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Molecular Docking interaction of Kaempferol-3-o-α-l-rhamnoside isolated from *Cardiospermum halicacabum* Linn with molecular targets involved in blood glucose homeostasis

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Abstract:

Aim: Molecular docking has become a progressively vital tool for drug discovery and development area. This technique is widely used to screen most potent drugs. The potency of isolated compounds from medicinal plants were assessing through docking. Diabetes mellitus being a most common endocrine disorder with the highest rates of prevalence and mortality, supplement of isolated compounds from herbal plants will be effective in the management of the disease. Hence in the present study the efficiency of the derivative of Kaempferol, a well-known flavonoid isolated from *Cardiospermum halicacabum* leaf extract in the management of diabetes been assessed through docking with various blood glucose regulating enzymes.

Methods: Molecular docking studies of the ligand with selected proteins were performed using PatchDock techniques used in Computer Vision.

Results: In the current research Kaempferol- 3- 0- α- l- rhamnoside showed good affinity with all the proteins. It showed highest binding affinity with much lesser binding energy with glycogen synthase kinase and glycogen phosphorylase. It also showed good quality of binding with Glucokinase, α-amylase and aldose reductase with relatively less binding energy.

Conclusion: The present study provides scientific evidence for the antidiabetic potential of Kaempferol- 3- 0- α- l- rhamnoside and anticipated to be useful in guiding the rational design of novel and robust drug for the treatment of diabetes.

Key words: Diabetes mellitus, blood glucose regulating enzymes, Kaempferol- 3- 0- α- l- rhamnoside, Molecular docking.

INTRODUCTION:

Molecular docking has become a progressively vital tool for drug discovery and development area. It provides detailed view of drug receptor interactions and also a new approach to drug design. This technique is employed for predicting and analysing the interactions between protein receptors and ligands by the orientation of one molecule to a second when bound to each other to form a stable complex [1]. Docking mechanism includes Searching Conformational Space, in which all possible orientations and conformations of the protein paired with ligand will be computed. Further the Scoring Functions, wherein the energy of the pose (protein-ligand pair) is estimated. Low energy indicates stable system and thus a likely binding interaction. A binding interaction between the ligand and the protein (enzyme) may result in activation or inhibition of the enzyme. In case of receptor proteins, ligand binding may result in agonism or antagonism. This technique is widely used to screen most potent drugs [2]. Currently the potency of isolated compounds from medicinal plants were assessing through docking.

Diabetes mellitus (DM) is the most common endocrine disorder with the highest rates of prevalence and mortality. It is the most common non-communicable disease worldwide [3]. Since the side effects of modern medicines were well-known, herbal supplements and other alternative medicines have gradually increased in treatment of diabetic disorders because of their safety and efficacy [4]. Compounds isolated from medicinal plants are potentate than crude extract. Hence the supplement of isolated compounds will be effective in the management of the disease. The potent antidiabetic compounds can be evaluated by docking it with the enzymes involved in blood glucose homeostasis.

Kaempferol, a natural polyphenol belonging to the flavonoid group, identified at high levels in broccoli, chives, tea, grapes, tomatoes and strawberries. It is isolated from various parts of the plant like flower, leaf or seeds. Studies have shown that kaempferol can help in the treatment of cancers, cardiovascular disease and neurological disorders [5]. Derivatives of kaempferol isolated from different plants showed significant decrease in blood glucose level, lipid profiles and improved activity of enzymatic and non-enzymatic antioxidants [6]. Although many studies have revealed the antidiabetic effect of kaempferol the potency of this compound can be evaluated by docking analysis. Hence in the present study the efficiency of Kaempferol- 3- o- α- l- rhamnoside isolated from *Cardiospermum halicacabum (C. halicacabum)* leaf extract in the management of diabetes been assessed through docking with various blood glucose regulating enzymes.

METHODS:

Selection of Target proteins

The Protein Data Bank (PDB) is a crystallographic database for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids.

The three-dimensional structure of selected proteins, Glucokinase (GK), α-amylase, aldose reductase, glycogen phosphorylse and Glycogen Synthase Kinase (GSK) were downloaded from the RCSB protein Data Bank. [Last accessed on 2014 Aug 15]. Available from: http://www.rcsb.org/pdb/home/home.do

Molecular Docking

Molecular docking studies of the ligand Kaempferol-3-o-α-l- rhamnoside with selected proteins GK, α-amylase, aldose reductase, glycogen phosphorylse and GSK was performed using PatchDock. PatchDock is an algorithm for molecular docking inspired by object recognition and image segmentation techniques used in Computer Vision. Given two molecules, their surfaces are divided into patches according to the surface shape. These patches correspond to patterns that visually distinguish between puzzle pieces. Once the patches are identified, they can be superimposed using shape matching algorithms. The algorithm has three major stages: 1. Molecular Shape Representation, 2. Surface Patch Matching, 3. Filtering and Scoring [7, 8]. The Ligand "Kaempferol-3-o-α-l-rhamnoside" and selected target proteins (GK, α-amylase, aldose reductase, glycogen phosphorylse and GSK) are uploaded in '.pdb' format in PatchDock to predict the predominant binding models. The 3D structures of the ligands were obtained in sdf file format and were converted into pdb files using Maestro. The structure was viewed by RasMol. Docking simulations were performed and docking scores were calculated for each docked conformations. The obtained docked complexes were run through Fire dock (Fast Interaction Refinement in Molecular Docking) to produce the refinement and rescoring of rigid body protein-ligand solutions.

Analysis of the Best docked conformations

The best refined docked protein-ligand complexes from Fire dock are then analyzed and complete illustration is generated using PDBsum. It is a database that provides an overview of the contents of each 3D macromolecular structure deposited in the Protein Data Bank. The best solution obtained from the Fire Dock is uploaded to PDBSum Generate in '.pdb' format and the interactions between the ligand and target proteins are analysed [Figure-1].

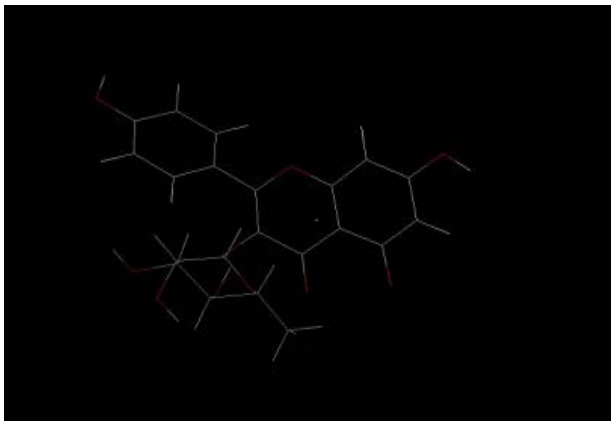
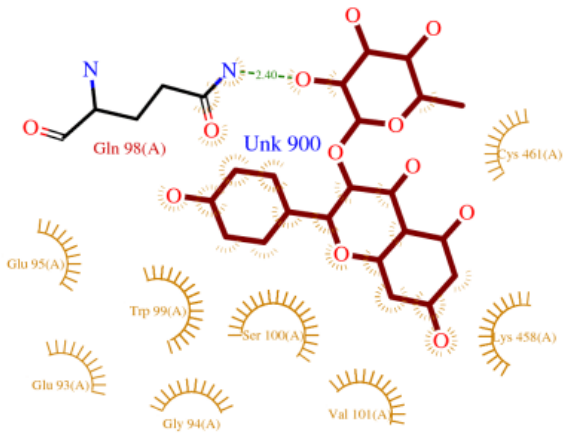


Figure: 1. 3D structure of Kaempferol- 3- 0- α- l- rhamnoside

RESULTS AND DISCUSSION:

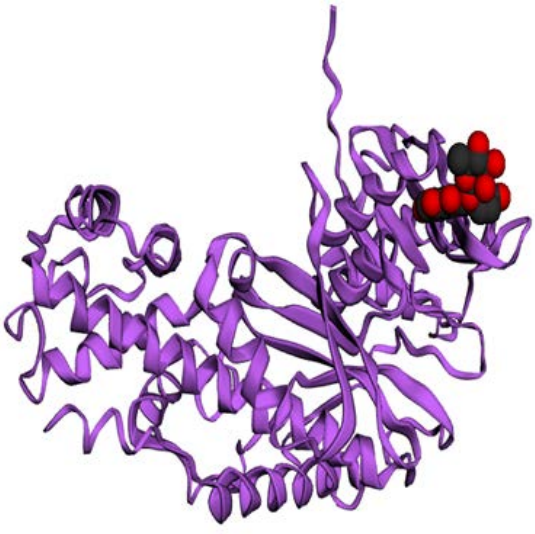
Docking interactions of Kaempferol- 3- 0- α- l- rhamnoside with Glucokinase:

Kaempferol- 3- 0- α- l- rhamnoside exhibited better docking interactions with glucokinase with a binding energy of -33.19 Kcal/mol. The interactions are favoured by the formation of H- bond with Gly 98 and hydrophobic interactions with Cys 461, Glu 95, Trp 99, Ser 100, Lys 458, Val 101, Gly 94 and Glu 93 (Figure 1). In addition to that Kaempferol showed 73 non bonded interactions with glucokinase. According to Ramachandran plot and statistics, Kaempferol- 3- 0- α- l- rhamnoside showed 73.9% of binding with the protein in the most favoured regions [A, B, L] [Figure 2 and 3].



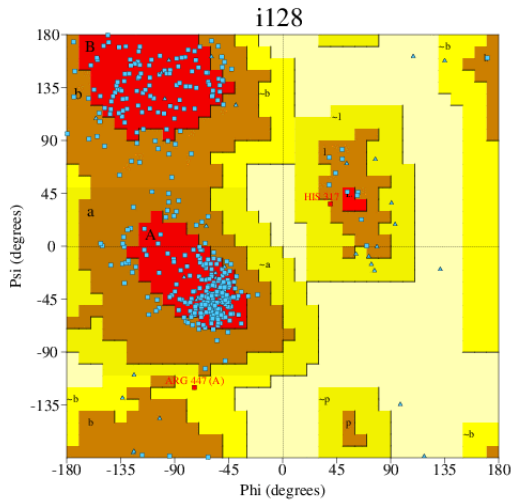
i128: Ligplot of interactions with UNK

(a). Hydrophobic and hydrophilic interactions of Kaempferol- 3- 0- α- l- rhamnoside with Glucokinase. Brown colored half circle indicates the hydrophobic reactions of Kaempferol- 3- 0- α- l- rhamnoside with the target enzymes Glucokinase. Green dotted lines indicate the hydrogen bond while green colored value indicates their bond length.



(b). Binding pose of Kaempferol- 3- 0- α- l- rhamnoside with Glucokinase.

Figure: 2.



(a) Ramachandran plot for Glucokinase

		No. of residues	%-tage
Most favoured regions	[A,B,L]	278	73.9%**
Additional allowed regions	[a,b,l,p]	96	25.5%
Generously allowed regions	[~a,~b,~l,~p]	2	0.5%
Disallowed regions	[XX]	0	0.0%
Non-glycine and non-proline residues		376	100.0%
End-residues (excl. Gly and Pro)		8	
Glycine residues		35	
Proline residues		9	
Total number of residues		428	

(b) Ramachandran plot statistics

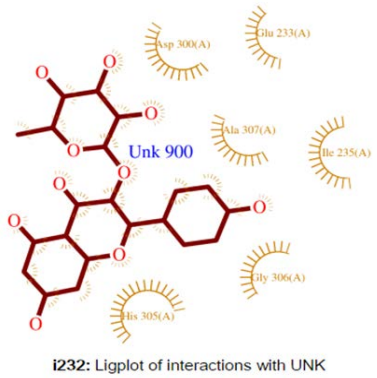
Figure: 3

Docking interactions of Kaempferol- 3- o- α- l- rhamnoside with α-amylase:

Kaempferol- 3- 0- α- l- rhamnoside binds with α-amylase with the binding energy of -35.03 Kcal/mol. It is observed that no H-bond between Kaempferol and α-amylase complex. Along with 79 non bonded interactions the following hydrophobic interactions were found between the enzyme and the ligand complex; Asp 300, Glu 233, ala 307, Ile 235, Gly 306 and His 305 (Figure 3). Binding efficiency of the ligand with the protein in the most favoured regions [A, B, L] was 91.0% (Figure 4 and 5).

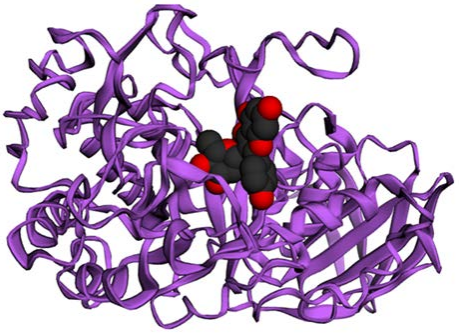
Docking interactions of Kaempferol- 3- o- α- l- rhamnoside with Aldose reductase

The docking interaction of Kaempferol- 3- o- α- l- rhamnoside with Aldose reductase showed the binding energy of -18.53 Kcal/mol. No H-bonds were seen between the ligand-protein complex (Figure 5). The interactions were favoured by the hydrophobic bonds at the positions of His 312, Glu 313, Phe 311, Glu 314 and 65 non bonded contacts. Kaempferol showed 91.4% of binding efficiency with aldose reductase in the most favoured regions [A,B,L] (Figure 6 and 7).



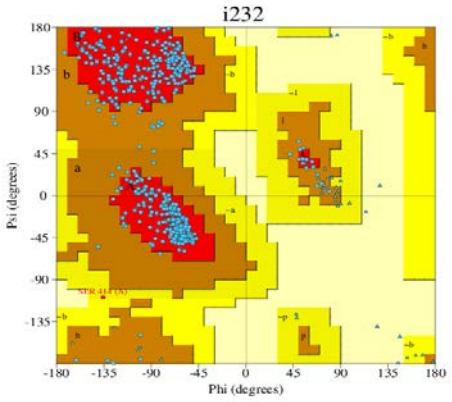
i232: Ligplot of interactions with UNK

(a) Hydrophobic and hydrophilic interactions of Kaempferol- 3- 0- α- l- rhamnoside with α-amylase. Brown colored half circle indicates the hydrophobic reactions of Kaempferol- 3- 0- α- l- rhamnoside with the target enzymes α-amylase.



(b) Binding pose of Kaempferol- 3- 0- α- l- rhamnoside with α-amylase.

Figure: 4

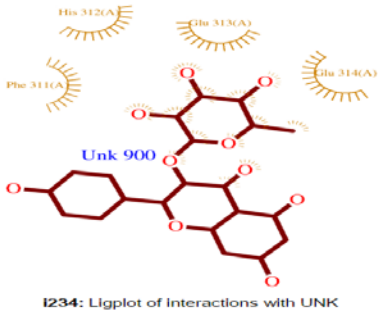


(a). Ramachandran plot for α-amylase

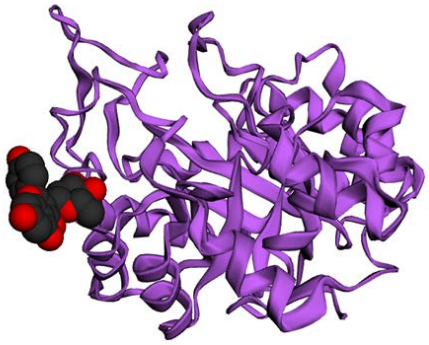
		No. of residues	%-tage
Most favoured regions	[A,B,L]	382	91.0%
Additional allowed regions	[a,b,l,p]	37	8.8%
Generously allowed regions	[~a,~b,~l,~p]	1	0.2%
Disallowed regions	[XX]	0	0.0%
Non-glycine and non-proline residues		420	100.0%
End-residues (excl. Gly and Pro)		5	
Glycine residues		50	
Proline residues		23	
Total number of residues		498	

(b). Ramachandran plot statistics

Figure: 5

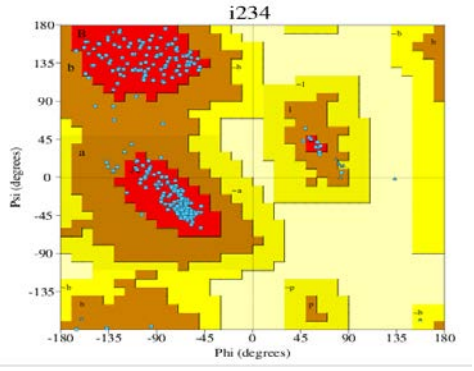


(a). Hydrophobic and hydrophilic interactions of Kaempferol- 3- 0- α- l- rhamnoside with Aldose reductase. Brown colored half circle indicates the hydrophobic reactions of Kaempferol- 3- 0- α- l- rhamnoside with the target enzymes Aldose reductase.



(b). Binding pose of Kaempferol- 3- 0- α- l- rhamnoside with Aldose reductase.

Figure: 6



(a) Ramachandran plot for Aldose reductase

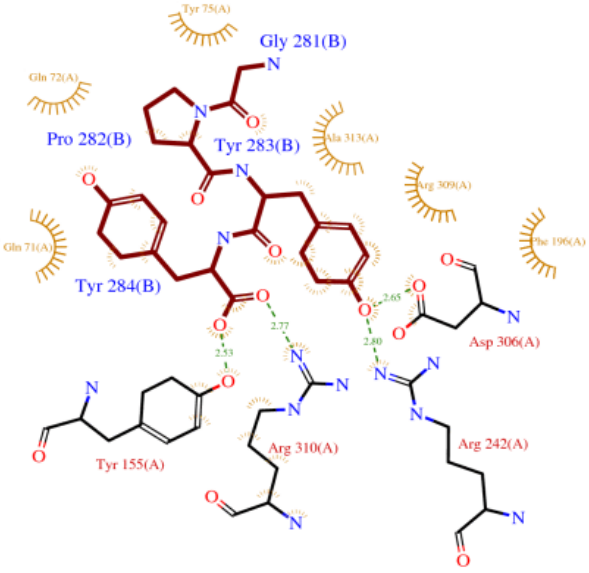
		No. of residues	%-tage
Most favoured regions	[A,B,L]	254	91.4%
Additional allowed regions	[a,b,l,p]	24	8.6%
Generously allowed regions	[~a,~b,~l,~p]	0	0.0%
Disallowed regions	[XX]	0	0.0%
Non-glycine and non-proline residues		278	100.0%
End-residues (excl. Gly and Pro)		5	
Glycine residues		16	
Proline residues		20	
Total number of residues		319	

(b) Ramachandran plot statistics

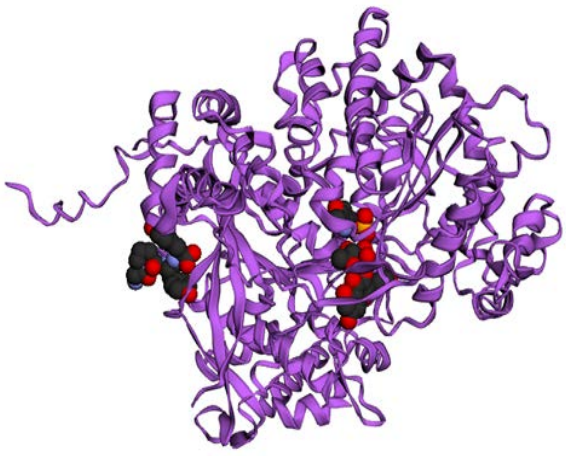
Figure: 7

Docking interactions of Kaempferol- 3- o- α- l- rhamnoside with Glycogen phosphorylase

Kaempferol- 3- o- α- l- rhamnoside showed highest binding affinity with lesser binding energy of -51.30 kcal/mol with the enzyme glycogen phosphorylase. The binding interactions were favoured by H-bonds at the positions of Tyr 155, Arg 242, Asp 306 and Arg 310, hydrophobic bonds at the positions of Gln 72, Gln 71, Tyr 75, Ala 313, Arg 309, Phe 196 and 48 non bonded contacts. It showed 80.7% of binding efficiency with the protein in the most favoured regions [A, B, L] [Figure 8 and 9].

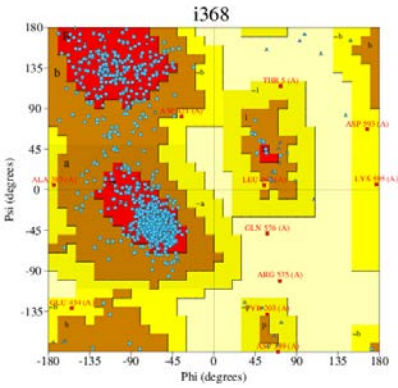


(a). Hydrophobic and hydrophilic interactions of Kaempferol- 3- 0- α- l- rhamnoside with Glycogen phosphorylase. Brown colored half circle indicates the hydrophobic reactions of Kaempferol- 3- 0- α- l- rhamnoside with the target enzymes DPP. Green dotted lines indicate the hydrogen bond while green colored value indicates their bond length.



(b). Binding pose of Kaempferol- 3- 0- α- l- rhamnoside with Glycogen phosphorylase.

Figure: 8



(a) Ramachandran plot for Glycogen phosphorylase

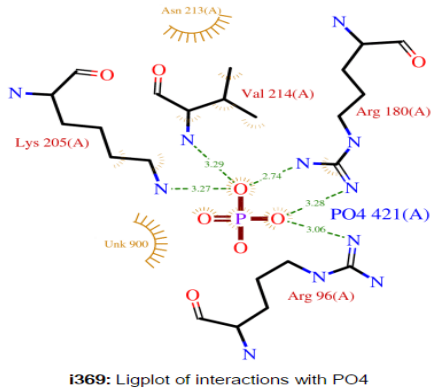
		No. of residues	%-tage
Most favoured regions	[A,B,L]	585	80.7%*
Additional allowed regions	[a,b,l,p]	129	17.8%
Generously allowed regions	[~a,~b,~l,~p]	9	1.2%
Disallowed regions	[XX]	2	0.3%*
Non-glycine and non-proline residues		725	100.0%
End-residues (excl. Gly and Pro)		11	
Glycine residues		44	
Proline residues		32	
Total number of residues		812	

(b) Ramachandran plot statistics

Figure: 9

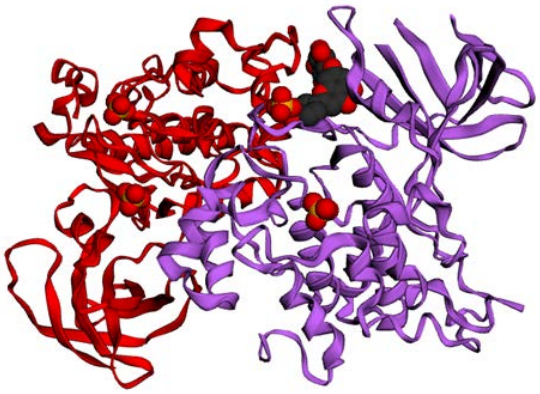
Docking interactions of Kaempferol- 3- o- α- l- rhamnoside with Glycogen synthase kinase

Kaempferol- 3- o- α- l- rhamnoside exhibited notable docking interactions with Glycogen synthase kinase with a binding energy of -46.05 Kcal/mol. The interactions are favoured by the formation of 5 H- bonds; Arg 96, Arg 180, Arg 180, Lys 205, Val 214 and hydrophobic interaction with Asn 213 (Figure 9). Kaempferol showed 21 non bonded interactions with Glycogen synthase kinase. From Ramachandran plot and statistics it was estimated that Kaempferol- 3- 0- α- l- rhamnoside showed 83.9% of binding with the protein in the most favoured regions [A,B,L] (Figure 10 and 11).



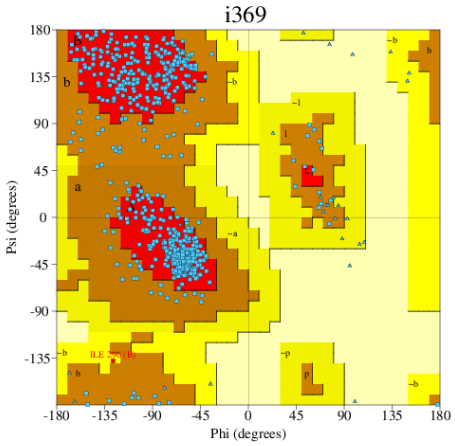
i369: Ligplot of interactions with PO4

(a). Hydrophobic and hydrophilic interactions of Kaempferol- 3- 0- α- l- rhamnoside with Glycogen synthase kinase. Brown colored half circle indicates the hydrophobic reactions of Kaempferol- 3- 0- α- l- rhamnoside with the target enzymes DPP. Green dotted lines indicate the hydrogen bond while green colored value indicates their bond length.



(b). Binding pose of Kaempferol- 3- 0- α- l- rhamnoside with Glycogen synthase kinase**.**

Figure: 10



(a). Ramachandran plot for Glycogen synthase kinase

		No. of residues	%-tage
Most favoured regions	[A,B,L]	491	83.9%*
Additional allowed regions	[a,b,l,p]	93	15.9%
Generously allowed regions	[~a,~b,~l,~p]	1	0.2%
Disallowed regions	[XX]	0	0.0%
Non-glycine and non-proline residues		585	100.0%
End-residues (excl. Gly and Pro)		15	
Glycine residues		34	
Proline residues		50	
Total number of residues		684	

(b) Ramachandran plot statistics

Figure: 11

DISCUSSION:

The complications of Diabetes Mellitus are mainly due to imbalance in blood glucose homeostasis. The consequences of long-term usage of hypoglycemic allopathic medicines are resistance to that medicine and also several side effects [9]. Currently herbal medicines and their isolates are in use in the treatment of various chronic diseases. Diabetes is not an exception as herbal extracts were used in the management of diabetes since ancient period [10,11]. In this scenario, the needs for the

additional finding of effective compounds from the medicinal plants are require retarding the rising complications of diabetes.

Numerous pharmacological studies have been reported with Kaempferol and its derivatives [12]**.** In the current research we have explored the effective docking of Kaempferol- 3- 0- α- l- rhamnoside with selective target proteins involved in blood glucose homeostasis. In general, Ligands with lesser binding energy, higher percentage of binding and good interaction with proteins through hydrogen bonds are considered as effectively docked with the respective protein. Kaempferol- 3- 0- α- l- rhamnoside showed good affinity with all the proteins. It showed highest binding affinity with much lesser binding energy with glycogen synthase kinase and glycogen phosphorylase respectively. It also showed good quality of binding with GK, α-amylase and aldose reductase with relatively less binding energy. According to Ramachandran statistics, greater than 90% of binding of the ligand with the protein in the most favoured regions [A,B,L] is considered as a good quality model. Kaempferol- 3- 0- α- l- rhamnoside showed good percentage (>90%) of binding with α-amylase, aldose reductase compared to GK, α-amylase and aldose reductase. The results clearly suggests that the binding of Kaempferol- 3- 0- α- l- rhamnoside with the target proteins effectively regulates their activity. This observation is in good agreement with the observation with our pervious study [13]. Regarding the antidiabetic activity of Kaempferol- 3- 0- α- l- rhamnoside in animal models.

CONCLUSION:

As conclusion, the results of the present study offer scientific evidence for the antidiabetic potential of Kaempferol- 3- 0- α- l- rhamnoside and anticipated to be useful in guiding the rational design of novel and robust drug for the treatment of diabetes.

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