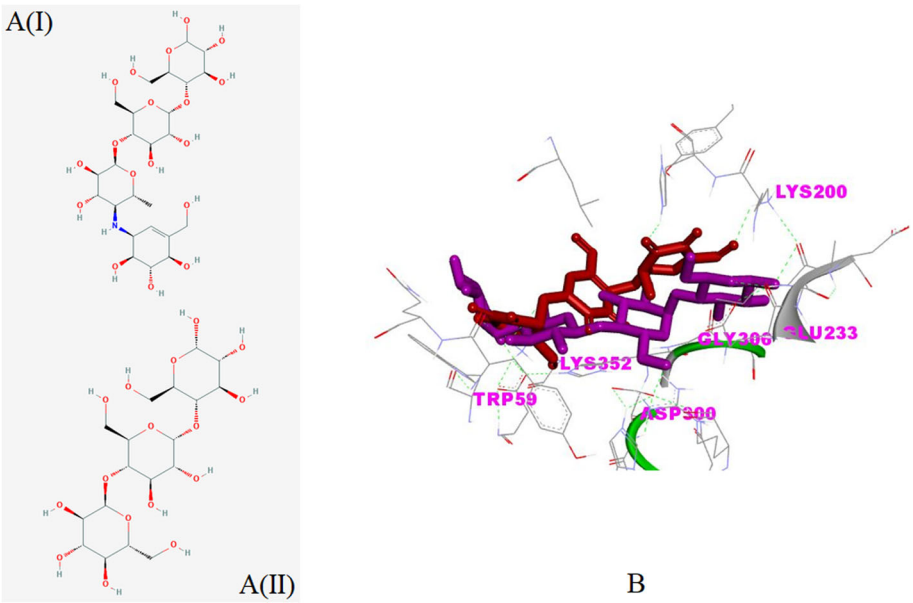

Fig. 4 Molecular docking simultaneous interaction pattern of amylose and acarbose with the active site of HSA (PDB ID: 1SMD) generated using AutoDock Tools 4.2 platform [**A(I)**] 2D structure of acarbose (PubChem CID: 41774) [**A (II)**] 2D structure of amylose (PubChem CID: 192826) (**B**) 3D docking representation of simultaneously docked compounds (amylose- substrate in red and acarbose- inhibitor in magenta) in the binding pocket showing the hydrogen bond interactions

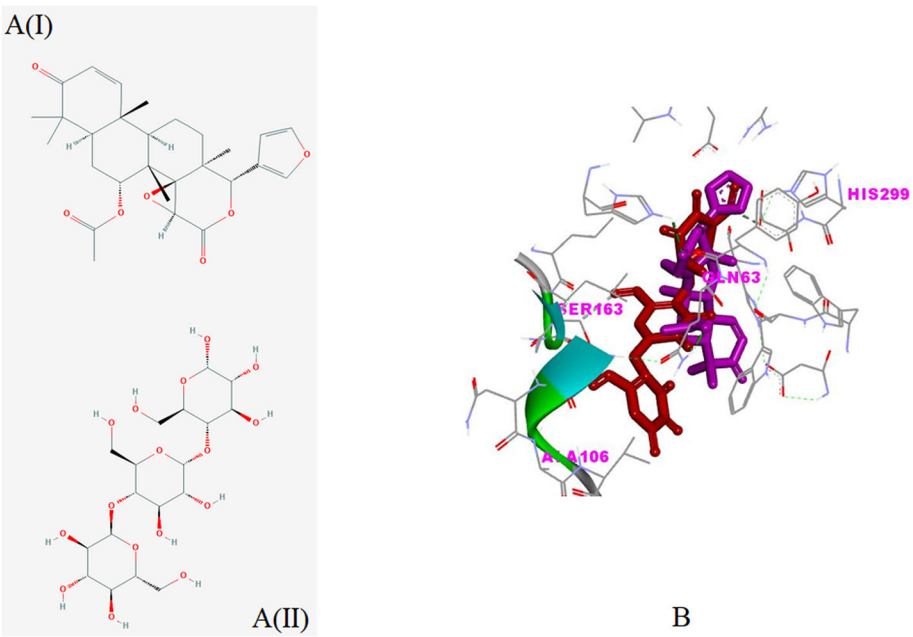

Fig. 5 Molecular docking simultaneous interaction pattern of amylose and gedunin with the active site of HSA (PDB ID: 1SMD) generated using AutoDock Tools 4.2 platform [**A(I)**] 2D structure of gedunin (PubChem CID: 12004512) [**A (II)**] 2D structure of amylose (PubChem CID: 192826) (**B**) 3D docking representation of simultaneously docked compounds (amylose- substrate in red and gedunin- inhibitor in magenta) in the binding pocket showing the hydrogen bond interactions

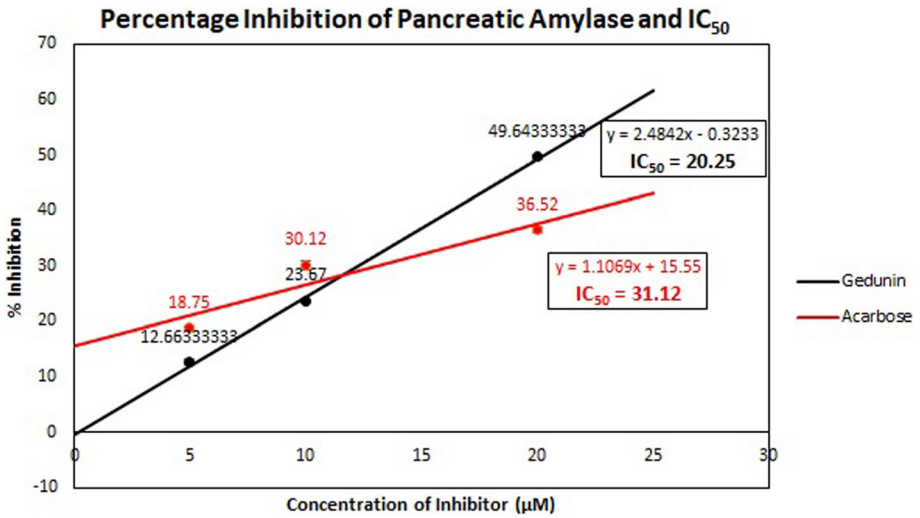

Fig. 6 Percentage inhibition of pancreatic alpha amylase at different concentrations of gedunin and acarbose (standard drug) and IC_{50}. Values represent mean ± SD for three independent experiments and error bars represent standard error (SE)

consequential two-fold increase in (36.98%) inhibition. The IC_{50} values of gedunin and acarbose were found to be 20.25 μM and 31.12 μM respectively.

Salivary α- amylase inhibition

Results of salivary alpha amylase inhibition by acarbose and gedunin are presented in Fig. 7. A concentration dependent inhibition of salivary amylase activity was observed for both. The results showed SA inhibition of 27.48%, 32.6% and 38.27% by 5 μM,10 μM and 20 μM gedunin respectively. Though these values showed a striking similarity in the pattern of inhibition with standard drug, the IC_{50} value of acarbose was 15.74 μM and the IC_{50} value of gedunin was found to be 36.34 μM.

Glucose uptake by yeast cells

The data obtained clearly shows that gedunin effectively inhibited initial glucose uptake in contrast to standard drug metformin which increased the uptake as known to do so. The initial glucose uptake in the presence of 10 and 20 μM gedunin was 30.18% and 21.76% whereas it was 51.17% and 41.40% in presence of metformin having same concentrations against control value of 35.63%. (Fig. 8).

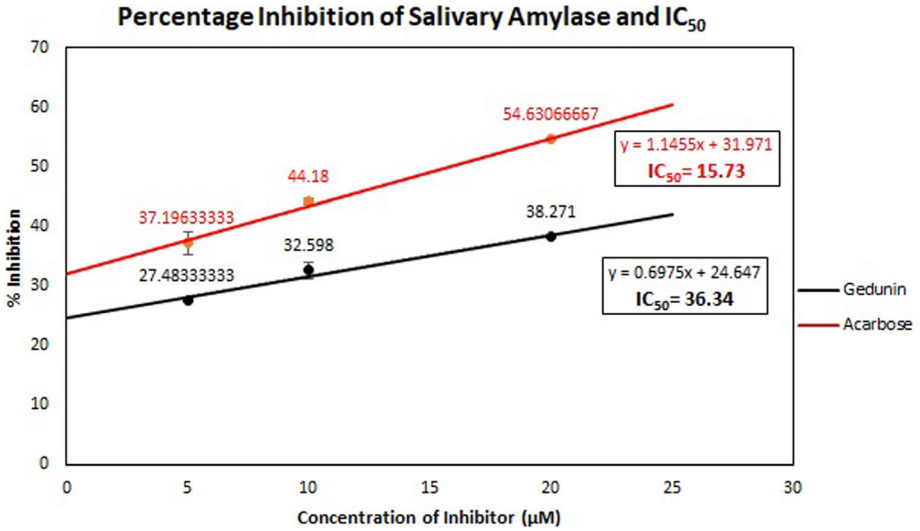

Fig. 7 Percentage inhibition of salivary alpha amylase at different concentrations of gedunin and acarbose (standard drug) and IC_{50}. Values represents mean ± SD for three independent experiments and error bars represent standard error (SE)

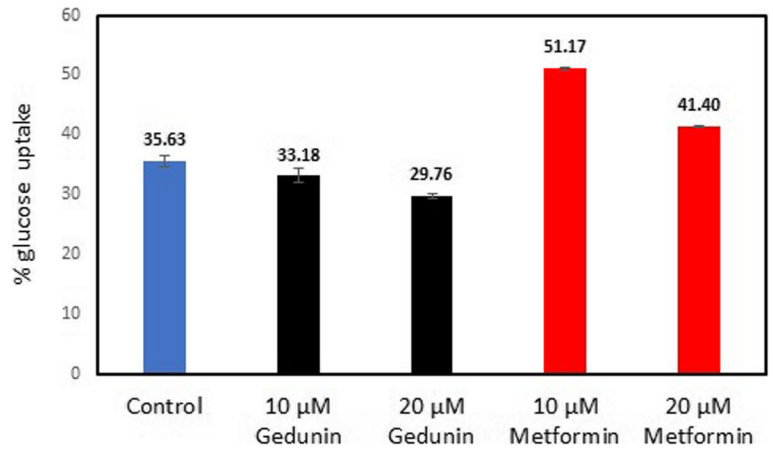

Fig. 8 Percentage glucose (10 mM) uptake by yeast cell at different concentrations of gedunin and acarbose (standard drug). Values represents mean ± SD for three independent experiments and error bars represent standard error (SE)

Discussion

Molecular docking study

Gedunin is one of the limonoids of neem and has been documented to have several therapeutic benefits. The initial interest in gedunin in the present study arose from the fact that it is a known molecular chaperone Hsp90 inhibitor [36]. Hsp90 modulation has been shown to be a new line of treatment in metabolic disorders like diabetes [37, 38]. The reasoned mode of action of gedunin is through the modulation of the chaperone Hsp90. However, it was interesting to learn that gedunin in itself could interact with diabetic targets resulting in therapeutic outcomes [19]. These proof of concept studies led us to evaluate the potential of gedunin as an independent anti-hyperglycaemic agent with the help *of* in silico docking and in vitro studies.

Conventional docking studies focus on the binding of one ligand at a time within the protein site and hence the conformations of simultaneous binding mode information cannot not be precisely defined. In the present study, we have used multiple ligand simultaneous docking approach (MLSD) to enhance the conformational search and sampling.

As evident from our results, gedunin and acarbose both share some common H-bonding amino acid residues like Glu63, Trp59 and His201 with HPA and Glu63, His101 and Trp58 with HSA in presence of amylose (Figs. 4 and 5). This implies that gedunin can recognize and generate a stable conformation near the binding site of enzyme.

It has to be noted that when both gedunin and amylose bind simultaneously at HPA site, it showed a promising stability of the complex in terms of energy and inhibition in terms of Ki value which are even better than standard inhibitor acarbose (Fig. 5). In the present study we have suggested a non-competitive binding of gedunin to the HPA protein site as it occupies slightly different conformations or positions at the site of binding. Gedunin binds partially in the vicinity of the active site proteins.

Inhibition of both the enzymes by gedunin interaction is predicted with fair amount of confidence based on the observed interaction of gedunin with important amino acid residues of the enzymes. These include the Trp58, 59 that play an important role in substrate binding and hydrolytic activity [39], His201 which is a calcium binding residue, Asp197, a likely nucleophile and Asp300 which is essential for catalytic activity [40]. Therefore, a non-competitive, mixed type of inhibition is reasoned as also reported in a previous study [19].

The differences in binding and inhibitory effect of gedunin with respect to pancreatic and salivary amylase can be explained through observation of the single docked and multiple ligand complexes. Differential binding with respect to amino acid residues Glu63, GLy-306 and His305 in the two enzymes might explain the less inhibitory effect of gedunin on HSA as evident from in vitro experiment. In addition, the substitution of Thr163 in HPA with Ser163 of HSA may have some steric effect due to bulkier side chain that makes the gedunin differentially susceptible to the two enzymes.

Pancreatic and salivary α- amylase inhibition

In vitro alpha amylase and alpha glucosidase inhibition of limonoids and tetratriterpenoids of neem have been reported previously [41]. While reports of a similar effect of gedunin are also documented as mentioned earlier, these represent work using semi-purified extracted

gedunin [19]. We used 98% pure gedunin and attempted to elucidate the role of this compound independent of the other extract components. Our investigations showed that pure gedunin was able to inhibit pancreatic alpha amylase in vitro almost linearly with increasing molar concentration. A 36.98% increase in enzyme inhibition between the lowest and highest concentration of gedunin was observed. The IC_{50} values of ovine pancreatic amylase inhibition were consistent with those found for meliacinolin which is also an *A. indica* tetratriterpenoids [42]. Our results are strongly supported by in silico findings that indicate stable binding with human pancreatic alpha amylase. A comparison of the inhibitory effect with the standard drug acarbose revealed a more consistent effect and a lower IC_{50} value. Acarbose is a pseudo tetrasaccharide containing a non-hydrolysable nitrogen-linked bond that suppresses alpha amylase activity by competitive, reversible inhibition [43]. Gedunin also showed distinct salivary amylase inhibition in a dose dependent manner albeit IC_{50} values were higher than acarbose. It is difficult to explain the variation in inhibitory effect of gedunin in the two types of amylases considering that salivary amylase has almost 97% similarity with the pancreatic amylase [25]. And yet our in vitro results corroborate with our *in-silico* observations that clearly indicate distinction in molecular interactions of the two enzymes with the ligands. Amino acid substitutions, positional shifting of participating amino acids and existence of HSA in glycosylated state may all contribute to the functional differences observed for these enzymes. Besides, a previous in vitro study has also shown greater sensitivity of pancreatic amylase to inhibitors as compared to salivary amylase [44, 45].

Glucose uptake by yeast cells

Contrary to the results documented for several anti-hyperglycaemic lead compounds, gedunin exhibited distinct inhibition of glucose uptake by yeast cells. The mechanism of glucose transport across the yeast cell membrane has been receiving attention as in vitro screening method for hypoglycaemic effect of various compounds/ medicinal plants [46] Glucose uptake enhancement is considered to be a desirable attribute as it is reasoned to lead to better management of blood sugar levels and combat hyperglycaemia. However, such uptake by intestinal mucosa would cause increased intestinal glucose absorption and higher blood sugar levels instead. Intestinal absorption of glucose, fructose and other monosaccharides is brought about by the intestinal sugar transporter GLUT2. Studies have shown that the orientation of GLUT2 towards the apical side of the cells is most appropriate for modulation by luminal compounds. An inhibition of glucose uptake would mean interaction and most likely antagonization of the transporter activity. Although glucose transport in *S. cerevisae* takes place by facilitated diffusion, the glucose transport proteins belong to the Hxt family that is distinct from the mammalian transporters. Only a direct study of the interaction of gedunin with the GLUT2 transport protein will be able to shed light on the exact effect of gedunin with respect to glucose uptake. Meanwhile flavonoids especially quercetin have been reported to exhibit robust inhibition of GLUT 2 expressed in xenopus oocytes [47]. In rat intestine metformin was found to slow down glucose absorption mainly by enhancing the secretion of glucagon like peptide 1(GLP1) [48].

There have been substantial attempts over the past few years to use herbal medicines for the treatment of diabetes. Natural compounds have been evaluated for a number of mechanisms that spell anti-diabetic, including inhibition of alpha glucosidase and alpha amylase, the effects on glucose uptake and glucose transporters, the enhancement of insulin secretion and of pancreatic β-cell proliferation, the inhibition of protein tyrosine phosphatase 1B activity and the antioxidant activity [49]. However, the clinical effectiveness of medicinal plants in the treatment and management of diabetes still remains obscure and lacking in evidence-based data. Variability of the raw herbal preparations and extracts could account for the clinical inconsistency and to some extent the non-reproducibility of the study results. Our study was an attempt to verify the clinical potential of gedunin, and the employment of pure gedunin was a deliberate choice to uncover its effect alone, free from several other bioactive components of the neem tree extract.

Conclusion

Post prandial hyperglycaemia is the major cause of diabetic complications. Delayed carbohydrate digestion and glucose absorption helps control blood glucose levels. The present study showed that gedunin distinctly inhibits pancreatic and salivary amylase with improved inhibition efficacy and kinetics compared to acarbose. From the observed effects on glucose uptake in yeast cells, one cannot exclude the possibility that gedunin could be interacting and influencing the activity of the glucose transport protein GLUT2 in the intestinal mucosal cells. This aspect justifies further experimental scrutiny. Considering the multi-target property of the anti-hyperglycaemic effect of gedunin observed in this study, further supplemented by its herbal and hence less toxic nature, gedunin is definitely a good candidate to be added to the arsenal of anti-diabetic drugs. Ours is the first study to the best of our knowledge to describe the effect of gedunin in glucose uptake and we are currently testing the mechanism of action through in silico studies.

Abbreviations

AI: *Azadirachta indica*; DMSO: Dimethyl sulfoxide;; DNS: 3,5-dinitrosalicylic acid; HPA: Human pancreatic α-amylase; HAS: Human salivary α-amylase; MLSD: Multiple ligand simultaneous docking; NIDDM: Non-insulin dependent diabetes mellitus; PPHG: Post prandial hyperglycemia; GLUT: Glucose transporter

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Authors' contributions

SM performed this research, contributed to study design, data analysis and manuscript writing and critically reviewed the final draft. TM contributed to study design, data analysis and manuscript writing and critically reviewed the final draft. JP contributed to study design, data analysis and manuscript writing and critically reviewed the final draft and is the corresponding author. SD, VZ and DS performed in silico studies and contributed to in silico data analysis and manuscript writing and reviewing. The author(s) read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed in this study are included in this published article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Competing interests

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