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Beneficial protective effect of 2-allyl amino 4-methyl sulfanyl butyric acid on glucose metabolism, glycoprotein components and molecular modeling in streptozotocin induced diabetic rats

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In the present study the potential effect of 2-allyl amino 4-methyl sulfanyl butyric acid (AMSB) on glucose metabolism and glycoprotein components in streptozotocin (STZ) induced experimental diabetic rats was carried out. Further, molecular modeling was done to investigate the modes of AMSB interaction with insulin receptor active sites. STZ induced diabetic rats were measured for blood glucose and plasma insulin levels, whereas the glucose metabolism and glycoprotein components were analyzed in the plasma and tissues. After oral treatment of AMSB there was a significant reduction in blood glucose, glucose-6-phosphatase, fructose-1,6-bisphosphatase and glycogen phosphorylase. On the other hand the activity of glycoproteins levels such as hexose, hexosamine, fucose and sialic acid were significantly reduced. In addition, significant elevation in plasma insulin, hexokinase, glycogen and glycogen synthase were also observed in AMSB treated rats. Molecular modeling study revealed that AMSB has a stable binding pattern to the active site of insulin with a Gscore value of -7.34 Kcal/mol. From this study we conclude that the AMSB has potent antidiabetic activity in addition to its protective effect on glycoprotein metabolism.

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1. Introduction

Diabetes mellitus is a metabolic disorder, characterized by hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The burden of diabetes is increasing globally and estimates suggest that there will be 366 million cases by the year 2030, with increases particularly in developing countries.[1,2] In experimental diabetes, enzymes of carbohydrate metabolism are markedly altered and produce hyperglycemia that ends up pathologic process of diabetic complications.[3] Defects in glucose machinery and consistent efforts of the physiological systems to correct the imbalance in sugar metabolism create an over effort on the endocrine system resulting in the deterioration of endocrine control.[4,5] Continued deterioration of endocrine management exacerbates the metabolic disturbances by shifting glucose metabolic enzymes and leads primarily to hyperglycemia symptom.[6,7]

Glycoproteins are conjugated proteins that contain one or more covalently linked carbohydrate chains which contribute to the structure of extracellular matrix in animal cells.[8] Alterations in glycoprotein level leads to the pathogenesis of diabetes mellitus.[9] Various studies have suggested that alteration in glycoprotein components could be a consequence of impaired carbohydrate metabolism.[10,11]

The AMSB (Fig. 1) could be a well known compound of antidiabetic (α-amylase and α-glucosidase) properties. Recently, we have found that AMSB shows higher antidiabetic and antioxidant activity.[12] The present study was undertaken to scientifically investigate the impact of AMSB on carbohydrate and glycoprotein metabolism in STZ induced diabetic rats. The effectuality was compared with a typical antidiabetic drug (glibenclamide). Additionally,

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molecular docking was performed to achieve insight into the binding mode of AMSB with the active site of insulin.

2. Materials and methods

2.1. Chemicals

STZ and biochemicals were purchased from Sigma-Aldrich Chemical Corporation, USA. Commercial diagnostic kits were obtained from Qualigens Diagnostics (Mumbai, India) for determination of blood glucose and plasma insulin. All other chemicals used were of analytical grade.

2.2. Experimental animals

Healthy adult male wistar rats weighing 160-180 gm were chosen for the study. The experimental design and protocol were approved by the institutional animal ethics committee (Registration No. PU-TAEC/JULY 2011/06) of Periyar university, Salem. The rats were kept in customary polypropylene cage and maintained under standard laboratory conditions with relevancy temperature (22 ± 2 ºC), relative humidity (50 ± 15%), light–dark cycle (12 hrs), customary diet and water *ad libitum*.

2.3. Dose fixation study

The acute oral toxicity study was performed according to guidelines of the Organization for Economic Cooperation and Development (OECD), especially the acute toxicity fixed dose procedure 423 guidelines.[13] In all cases, 1000 mg/kg oral dose of the AMSB was found to be safe as no mortality and food consumption were observed during the study. Also, no gross pathological changes were seen. On the basis of these studies, the dose of 170 mg/kg was selected for AMSB.[14]

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2.4. Induction of experimental diabetes

Diabetes was induced in rats by intra peritoneal (i.p) injection of STZ at a dose of 55 mg/kg b.w, dissolved in 0.1 M citrate buffer (pH 4.5).[15] Diabetes was confirmed 72 hrs after induction by measurement of tail vein blood glucose levels using glucose meter. After 72 hrs the rats with marked hyperglycaemia blood glucose level above 250 mg/dl was considered diabetic and were used in the experiment.

2.5. Chronic experimental design

After the successful induction of experimental diabetes, the rats were divided into four groups comprising of six animals in each groups as given below.

Group I: Normal control rats.

Group II: Diabetic control rats (STZ in single dose of 55 mg/kg/b.w).

Group III: STZ treated rats was orally given AMSB (170 mg/kg b.w) and

Group IV: STZ treated rats orally given gilbenclamide (1 0 mg/kg b.w) for a period of 45 days.[16] Blood glucose level was measured periodically. At the end of the 45th day the experimental animals were deprived of food overnight, anaesthetized and sacrificed by cervical decapitation. Plasma and tissues were dissected out and rinsed with ice cold saline and stored at −80ºC for further studies. Samples were collected estimation of various biochemical analyses.

2.6. Measurement of carbohydrate metabolic enzymes

A plasma insulin assay was carried out using an enzyme linked immunosorbent assay kit. For enzyme assays, a portion of the liver tissue was dissected out, washed with ice-cold saline immediately and the liver tissue was homogenized in 0.1 M Tris-HCl (pH 7.4) and supernatant was quantified for hexokinase,[17] glucose-6-phosphatase,[18] fructose-1,6-bisphosphatase[19] and

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hepatic glycogen content by that of Morales et al., (1973)[20] whereas glycogen synthase[21] and phosphorylase were also assayed according to the standard method.[22]

2.7. Determination of glycoprotein levels

Hexose content (plasma and tissues) was estimated by the method of Niebes, (1972),[23] sialic acid in plasma and tissues were estimated by the method of Warren, (1959)[24] and hexosamine by the method of Wagner (1979).[25] Fucose was estimated by the method of Dische and Shettles (1948),[26] respectively.

2.8. Protein structure and binding site prediction

The 3-Dimensional structure of insulin (PDB ID: 2DTG) and commercially available synthetic and phytochemical drugs/compounds were retrieved from protein data bank (www.rcsb.org/pdb) and pubchem (http://www.ncbi.nlm.nih.gov/pccompound) data base respectively.

The active site of the insulin (PDB ID: 2DTG) was identified by the online server Q-site finder (http://www.modelling.leeds.ac.uk/qsitefinder).

2.9. Molecular docking

Docking of insulin protein with retrieved AMSB, synthetic and phytochemical drugs/ compounds were performed using Schrodinger glide module (http://schrodinger.com/). Protein structure refinement, optimization, energy minimization and partial atomic charges were done using protein preparation wizard. The AMSB, synthetic and phytochemical drugs/compounds were prepared using LigPrep, besides the drugs and compounds tautomers were generated and optimized. Grid box were generated for all the residues. All the prepared compounds and drugs were then subjected to docking studies against the molecular targets insulin using Glide extra-precision method. Glidexp mode determines all reasonable conformations for each low-energy conformer in the designated binding site. In the process torsional degrees of each ligand are

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relaxed, though the protein conformation is fixed. The glide scoring function (Gscore) was used to select the best conformation for each ligand.

2.10. Statistical analysis

The results were expressed as mean ± SD for 6 animals in each group. Values were statistically analyzed using one way ANOVA followed by Student's Duncan's test by using statistical package of social science (SPSS) 16.0 for windows. The results were considered statistically significant was set at $P<0.05$.[27]

3. Results

Table 1 shows the effect of blood glucose in experimental group of rats on day 0, 3, 20, 35 and 45. Diabetic rats showed a significantly elevated blood glucose level compared with normal rats. After oral administration of AMSB and glibenclamide respectively tended to bring blood glucose towards near normal levels which comparable with diabetic rats.

We can observe in Table 2 that the activities of plasma insulin and hepatic hexokinase levels were found to be significantly reduced where as glucose-6-phosphatase and fructose-1,6-bisphoshatase enzyme levels were increased in STZ induced diabetic rats than those of non diabetic rats (group I). After oral treatment of AMSB and glibenclamide to the diabetic rats for 45 days the activities tended to bring the values back to normal.

Table 3 exemplifies the effect of AMSB on glycogen, glycogen synthase and glycogen phosphorylase in experimental group of rats. Significant reduction was observed in hepatic glycogen and glycogen synthase with increase in the activity of glycogen phosphorylase in STZ induced rats when compared to control rats. By administration of AMSB and glibenclamide the hepatic enzyme levels were normalized compared with diabetic control rats.

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The effect of AMSB on plasma glycoprotein levels of STZ induced diabetic rats are presented in Table 4. The STZ induced diabetic rats showed significant elevation of glycoprotein such as hexose, hexosamine, fucose and sialic acid compared to non diabetic rats. In diabetic rats administration of AMSB and gilbenclamide showed significant reduction of plasma glycoprotein which was comparable with STZ induced diabetic control rats.

The effect of treatments for 45 days on glycoprotein components were demonstrated in the hepatic and renal tissues of the diabetic rats are shown in Table 5 and 6. In the diabetic condition the glycoprotein levels were increased compared to control rats. After oral treatment of AMSB and gilbenclamide the hexose, hexosamine, fucose and sialic acid levels were near to normal compared to diabetic control rats.

Three dimensional structure of insulin (PDB ID: 2DTG) was retrieved from protein data bank (Fig 2). The AMSB, synthetic and phytochemical drugs/compounds bound to the structure were removed further studies. The active site of the insulin protein chain E was found using Q site finder. The active site residues were PHE 503, MET 504, LEU 505, PHE 506, TYR 507, LYS 508, GLU 509, ALA 510, PRO 511, TYR 512, GLN 513, THR 516, GLU 517, PHE 518, ASP 519, GLY 520, VAL 532, ASP 533, ASP 535, ALA 563, ILE 564, PHE 565 and ILE 585 respectively. The active site volume of insulin receptor was found to be 465 $Å^3$ of the total 74739 $Å^3$ volume of the protein.

Insulin is a pentameric complex, among that chain E act as the receptor. Based on this chain E is selected for docking studies. AMSB, synthetic and phytochemical drugs/compounds docked with insulin protein chain E. Newly synthetic compound of AMSB has better interaction with the insulin receptor than the existing drugs and phytochemical compounds shown in Table 7. The glide score of the AMSB was lowest docking energy -7.34 Kcal/mol and form of five hydrogen

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bonds with the ASN 349, ASP 519, GLY 520, GLN 521 and GLU 353 residues of insulin receptor (Fig 3). The glide score of the synthetic drugs lies between 0.97 Kcal/mol to -5.84 Kcal/mol and phytochemical compounds was -1.31 Kcal/mol to - 4.18 Kcal/mol with receptor (Table 7).

4. Discussion

STZ administered hyperglycemia in rats is considered to be a good model for the preliminary screening of agents active against diabetes complications and is widely used.[28] In these model diabetic rats pancreatic β-islet cells are selectively destroyed and the resultant chronic hyperglycemia, reduction of insulin, abnormal changes, oxidative stress and inflammation reaction are collectively called as glucose toxicity. The mechanism forms development of diabetes complications.[29-32] Based on this report our results clearly indicated that the AMSB administered significantly reduced blood glucose and caused elevation of plasma insulin levels in diabetic animals.

The gluconeogenic enzymes like hexokinase, glucose-6-phosphatase and fructose 1,6-bisphosphatase play a very important role within the maintenance of glucose homeostasis.[33,34] The hexokinase considerably reduced besides glucose-6-phosphate and fructose 1,6-bisphosphatase were increased within the liver throughout diabetic state linked to hyperglycemia.[35,36] Previous reported literature indicates that the gluconeogenic enzymes were shown in near to normal level after treatment with diabetic rats.[37,38] Based on this report the treatment of AMSB significantly recovered gluconeogenic enzymes level in STZ induced diabetic rats. The extent of plasma insulin was found to enhance significantly in diabetic rats treated with AMSB, which may be potential reason for the significant recovered in the level of gluconeogenic enzymes.[39]

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The liver hepatic glycogen content was markedly reduced in diabetic animals, which is in proportion to insulin deficiency and this type of results most likely owing to the inactivation of glycogen synthetase system.[40,41] Treatment of diabetic rats with AMSB significantly restored the level of hepatic glycogen which may be due to the increased secretion of insulin from residual exocrine gland β-cells attributed to stimulation of glycogen synthase and inhibition of glycogen phosphorylase.

Generalized abnormalities in glycoprotein metabolism are observed in both naturally occurring and experimental diabetes.[41] Berenson and Radhakrishnamurthy Dalferes (1972)[43] reported that increased levels of hexose, hexosamine, fucose and sialic acid in the plasma and tissues of STZ induced diabetic rats. The secretion or shedding from cell membrane glycol conjugates into the circulation leads to the elevation of plasma glycoprotein components. STZ induced diabetic rats exhibited a significant modification in the connective tissue macromolecule.[43] Spiro and Spiro (1971)[44] reported that this may be due to the depressed utilization of glucose by insulin dependent pathways leading to the increased formation of hexose, hexosamine, sialic acid and fucose for the accumulation of glycoproteins. In our report administration of AMSB to STZ induced diabetic rats extensively reduced glycoproteins levels in plasma and tissues, which could be due to improved glycemic control.

Molecular modeling is one in all the leading powerful techniques to get novel ligands for receptors of apprehend structure and so play a key role in structure based drug design triggers.[45] The glide score obtained in the present docking study confirm that there is a stronger receptor ligand binding affinity between AMSB and insulin receptor. This might lead to the activation of tyrosine kinase and thereby led to autophosphorylation and phosphorylation of intracellular

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substrates that are essential for initiating other cellular responses to normalize the glucose level.[46,47]

5. Conclusion

From this study, we can conclude that AMSB has potential antidiabetic action. The present result shows significant effect of AMSB on carbohydrate and glycoprotein metabolism in addition to its antidiabetic properties. Molecular modeling of AMSB shows better interaction than existing drugs and phytochemical compounds. Hence, this study provides that AMSB may stimulate extant beta cells. These results support that the AMSB may be used as an antidiabetic drug.

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Legends of Figures

Fig. 1 Chemical structure of AMSB.

Fig. 2 Structure of Insulin Protein (PDB ID: 2DTG)

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Fig. 3 AMSB forms five hydrogen bonds with ASP 519, GLY 520, ASN 349, GLN 521, GLU 353 with insulin receptor (2DTG: Chain E) and the glide score was -7.34 Kcal/mol.

Legends of Tables

Table 1 Changes in the level of blood glucose experimental groups of rats.

Table 2 Effect of AMSB on plasma insulin, hexokinase, giucose-6-phosphatase and fructose 1,6 bisphosphatase in experimental animals.

Table 3 Effect of AMSB on glycogen, glycogen synthase and glycogen phosphorylase in experimental diabetic rats (mean± S.D, n = 6).

Table 4 Changes in the levels of plasma glycoproteins in control and experimental animals.

Table 5 Changes in the levels of liver glycoproteins in control and experimental animals.

Table 6 Changes in the levels of kidney glycoproteins in control and experimental animals.

Table 7 Docking of AMSB and synthetic/phytochemical compounds against insulin receptor (PDB ID: 2DTG)

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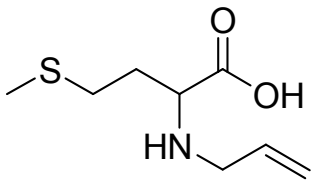

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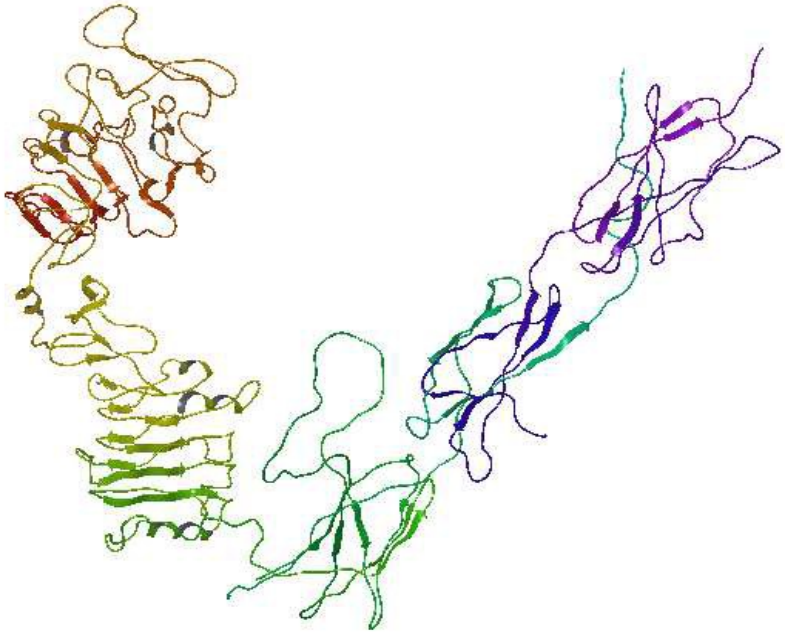

Fig. 2 Structure of Insulin Protein (PDB ID: 2DTG)

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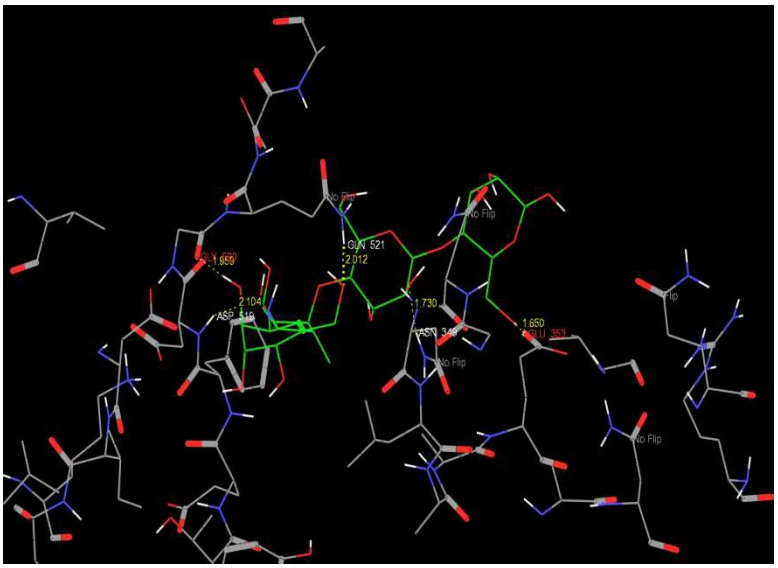

Fig. 3 AMSB forms five hydrogen bonds with ASP 519, GLY 520, ASN 349, GLN 521, GLU 353 with insulin receptor (2DTG: Chain E) and the glide score was -7.34 Kcal/mol.

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Table 1 Changes in the level of blood glucose experimental groups of rats.

Experimental Groups	Blood glucose (mg/dl)				
	0	3rd day	20th day	35th day	45th day
Group I: Control	92.38 ± 3.12	93.02 ± 2.43	96.07 ± 1.62	95.08 ± 3.681	95.103 ± 4.018
Group II: Diabetic control (STZ: 55 mg/kg)	91.05 ± 4.42[a*]	276.57 ± 1.30[a*]	283.67 ± 1.21[a*]	286.14 ± 0.56[a*]	288.17 ± 1.04[a*]
Group III: Diabetic + AMSB (170 mg/kg)	92.73 ± 1.74[b*]	283.03 ± 3.72[b*]	210.02 ± 0.04[b*]	133.72 ± 1.03[b*]	106.06 ± 3.02[b*]
Group IV: Diabetic + Glibenclamide (10 mg/kg)	91.83 ± 1.06[b*]	281.19 ± 3.08[b*]	201.52 ± 4.803[b*]	124.09 ± 0.61[b*]	101.15 ± 1.01[b*]

Each value represents mean ± S.D., n = 6.

[*a] *P<0.05* Diabetic control (Group II) compared with control (Group I)

[*b] *P<0.05* AMSB and glibenclamide compared with diabetic control (Group II) group.

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Table 2 Effect of AMSB on plasma insulin, hexokinase, giucose-6-phosphatase and fructose 1,6 bisphosphatase in experimental animals.

Experimental Groups	Plasma insulin (μU/ml)	Hexokinase Unit/mg protein/min	Glucose-6-phosphatase Unit/mg protein/min	Fructose-1,6-bisphosphatase Unit/mg protein/min
Group I: Control	15.76±0.83	263.71 ± 11.01	961.31 ± 31.01	460.25 ± 53.61
Group II: Diabetic control (STZ: 55 mg/kg)	3.95±0.11 [a*]	130.95 ± 7.64[a*]	1609.08 ± 117.63[a*]	768.26 ± 80.51[a*]
Group III: Diabetic + AMSB (150 mg/kg)	11.28±0.38 [b*]	257.08 ± 10.53[b*]	1008.95 ± 34.12[b*]	593.59 ± 57.32[b*]
Group IV: Diabetic + Glibenclamide (2.5 mg/kg)	13.60±0.52 [b*]	261.32 ± 10.94[b*]	992.53 ± 31.69[b*]	482.37 ± 57.83[b*]

Values are mean ± SD, for 6 rats in each group

[a] Significantly different from control ($^{*}P<0.05$).

[b] Significantly different from diabetic control ($^{*}P<0.05$).

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Table 3 Effect of AMSB on glycogen, glycogen synthase and glycogen phosphorylase in experimental diabetic rats (mean± S.D, n = 6).

Experimental Groups	Glycogen (mg/g tissue)	Glycogen synthase Unit/mg protein/min	Glycogen phosphorylase Unit/mg protein/min
Group I: Control	63.61 ± 2.13	859.09 ± 4.14	601.25 ± 1.57
Group II: Diabetic control (STZ: 55 mg/kg)	20.48 ± 0.46[a*]	457.92 ± 2.37[a*]	849.07 ± 2.60[a*]
Group III: Diabetic + AMSB (150 mg/kg)	51.73 ± 1.80[b*]	826.48 ± 1.40[b*]	706.34 ± 2.031[b*]
Group IV: Diabetic + Glibenclamide (2.5 mg/kg)	54.22 ± 0.42[b*]	839.71 ± 1.01[b*]	695.69 ± 1.84[b*]

[*a] *P<0.05* Diabetic control (Group II) compared with control (Group I)

[*b] *P<0.05* AMSB (Group III) and Glibenclamide (Group IV) compared with diabetic control (Group II) group.

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Table 4 Changes in the levels of plasma glycoproteins in control and experimental animals.

Experimental Groups	Hexoses mg/dl	Hexosamine mg/dl	Fucose mg/dl	Sialic acid mg/dl
Group I: Control	85.61±4.78	67.39±2.89	32.69±4.64	56.83±3.20
Group II: Diabetic control (STZ:55mg/kg)	137.72±7.94[a*]	88.61±5.71[a*]	49.78±7.03[a*]	80.12±6.73[a*]
Group III: Diabetic + AMSB(150mg/kg)	90.43±5.26[b*]	70.68±4.32[b*]	38.40±5.62[b*]	59.74±3.99[b*]
Group IV: Diabetic + Glibenclamide (2.5mg/kg)	86.49±4.97[b*]	69.03±3.16[b*]	35.13±5.001[b*]	57.36±3.17[b*]

Values are given as mean ± SD from six rats in each group

[*] $P<0.05$: [a]Significantly different from group I (Control)

[*] $P<0.05$: [b]Significantly different from group II (Diabetic control)

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Table 5 Changes in the levels of liver glycoproteins in control and experimental animals.

Experimental Groups	Hexoses mg/g	Hexosamine mg/g	Fucose mg/g	Sialic acid mg/g
Group I: Control	30.81±4.23	17.37±0.012	13±1.01	7.29±0.23
Group II: Diabetic control (STZ:55mg/kg)	59.68±6.12 [a*]	32.54±2.111 [a*]	25.87±0.30 [a*]	16.17±1.001 [a*]
Group III: Diabetic + AMSB(150mg/kg)	38.65±5.66 [b*]	19.21±0.30 [b*]	16.32±0.23 [b*]	8.46±0.84 [b*]
Group IV: Diabetic + Glibenclamide (2.5mg/kg)	33.41±4.27 [b*]	19.61±0.82 [b*]	14.29±1.86 [b*]	8.63±0.90 [b*]

Values are given as mean ± SD from six rats in each group

[*]$P<0.05$: [a]Significantly different from group I (Control)

[*]$P<0.05$: [b]Significantly different from group II (Diabetic control)

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Table 6 Changes in the levels of kidney glycoproteins in control and experimental animals.

Experimental Groups	Hexoses mg/g	Hexosamine mg/g	Fucose mg/g	Sialic acid mg/g
Group I: Control	25.19±2.45	12.78±1.01	11.0±0.92	7.94±1.38
Group II: Diabetic control (STZ:55mg/kg)	50.47±5.12 [a*]	29.03±3.45 [a*]	27.39±2.34 [a*]	12.38±2.74 [a*]
Group III: Diabetic + AMSB(150mg/kg)	29.69±3.11 [b*]	19.56±0.03 [b*]	13.65±1.12 [b*]	9.75±1.89 [b*]
Group IV: Diabetic + Glibenclamide (2.5mg/kg)	25.66±2.75 [b*]	15.32±1.21 [b*]	12.71±0.47 [b*]	7.62±1.46 [b*]

Values are given as mean ± SD from six rats in each group

[*]$P<0.05$: [a]Significantly different from group I (Control)

[*]$P<0.05$: [b]Significantly different from group II (Diabetic control).

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Table 7 Docking of AMSB and synthetic/phytochemical compounds against insulin receptor (PDB ID: 2DTG)

S.No	Compound name	Gscore Kcal/mol	Hydrogen bonds Distance (Å)	Interaction (D-H….A)
1	AMSB	-7.34	2.104	ASP519 (H….O)
			1.959	GLY520 (O….H)
			1.730	ASN349 (H….O)
			2.012	GLN521 (H….O)
			1.650	GLU353 (O….H)
2	Rosiglitazone	-5.84	1.880	GLN521 (H….O)
			1.997	THR530 (O….H)
			1.870	ASP522 (H….O)
3	Metformin	-5.21	2.041	GLN521 (H….O)
			2.580	ASP522 (H….O)
			2.060	ASP522 (O….H)
			1.922	ASP522 (O….H)
			2.060	ASP522 (O….H)
4	Gallic acid	-4.18	2.002	THR520 (O….H)
			1.995	THR520 (O….H)
			2.444	GLN521 (H….O)
			1.954	ASP522 (O….H)
5	Curcumin	-3.02	2.120	GLN521 (O….H)
			1.960	ASP522 (O….H)
			2.324	ALA523 (O….H)
			2.557	ASP522 (O….H)
6	Alliin	-2.73	2.234	LYS508 (H….O)
			2.183	GLU517 (O….H)
7	Glipizide	-2.59	2.003	SER526 (H….O)
			1.803	ASP522 (O….H)
			2.611	ASP522 (O….H)
			2.058	ASP519 (O….H)
8	Glyburide	-2.53	2.315	SER528 (H….O)
			2.036	SER526 (H….O)

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			2.005	ASP522 (O….H)
			2.194	ASP522 (O….H)
			2.170	ASP519 (H….O)
9	Furanthiazolidiones	-2.46	2.138	ASP519 (O….H)
			1.915	ASP522 (O….H)
			2.596	ASP522 (O….H)
10	N-acetyl-D-Glucosamine	-2.34	2.104	GLN521 (H….O)
			2.574	LYS508 (H….O)
11	Glimepiride	-2.01	2.340	ASP522 (O….H)
			1.901	ASP522 (O….H)
			1.783	ASP522 (O….H)
12	Gallocatechin	-1.31		
			2.306	GLN521 (O….H)
13	Genistein	-1.21	1.922	THR580 (O….H)
			2.383	GLY525 (O….H)
14	Miglitol	0.97	1.870	GLY525 (O….H)

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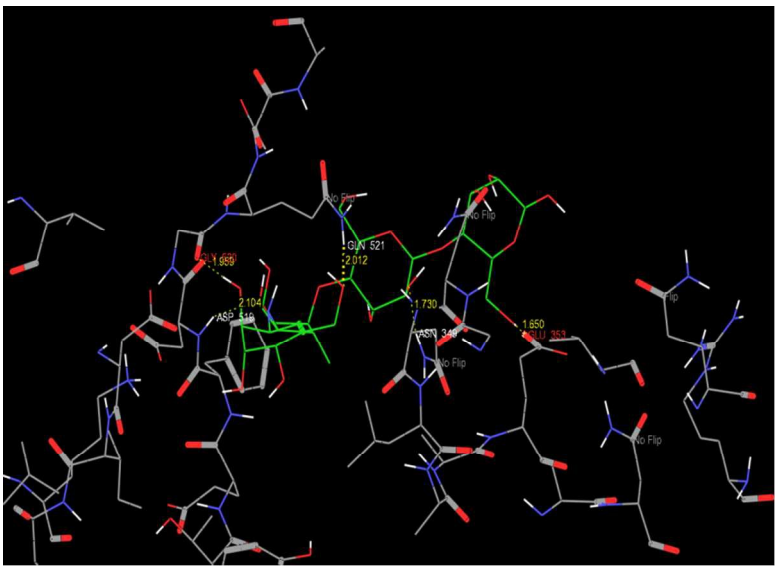

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