INVESTIGATION OF IN-VITRO AND IN-SILICO ANTI-DIABETIC POTENTIAL OF DIETHYL PHTHALATE AND NANO PARTICLES (SILVER AND GOLD) WITH DIETHYL PHTHALATE

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Abstract

Impaired insulin synthesis (inhibitory action) or the cells' inability to utilise insulin that has already been created are the two main causes of diabetes, a chronic condition of the metabolism of carbohydrates, lipids, and proteins. Undiagnosed diabetes affects a large portion of population. It is now essential to screen for diabetes and to streamline the diagnostic procedures. Compared to synthetic drugs, herbal remedies are regarded to be safer and have fewer adverse effects. Certain plant-derived bioactive components, which are composed of various chemical components, have shown characteristics that favor their use in the treatment of non-insulin-dependent diabetic mellitus (NIDDM). According to the investigation, the enzymes amylase and glucosidase, which are linked to diabetes, were inhibited more effectively by silver nanoparticles with diethyl phthalate in the test samples. Through molecular docking against the target enzymes DPP4 complexed with syn-7aa (4n8d), Human pancreatic alpha-amylase (1HNY), and Human lysosomal alpha-glucosidase, the molecular interaction was assessed

(5KZW). In the midst of the above, the target diethyl phthalate (DP) showed a substantial interaction with human lysosomal alpha-glucosidase (5KZW), with an overall binding energy of -4.87 kcal/mol. This included details on the creation of altered metal nano drug carrier systems. The therapeutic window for anti-diabetic medications was extended by the more efficient inhibition of the target molecule by silver nanoparticles with DP.

Keywords: Diabetic mellitus (DM), Herbal based drug development, Invitro enzymatic inhibition assays (Alpha-aymylase and glcosidase) and Molecular docking.

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Introduction

Diabetes is a chronic disorder of the metabolism of carbohydrates, lipids, and proteins brought on either by insufficient insulin production (inhibitory action) or by the cells' inability to use insulin that has already been produced (Gupta, A *et al*., 2014). Because of bad human behaviour, it has spread throughout the world. In India, where more than 30 million people have diabetes, the disease is also on the increase. Numerous members of society are impacted by undiagnosed diabetes. Both diabetes screening and the streamlining of diagnostic processes have become crucial (Wang, T, 2001). The two most prevalent kinds of diabetes are type-1 and type-2. Herbal medicines are thought to be safer and have fewer side effects than manufactured medications (Hux *et al*., 2002). Numerous active principles generated from plants that are made up of different chemical compounds have demonstrated properties that support their use in the management of non-insulin-dependent diabetes mellitus (NIDDM) (Mukherjee, P. K, 2006). In the search for suitable anti hyperglycaemic agents, which has recently focused on plants used in traditional medicine, natural products may be a better option than currently used medications (Frye, E. B., 1998).

Approximately 800 plants have historically been very used medicines, and many modern drugs are derived from them, according to ethnobotanical research. Although there are oral diabetic medications available, some still seek a long-term cure for the induction of insulin from a damaged pancreas, as in Type-1 diabetes (Cryer PE., 2010). Pharmaceuticals made from plants are regarded to be less dangerous and to have fewer side effects (Arul, M. *et al*., 2017). Okhuarobo, A. *et al*., (2014) evaluated the possible qualities, which may include anti-diabetic,

anti-infective, anti-angiogenic, hepato-renal protective, sex hormone modulatory, liver enzymes modulatory, and insecticidal actions.

The *Acanthaceae* family of *Andrographis paniculata* (Burm. F.) Wall. Ex Nees (AP), often known as Kalmegh or "King of Bitters." It's been used in Asia for millennia to treat Gastrointestinal and upper respiratory infections, fever, herpes, sore throat, and a range of other chronic and infectious disorders. Andrographis is a plant with an essential "cool quality" in Traditional Chinese Medicine (TCM) that is used to treat body heat in fevers and to remove toxins from the body. It is extensively used to prevent and treat common colds in Scandinavian nations. This review also focuses on its medicinal properties, phytochemistry, and phytochemistry, as well as the pharmacological effects of its many extracts and components. Several of this species' toxicological traits are investigated in this study. The main active constituent, andrographolide, exhibits a wide range of biological activities, including hepatoprotective, anti-inflammatory, antibacterial, anticancer, and antidiabetic actions (Jarukamjorn K, Nemoto N. (2008.). The *Andrographis paniculata* plant was used in the current study to analyse its potential bioactive compound (Diethyl phthalate) modification in nano scale with metals (Silver and Gold) using chemical methods. Their bioactivity of diabetic associated enzymes inhibition also evaluated via in-vitro and in-sillico methods.

Materials and Methods

Sample Preparation

Andrographis paniculata leaf ethanolic crude extract was prepared used soxhlet extraction procedure and it has been further partially purified by column chromatography. Among the chromatographic fractions, fraction-4 was subjected to GC-MS identification and described for the presence of active compound namely Diethyl phthalate. Diethyl phthalate was in turn exploited for the synthesis of silver and gold nano particles using the reducing agent (Ascorbic acid). The synthesized samples were employed for the assessment of invitro-antidiabetic assays.

Invitro-Antidiabetic Activity

α – Amylase inhibition assay

The methodology was described by Thalapaneni NR *et al*., 2008; Heidari R *et al*., 2005 for estimating the property of α-amylase inhibition. A total of 500 µl of reaction mixture contains

test sample (1mg/ml) and standard drug (20-100µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic acid color reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm against positive control (Acarbose 50mg in 10 mL) as Stock solution.

The inhibitory percentage is calculated by the following equation Shai LJ *et al*., 2010.

$$\% \text{ of inhibition} = \frac{\text{OD of control} - \text{OD of sample}}{\text{OD of control}} \text{ X } 100$$

α-Glucosidase inhibition assay

The inhibition of α-glucosidase activity of test sample was determined using the modified method R. T. Dewi *et al.,* 2007. 1 mg of α-glucosidase was dissolved in 100 mL of phosphate buffer (pH 6.8) containing 200 mg of bovine serum albumin. The reaction mixture of 10 µL of test sample was premixed with 490 µL phosphate buffer pH 6.8 and 250 µL of 5 mM p -nitrophenyl α-D-glucopyranoside was added to the mixture. Followed by preincubating at 37 °C for 5 min, add 250 µL α-glucosidase (0.15 unit/mL) and incubated at 37°C for 15 min. The reaction was terminated by adding 2 mL 200 mM Na_2CO_3. α-glucosidase activity was determined spectrophotometrically at 400 nm by measuring the quantity of p -nitrophenol released from p-NPG. Acarbose was used as positive control of α-glucosidase inhibitor. The concentration of the test sample required to inhibiting the 50% of α-glucosidase activity under the assay conditions was defined as the IC_{50} value.

The inhibitory percentage is calculated by the following equation Shai LJ *et al*., 2010.

In-silico Evaluation of Computational Methods

Docking calculations were carried out using Docking Server (*Bikadi, Hazai, 2009*). Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were

merged, and rotatable bonds were defined. Docking calculations were carried out on *Diethyl Phthalate [USAN]* protein model. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools (*Morris, Goodsell et al., 1998*). Affinity (grid) maps of ×× Å grid points and 0.375 Å spacing were generated using the Autogrid program (*Morris, Goodsell et al., 1998*). AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method (*Solis and Wets, 1981*). Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 2 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.

Results

In-vitro Antidiabetic Activity

α – Amylase inhibition assay

α - Amylase is a digestive enzyme that breaks down polysaccharides into monosaccharides and is thus responsible for the elevation of blood glucose levels, which is a sign of diabetes. The inhibition or control of this metabolic enzyme unlocks the suppression of blood glucose. The inhibition property of -amylase was evaluated using study samples such as Andrographis paniculata leaf extract and the chromatographic fraction (diethyl phthalate) of crude leaf ethanolic extract and its nanocomposites (Silver and Gold). The available data showed the promising inhibition proficiency of DPAgNps (diethyl phthalate silver nanoparticles) with 89.72% at 100 µg/mL along with the IC 50 value of 7.55 µg/mL, showing the potential inhibitory effect against the enzyme. The second most active sample was DP gold nanoparticles (DPAuNPs), which demonstrated 87.78% activity at 100 µg/mL and an IC 50 value of 16.86 µg/mL. At 100 µg/mL, *Andrographis paniculata* leaf extract inhibited 84.17% of α -amylase activity and had an IC 50 value of 23.87 µg/mL. Diethyl phthalate showed the least activity at 100 µg/mL, with an IC 50 value of 618.8 µg/mL (**Table:1**). Furthermore, the one-tailed ANOVA

revealed that activity was significant among the sample groups at p<.05 in **Table:2 and Figure:1.**

Table: 1. The Inhibition percentage and ANOVA range of study samples

α-Amylase Inhibition Assay				
Concentration (µg/mL)	***Andrographis peniculata***	**DP**	**DPAgNP**	**DPAuNP**
100	84.17	8.08	89.72	87.78
80	76.67	4.44	80.28	78.61
60	64.17	2.5	67.5	67.78
40	51.67	1.67	63.33	55
20	36.39	0.83	51.67	43.89
IC50[a]	23.87	618.8	7.55	16.86
Mean[b]	62.614	3.504	70.5	66.612
SD[c]	19.18413	2.886941	14.83269	17.63894
SE[d]	2.493415	1.42003	5.066714	3.405554
ANOVA[e]	The f-ratio value is 22.14562. The p-value is < .00001. The result is significant at p < .05.			

[a] Inhibition concentration 50, [b] Mean average value, [c] Standard deviation, [d] Standard error and [e] Variance of the data group.

Table: 2. The Analysis of Variance among the study samples against α-Amylase Inhibition Assay

Summary of Data						
	Treatments					
	1	2	3	4	5	Total
N	5	5	5	5		20
∑X	313.07	17.52	352.5	333.06		1016.15
Mean	62.614	3.504	70.5	66.612		50.808
$\sum X^2$	21074.6877	94.7278	25731.2846	23430.321		70331.0211
Std.Dev.	19.1841	2.8869	14.8327	17.6389		31.3746
Result Details						
Source	***SS***	***df***	***MS***			
Between-treatments	15072.9567	3	5024.3189	*F* = 22.14562		
Within-treatments	3630.0233	16	226.8765			
Total	18702.98	19				

The f-ratio value is 22.14562. The p-value is < .00001. The result is significant at p < .05.

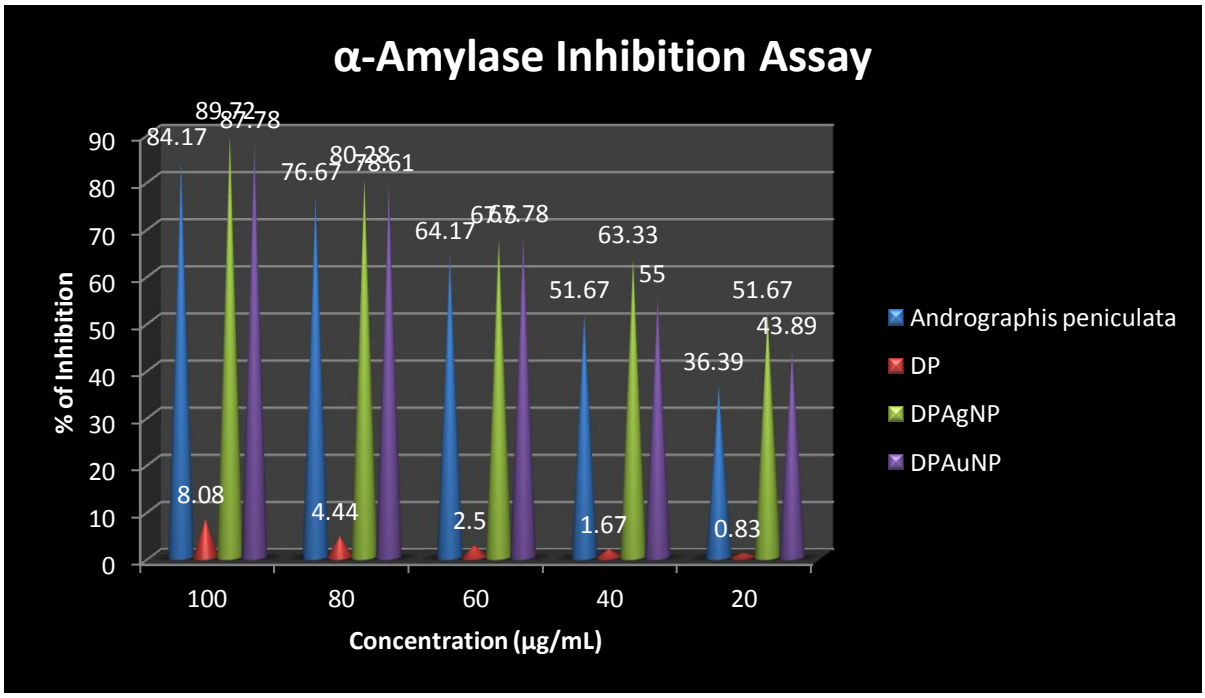

Figure: 1. α-Amylase Inhibition percentage of study samples

α – Glucosidase inhibition assay

α -Glucosidase is another digestive enzyme that is responsible for the elevation of blood glucose levels in diabetics by breaking down the 1-4 glycosidic linkage of disaccharides into monosaccharides. The inhibition or control of this metabolic enzyme unlocks the suppression of blood glucose. The inhibition property of -glucosidase was evaluated in the study samples, which included *Andrographis paniculata* leaf extract and the chromatographic fraction (diethyl phthalate) of crude leaf ethanolic extract and its nanocomposites (Silver and Gold). The results showed that Andrographis paniculata leaf extract inhibited the enzyme 70.62% at 100 µg/mL, with an IC 50 value of 61.37 µg/mL, indicating a potential inhibitory effect. The second most active sample was DP silver nanoparticles (DPAgNPs), which showed 69.62% activity at 100 g/mL and an IC 50 value of 34.81 µg/mL. At 100 µg/mL, DPAuNPs (DP gold nanoparticles) inhibited 33.2% of α -glucosidase activity and had an IC50 value of 150.6 µg/mL. Diethyl phthalate was estimated to have the least activity at 100 µg/mL, with an IC 50 value of 311.7 µg/mL described in **table: 3**. Furthermore in **table: 4**, the one-tailed ANOVA revealed that activity was significant among the sample groups at $p<.05$ **(Figure: 2)**.

Table: 3. The Inhibition percentage and ANOVA range of study samples

α-Glucosidase Inhibition Assay				
Concentration (µg/mL)	***Andrographis peniculata***	**DP**	**DPAgNP**	**DPAuNP**
100	70.62	16.04	69.62	33.2
80	49.76	12.57	65.86	30.73
60	42.6	9.69	60.63	26.67
40	17.98	5.41	52.94	19.86
20	7.11	2.53	44.48	13.63
IC50[a]	61.37	311.7	34.81	150.6
Mean[b]	37.614	9.248	58.706	24.818
SD[c]	25.38587	5.413503	10.11957	8.037299
SE[d]	4.321478	0.363703	1.843793	1.662595
ANOVA[e]	The f-ratio value is 10.40765. The p-value is .000485. The result is significant at $p < .05$.			

[a] Inhibition concentration 50, [b] Mean average value, [c] Standard deviation, [d] Standard error and [e] Variance of the data group.

Table: 4. The Analysis of Variance among the study samples against α-Glucosidase Inhibition Assay

Summary of Data						
	Treatments					
	1	2	3	4	5	Total
N	5	5	5	5		20
∑X	188.07	46.24	293.53	124.09		651.93
Mean	37.614	9.248	58.706	24.818		32.597
$\sum X^2$	9651.8345	544.8516	17641.5949	3338.0583		31176.3393
Std.Dev.	25.3859	5.4135	10.1196	8.0373		22.8562
Result Details						
Source		***SS***	***df***	***MS***		
Between-treatments		6562.6941	3	2187.5647		$F = 10.40765$
Within-treatments		3363.009	16	210.1881		
Total		9925.7031	19			

The f-ratio value is 10.40765. The p-value is .000485. The result is significant at $p < .05$.

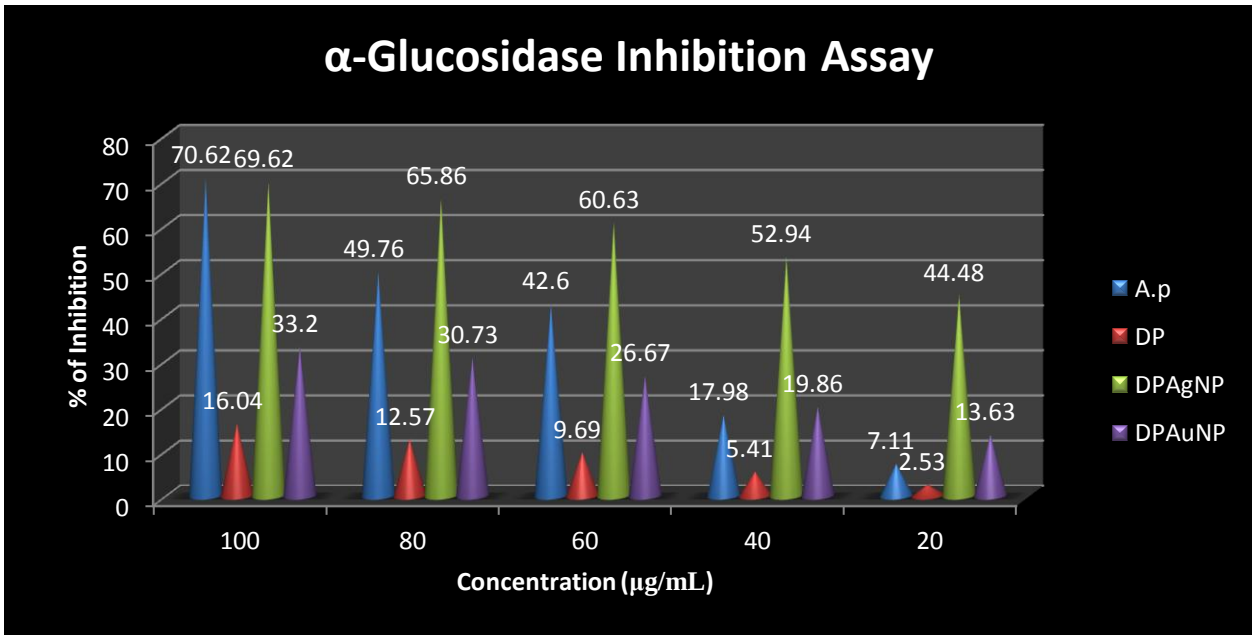

Figure: 2. α-Glucosidase Inhibition percentage of study samples

In-silico evaluation of Anti-diabetic activity

Docking calculations were carried out to evaluate the drug target interaction using computational algorithms, and the diethyl phthalate was examined for its interaction ability against the diabetic-associated carbohydrate metabolic enzymes such as DPP4 (Dipeptidyl-peptidase 4), complexed with syn-7aa (4n8d), human pancreatic alpha-amylase (1HNY), and human lysosomal alpha-glucosidase (5KZW) showed in **Figure: 3**. Diethyl phthathale shows some toxicity beyond the dosage limit, hence it can exhibit certain biological activity as here described in the interaction with the diabetic-associated metabolic enzymes. The drug (DP) had the best interaction with the target of human lysosomal alpha-glucosidase (5KZW), with a bonding free energy of -4.87 kcal/mol. It was further described in **Table: 5** with an estimated inhibition constant (Ki) of 269.87 M, surface interaction (558.439), and total intermolecular energy of -6.49 kcal/mol. The bonding was held against the amino acids of PRO217 (-0.5296), PHE129 (-0.5208), and PRO238 (-0.4811) through hydrophobic interaction **(Figure: 4)**.

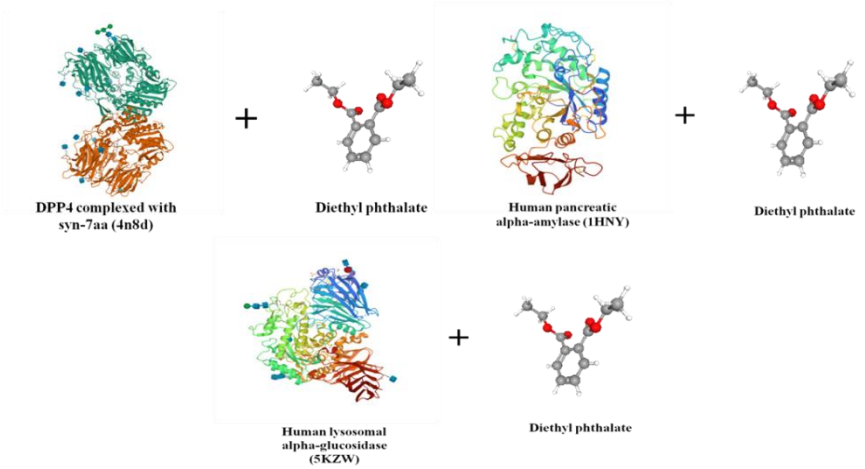

Figure: 3. The Structure Illustration of Target and Ligand Molecules

Table: 5. The Description value of Molecular Interaction between Target (human lysosomal alpha-glucosidase (5KZW) and Ligand (DP) Molecules

Rank	Estimated free energy on Binding	Estimated Inhibition Constant (Ki)	VdW + Hbond + Desolve Energy	Electrostatic Energy	Total Intermolecular Energy	Frequency	Surface Interaction
1	-4.87 kcal/mol	269.87 uM	-6.49 kcal/mol	+0.00 kcal/mol	-6.49 kcal/mol	50%	558.439
2	-4.31 kcal/mol	694.52 uM	-6.02 kcal/mol	-0.10 kcal/mol	-6.12 kcal/mol	50%	555.606

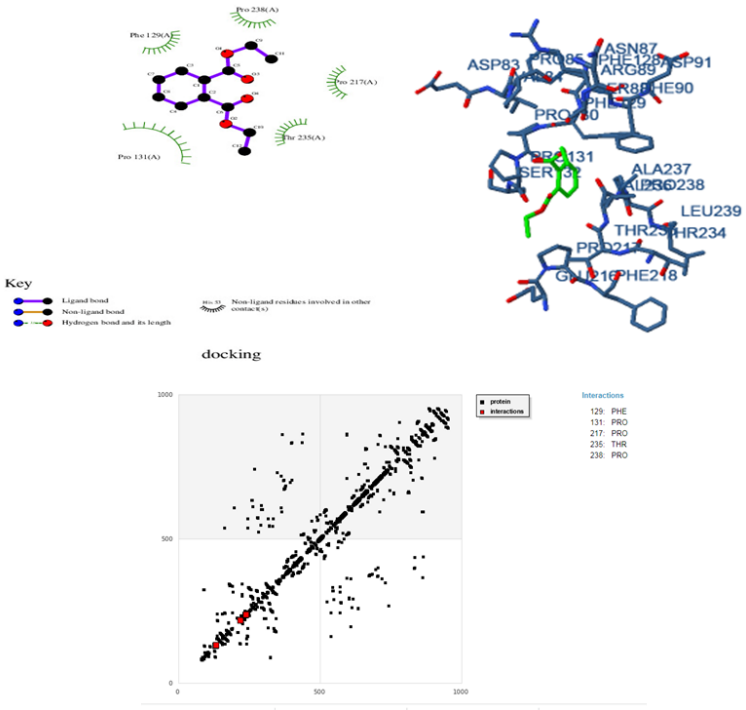

Figure: 4. Pictorial Illustration of human lysosomal alpha-glucosidase (5KZW) and DP Docking via possible Amino acids

While the interaction of diethyl phthalate with DPP4 (Dipeptidyl-peptidase 4) complexed with DPP4 syn-7aa (4n8d) was quite moderate, showing the bonding free energy of -3.73 kcal/mol, 1.85 mM, the estimated inhibition constant (Ki), and 511.74 of surface interaction with -5.42 kcal/mol of total intermolecular energy described in **Table: 6** The interaction was meant to be in SER106 (-0.5231) with hydrogen bonding, GLU117 (-0.603) with polar bonding, and TRP154 (-0.866), TYR132 (-0.5845), and VAL155 (-0.5179) with hydrophobic bonding **illustrated in Figure: 5**.

Table: 6. The Description value of Molecular Interaction between Target (DPP4 syn-7aa (4n8d) and Ligand (DP) Molecules

Rank	Estimated free energy on Binding	Estimated Inhibition Constant (Ki)	VdW + Hbond + Desolve Energy	Electrostatic Energy	Total Intermolecular Energy	Frequency	Surface Interaction
1	-3.73 Kcal/Mol	1.85 mM	-5.35 kcal/Mol	-0.07 kcal/Mol	-5.42 kcal/Mol	50%	511.74

2	-3.56 Kcal/Mol	2.46 mM	-5.14 kcal/Mol	-0.09 kcal/Mol	-5.23 kcal/Mol	50%	529.591

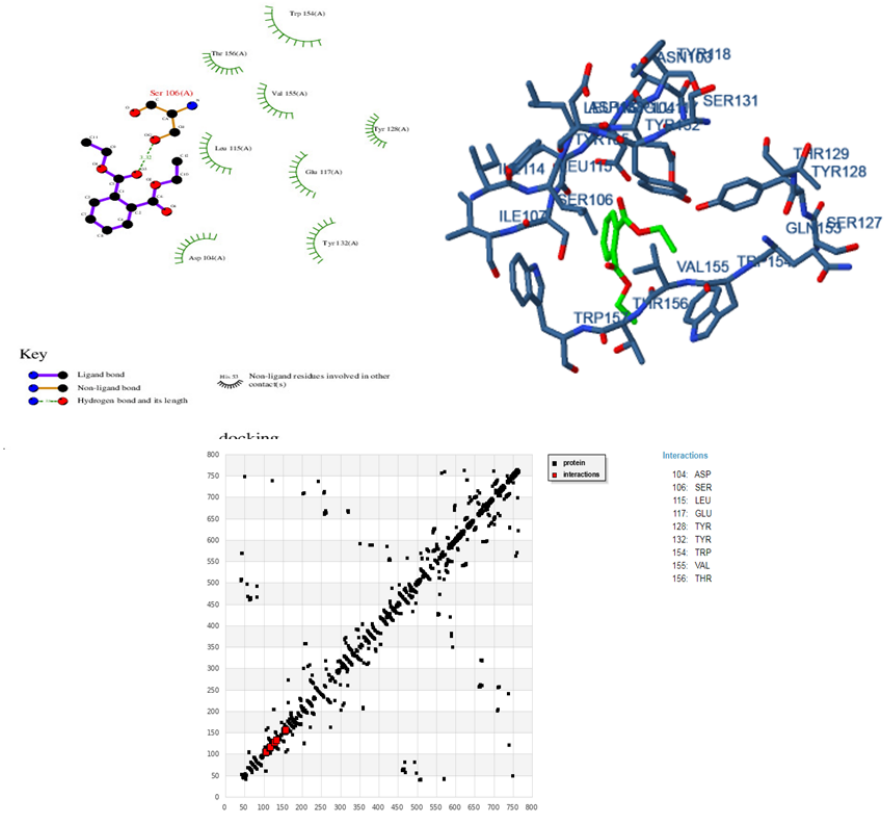

Figure: 5. Pictorial Illustration of DPP4 syn-7aa (4n8d) and DP Docking via possible Amino acids

In Table, the least-binding free energy was calculated for human pancreatic alpha-amylase (1HNY): -3.02 kcal/mol and -4.74 kcal/mol total intermolecular energy. The estimated inhibition constant (Ki) was 6.11 mM and overall surface interaction is about 567.393. The interaction setup for DP and 1HNY was shown in the **figure: 6** as hydrophobic bonding in TRP59 (-2.2474), TYR62 (-1.0412), HIS305 (-0.2745), and polar interaction in ASP300 (-0.2528).

Table: 7. The Description value of Molecular Interaction between Target (human pancreatic alpha-amylase (1HNY) and Ligand (DP) Molecules

Rank	Estimated free energy on Binding	Estimated Inhibition Constant	VdW + Hbond + Desolve	Electrostatic Energy	Total Intermolecular Energy	Frequency	Surface Interaction

		(Ki)	**Energy**				
1	-3.02 kcal/mol	6.11 mM	-4.76 kcal/mol	+0.02 kcal/mol	-4.74 kcal/mol	50%	567.393
2	-2.74 kcal/mol	9.89 mM	-4.46 kcal/mol	-0.02 kcal/mol	-4.49 kcal/mol	50%	479.724

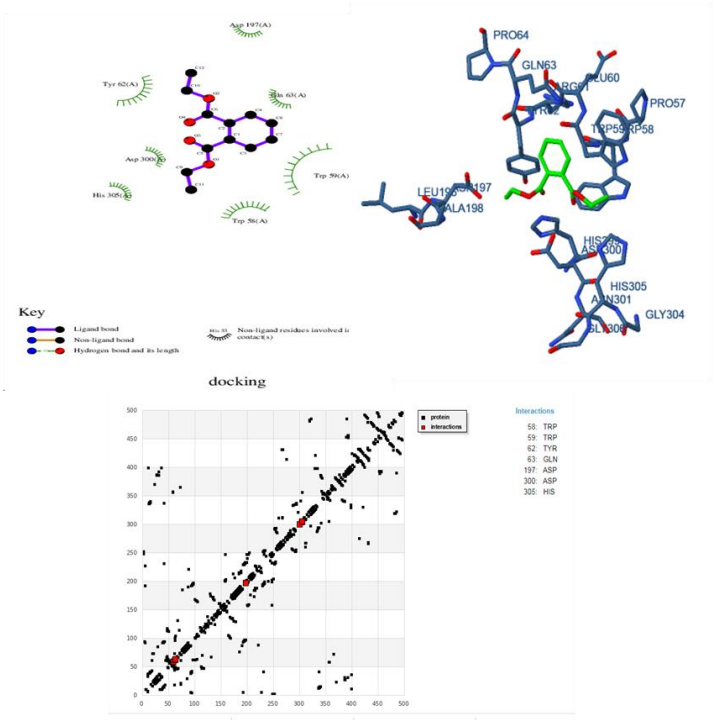

Figure: 6. Pictorial Illustration of human pancreatic alpha-amylase (1HNY) and DP Docking via possible Amino acids

Discussion

The Indian herb Andrographis paniculata (Burm. f.) Nees has been used for a variety of things, but its main usage has been to prevent diabetic mellitus (DM) (Niranjan A. et al., 2010). According to Zhang and Tan, the ethanolic extracts are effective at lowering blood glucose levels in rats with type 1 DM who have been given the drug streptozotocin (STZ). Additionally, the type 1 DM rats' superoxide dismutase (SOD) and catalase activities are increased by the water soluble extract, demonstrating its antioxidant properties (Dandu AM. *et al*., 2009). In comparison to controls, the pure extract and andrographolide considerably (P 0.05) reduced the levels of blood glucose, triglyceride, and LDL. Serum cholesterol and rat body weight did not alter,

though. Similar results were seen with metformin on these metrics (Nugroho, A. E. *et al*., 2012). It was discovered that *A. paniculata* leaf extract was extremely successful in bringing the abnormal metabolic profile of obdb rats back to normal settings. Additional genetic research and cutting-edge molecular tools may provide light on the molecular pathways behind diabetes mellitus aetiology (Akhtar, M. T. *et al*., 2016).

Andrographis paniculata and *Eugenia polyantha* leaf may play a beneficial effect in type 2 diabetes patients, according to a preliminary research by N I Ischak and D N Botutihe (2020). These medicinal herbs were supplied as a pill containing 300 mg. The findings indicated that, among 20 responders from each group, the fasting blood glucose level reduced by 70% and 80%, respectively. The research of Roy, B. (2007) revealed that both glucose-loaded and diabetic rats responded well to the hot water and ethanol extracts of Andrographis paniculata, also known as Kalomegh locally. Blood sugar levels were elevated by oral glucose treatment (1.5 g/kg body weight) and elevated by intraperitoneal alloxan injection (40 mg/kg).

According to in vitro assays of study samples against the inhibitory effect of diabetic-associated enzymes such as α -amylase, it demonstrated 89.72 percent activity at 100 μg/mL with an IC 50 value of 7.55 μg/mL. While the α -glucosidase enzyme was inhibited by the leaf extract of *Andrographis peniculata*, the inhibition percent was approximately 70.62 μg/mL, with an IC 50 value of 61.37 μg/mL. As well, among the target proteins, the finest molecular interaction showed against Human lysosomal alpha-glucosidase (5KZW) with the overall binding energy of -4.87 kcal/mol through the amino acids of PRO217 (-0.5296), PHE129 (-0.5208), and PRO238 (-0.4811) through hydrophobic interaction.

Conclusion

Among the test samples, silver nanoparticles with diethyl phthalate exhibited enhanced inhibitory effects on the diabetic-associated enzymes -amylase and -glucosidase. The molecular interaction was evaluated through molecular docking against the target enzymes such as DPP4 complexed with syn-7aa (4n8d), Human pancreatic alpha-amylase (1HNY) and Human lysosomal alpha-glucosidase (5KZW). Target diethyl phthalate (DP) demonstrated a significant interaction against human lysosomal alpha-glucosidase (5KZW) with an overall binding energy of -4.87 kcal/mol in the middle of the above. This provided information about the development

of modified metal nano drug carrier systems. Silver nanoparticles with DP inhibited the target molecule more effectively, broadening the therapeutic window for anti-diabetic drugs.

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