Saudi Journal of Biological Sciences 27 (2020) 3334–3341

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Down-regulation of hepatic G-6-Pase expression in hyperglycemic rats: Intervention with biogenic gold nanoconjugate

Sengani Manimegalai [a], Shahid Mahboob [b], Khalid A. Al-Ghanim [b], Fahad Al-Misned [b], Marimuthu Govindarajan [c,d], Krishnan Anbarasu [e], Vijayarangan Devi Rajeswari [a,*]

[a] Department of Bio-Medical Sciences, School of BioSciences and Technology, Vellore Institute of Technology (VIT), Vellore 632 014, Tamil Nadu, India
[b] Department of Zoology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia
[c] Unit of Vector Control, Phytochemistry and Nanotechnology, Department of Zoology, Annamalai University, Annamalainagar 608 002, Tamil Nadu, India
[d] Department of Zoology, Government College for Women (Autonomous), Kumbakonam 612 001, Tamil Nadu, India
[e] Department of Bioinformatics, School of Life Sciences, Vels Institute of Science, Technology & Advanced Studies, Pallavaram, Chennai 600 117, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 22 July 2020
Revised 30 August 2020
Accepted 13 September 2020
Available online 22 September 2020

Keywords:
Diabetes
Glucose 6 phosphatase
Couroupita guianensis
Gold nanoparticles
Quantitative RT-PCR

ABSTRACT

Chronic diabetes extensively complicates the glucose metabolism to onset and progress the complication. Concurrently, several contemporary medicines, especially organo-metallic formulations, are emerging to treat hyperglycemia. The current study aims to emphasize the gold nanoparticles (GNPs) potential for glucose metabolism regulation in Streptozotocin (STZ) induced diabetes. Quantitative real-time polymerase chain reaction (RT-PCR) was carried out to detect the mRNA expression of Glucose transporters 2 (GLUT2), Glucokinase (GK) and Glucose 6 Phosphatase (G-6-Pase). The study shows remarkable results such as the prognostic effect of GNPs in reinforcing the repression of enzyme complex G-6-Pase about 13.3-fold when compared to diabetes control. Also, molecular docking studies showed significant inhibition of G-6-Pase by the terpenoid ligands with alpha and beta amyrin from leaf extract of *Couroupita guianensis*. Thus the study explored the novel mechanism of G-6-Pase downregulated by GNPs intervention that majorly contributes to the regulation of circulatory glucose homeostasis during diabetes.

1. Introduction

Diabetes mellitus is one of the deadliest disorder that challenges the healthcare system by requiring lifetime treatment (Clarke et al., 2004). High circulatory blood glucose levels represent it, eventually resulting in microvascular abnormalities observed in the retina, glomerulus, and lower extremities of the brain nervous (American Diabetes Association, 2017). Usually, microvascular abnormalities directly accelerate atherosclerosis and consequently increase the risk of myocardial infection (Peluso and Serafini, 2017). Substantially, there is an essential need for new pharmaceutical interventions to discover a cure for hyperglycemia and associated complications. In general, organic anti-oxidants are capable of controlling oxidative stress, which constrains the complication progression in all types of diabetes (Sies, 1997). Many studies reported that plants and their secondary metabolites are potential biological anti-oxidants and also possess anti-diabetic activity (Martel et al., 2016). Specifically, anthraquinones and its derivatives are proved as potent inhibitors of the critical hydrolyzing enzyme, Glucose 6 phosphatase (G-6-P ase) (Westergaard and Madsen, 2001). The herbal plant *Couroupita guianensis* is majorly encompassed with triterpenes and anthraquinone isatin, which acts as a specific, reversible and competitive G-6-P translocase inhibitor (Shekhawat and Manokari, 2016). The leaf extract suppresses the G-6-Pase expression and declines the hepatic glucose production during gluconeogenesis.

Similarly, 7-hydroxy-5-methoxy-6,8-dimethyl flavanone from *C. guianensis* protects pancreatic β cells from hyperglycemia-induced glucotoxicity (Hu et al., 2012). Altogether, polyphenols effectively enhance glucose uptake and reduce hepatic glucose output by increasing insulin activity (Kumar et al., 2011; Martínez et al., 2012). At the same time, these organic sources are found to be lacking in parameters like dissolution, bioavailability, and sufficient dosage (Yang et al., 2008).

* Corresponding author.
E-mail address: vdevirajeswari@vit.ac.in (V. Devi Rajeswari).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2020.09.026
1319-562X/

To overcome this limitation, Metals combined with polyphenols confirmed the greater control of glucose homeostasis and used to treat various diseases and disorders in the ancient science of Ayurveda (Bailey and Day, 1989). The mechanism of converting metals into non-toxic, stable colloids through herbals restrain dosage and increase availability to the human system (Karthick et al., 2014). Polyphenol coated colloids actively reduce the reactive oxygen species (ROS), resulting in the reduction of oxidative stress (BarathManiKanth et al., 2010). Thus, the integrated paradigm provides a new conceptual framework for the drug delivery aspect.

The mechanism of glucose influx from the bloodstream to the liver acts as a reinforcing step in carbohydrate metabolism and glycogen storage. The enhancement of glucose transportation step by GLUT2 through converting glucose into Glucose 6 phosphate (G-6-P) by GKase and attenuated opposite reaction by reducing G-6-Pase will facilitate hepatic glycogen storage (Ashmore and Weber, 1959). Hence, the current research aims to analyze the possible mechanism of *C. guianensis* synthesized gold nanoparticles (GNPs) targeted G-6-Pase inhibition that will positively control the circulatory blood glucose level.

2. Material and methods

2.1. Synthesis and characterization of GNPs

The shade dried *Couroupita guianensis* leaves and gold (III) chloride hydrate $(HAuCl)_4$ (0.001 M) were used for the preparation of GNPs. The detailed synthesis and characterization were described in the previous study (Sengani, 2017).

2.2. Ethical clearance and animal management

All *in-vivo* experiments were carried out in compliance with the animal ethics committee guidelines (VIT/IAEC/12/July 23/27). Thirty-five albino male Wistar rats were weighed (150–200 g) and housed together for a week for acclimatization. All the experimental rats were allowed free access to water and pelleted feed. The temperature (23–25 °C) and the relative humidity level (41–60%) were maintained in the animal house throughout the study.

Table 1
The list of oligonucleotides (primers) used in this study.

S. No.	Gene name	F′ primer and R′ primer
1	Glucose transporter-2 (243 bp)	5′-TTA GCA ACT GGG TCT GCA AT-3′ 5′-TCT CTG AAG ACG CCA GGA AT-3′
2	Glucokinase (162 bp)	5′-CAC CCA ACT GCG AAA TCA CC-3′ 5′-CAT TTG TGG GGT GTG GAG TC-3′
3	GAPDH (215 bp)	5′-CCC GTA GAC AAA ATG GTG AAG GTC-3′ 5′-GCC AAA GTT GTC ATG GAT GAC C-3′
4	G-6-Pase	5′-AAA GAG ACT GTC GGC ATC AAT C-3′ 5′-AAG AGG CTG GCA AAG GGT GTA G-3′

2.3. Experimental design

Experimental rats were randomly classified into seven groups. In each group, six animals were placed (n = 6).

Group A: conventional rats without any treatment (normal control, NC),
Group B: normal control Wistar rats fed with aqueous leaf extract of *C.guianensis* (100 mg (kg/bw) dissolved in 12 ml of double-distilled water through oral administration for 15 days (CP),
Group C: normal control Wistar rats treated with biosynthesized GNPs suspended in deionized water at a dosage of 2.5 mg (kg /b.wt) per day using SONICS VCX 500 model sonicator by oral administration for 15 days (CN),
Group D, E, F and G experimental rats were injected with Streptozotocin (40 mg/kg of b.wt) through a single dose of intraperitoneal injection to induce diabetes.
Group D: diabetic control (DC); throughout the study, these animals remained without any treatment.
Group E: diabetic Wistar rats were orally administered with aqueous leaf extract (100 mg (kg/bw) dissolved in 12 ml of double-distilled water through oral administration for 15 days (DP),
Group F: diabetic animals orally administered with GNPs as per the dosage mentioned in group 3 (DN) and
Group G: diabetic animals orally administered with glibenclamide (600 μg/kg b.w.) (DG).

2.4. Examination of glucokinase, glucose 6 phosphatase activity, and hepatic glycogen storage

During the experimental period, plasma markers were evaluated from the experimental rats. At the end of the study, the rats were sacrificed and autopsied; liver tissue was dissected and immediately stored at −80 °C. Hepatic GKase activity was measured through the standard method (Davidson and Arion, 1987). In the liver homogenate, the reduction of NADP + form glucose-6-phosphate in the presence of glucose-6-phosphate dehydrogenase was measured. The released Glucose-6-phosphatase was predicted as specified by the standard method by estimating the amount of inorganic phosphate. Glucose-6-phosphate produced by the GKase accelerates the phosphorylation could potentially elevate the glycogen content of the liver and muscle (Glock and McLean, 1953). The hepatic glycogen content was estimated from the liver homogenize with little modification (Van der Vies, 1954).

2.5. RNA extraction and cDNA synthesis

The preserved hepatic tissue homogenized with liquid nitrogen to isolate total RNA using Hipura. Total RNA Miniprep Purification Kit (Himedia). Quality and quantity were checked through the nanodrop instrument and 1% agarose gel electrophoresis. The total RNA was reverse transcribed into first-strand cDNA by Maxima H

Table 2
Effect of GNPs on glycogen storage, GKase and G-6-Pase enzyme activity.

Parameters	NC	CP	CN	DC	DP	DN	DG
Glycogen (μg/glucose/mg tissue)	32.04 ± 0.13	32.17 ± 0.25	32.31 ± 0.21	20.63 ± 0.23	22.11 ± 0.61*	28.88 ± 0.29***	30.61 ± 0.3
Glucokinase (U/h/mg of protein)	0.257 ± 0.001	0.258 ± 0.001	0.256 ± 0.002	0.075 ± 0.0007	0.087 ± 0.002***	0.110 ± 0.0006***	0.134 ± 0.006
G6pase (U/min/mg of protein)	0.160 ± 0.002	0.162 ± 0.001	0.160 ±0.002	0.449 ± 0.004	0.383 ± 0.003***	0.305 ± 0.02***	0.300 ± 0.004

Values are mean ± SD of 6 rats from each group, NC: Normal Control; CP: Control animal treated with plant extract; CN: Control animal treated with nanoparticles; DC: Diabetic control without treatment; DP: Diabetic animal treated with plant extract; DN: Diabetic animal treated with nanoparticles; DG: Diabetic animal treated with glibenclamide. *Values are statistically significant at $p < 0.05$ as compared with the diabetic control (DC), **Values are statistically significant at $p < 0.01$ as compared with the diabetic control (DC), ***Values are statistically significant at $p < 0.001$ as compared with the diabetic control (DC).

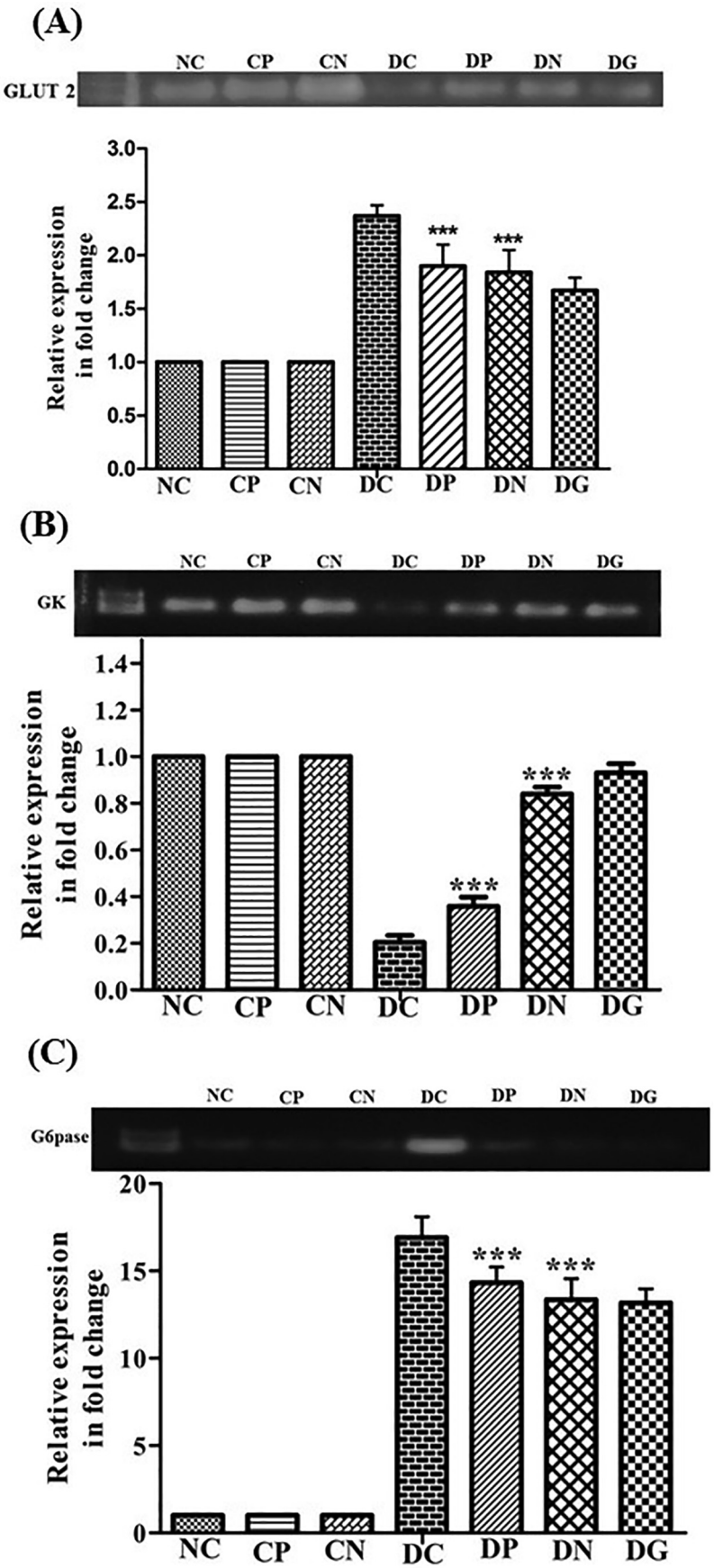

Fig. 1. Qualitative and quantitative gene expression. (A) Relative gene expression of GLUT 2, (B) Relative gene expression of Glucokinase (GKase), and (C) Relative gene expression of Glucose 6 phosphatase (G-6-Pase).

minus cDNA synthesis kit (Thermo Scientific) utilizing oligo $(dT)_{18}$ primers. Each reaction consisted of total RNA 1 μg, 1 μl (100 pmol) primer oligo $(dT)_{18}$, 1 μl of dNTP mix (0.5 mM) and nuclease-free water 15 μl was added, centrifuged briefly and incubated at 65 °C for 5 min. Further, components added with RT buffer 4 μl and maxima H minus enzyme 1 μl. The transcription was carried out for 30 min at 50 °C. Further, the reaction was restricted at 85 °C for 5 mins.

2.6. Quantitative PCR with SYBR green method

Using this first-strand cDNA as template PCR amplification was performed using Ampli Taq Gold 360 master mix (Thermo Fisher) with primers (Sigma-Aldrich, India) were listed (Table 1). Quality and quantity of all cDNAs were checked with rat GAPDH mRNA primer as a positive control and a negative control reaction was set without enzyme reverse transcriptase to ensure the absence of whole genomic DNA. The amplified PCR products were visualized with 1% agarose gel electrophoresis. GAPDH primer and fast SYBER® green master mix (Thermo Fisher Scientific) was used to perform real-time polymerase chain reaction. Individual reactions were prepared using each target primers to construct a melting curve to get gene-specific peaks. The baseline adjustment was made with Step one 2.3 version software to derive Ct value. Expression levels of GLUT2, GKase and G-6-Pase genes using GAPDH as endogenous control were evaluated by underlined methodology.

2.7. Statistical analysis

All the data were expressed as mean ± S.E (n = 6). The statistical analysis includes Student Newman-Keul's test and one-way ANOVA was used to compare test and control groups were performed through InStat Graph pad prism version 3.

2.8. Molecular modeling and validation

The structural analysis of human G-6-Pase was performed by molecular modeling due to limitations in performing experimental 3D structure. The protein sequence of human G-6-Pase with 357 aa and downloaded from the Uniprot/Swiss-Prot (Accession no: P35575) for initial analysis. 3D structure prediction of the G-6-Pase was performed by best modeling server I-TASSER based on the threading/fold recognition method (Zhang, 2008). The accurate model of G-6-Pase was identified based on the evaluation parameter C-score derived from the concept of relative clustering, consensus significance and structural density of protein templates. Additionally, structural parameters like TM score and RMSD with template majorly used for evaluating the accuracy of the predicted best model. 3D structural model of G-6-Pase was validated by the construction of the Ramachandran plot using phi/psi angles.

2.9. Molecular docking

C. guianensis leaves majorly composed mixture of pentacyclic triterpenes and anthraquinones. Ligands are chosen based upon the literature. The mechanism of G-6-Pase inhibition by a specific ligand dataset was studied by molecular docking approach using AutoDock Vina (Trott and Olson, 2010). Blind docking approach was performed with query ligands and 3D structural models of G-6-Pase. After docking runs, the best conformation which possessed the least binding affinity (kcal/mol) with G-6-Pase was selected for further analysis. The docked complex was visualized by LigPlot^{+} to predict hydrogen bond interactions and hydrophobic interactions (Laskowski and Swindells, 2011).

3. Results and discussion

3.1. Effect of GNPs on glycogen storage GKase, G-6-Pase enzyme activity

The described methodologies were evaluated for hepatic enzyme activity and glycogen storage in STZ induced diabetic rats. Our experimental results in table 2 showed the activity of GKase was increased in DN group ($p < 0.001$) compared with DC group. Whereas glucose-6 phosphatase increased in diabetic control (DC) rats compared to the normal untreated (NC) and normal leaf extract and GNPs supplemented groups (CP and CN). Nevertheless, diabetic animals supplemented with GNPs showed decreased glucose-6-phosphatase level and thereby decreased gluconeogenesis in diabetic rats. Our experimental evidence showed supplement with GNPs increased glycogen storage in diabetic animals (DN). In general, enzyme activity in hepatic tissues showed increased GKase accompanied by reduced G-6-Pase. In the experiment, glibenclamide was used as a positive control drug (DG). UK Prospective Diabetes Study (UKPDS) Group suggested glibenclamide clinically used to treat high blood glucose by promoting intracellular calcium, thereby induce insulin secretion (1998). However, its sulfonylurea derivative associated with severe hypoglycemia. Fortunately, studies reported that alpha amyrin and beta amyrin from the Phyto origin constrained the effect with hypoglycemia (Nair et al., 2014). Similarly, triterpenoids enhance glucose uptake and insulin sensitivity (Tuan et al., 2009). Further, Mosa et al. (2015) observed in their study that improved GKase and glucose tolerance when diabetic rats treated with triterpene. Based on this strategy, GNPs reduced by triterpene rich *C. guianensis* used to treat diabetic animals (DP and DN), thus increasing the release of the insulin.

3.2. Effect of GNPs on GLUT 2, GKase and G-6-Pase expression

Long term clinical manifestation of high circulatory blood glucose increases complications of diabetes. The primary aim was to control circulatory blood glucose, and it was achieved by GLUT 2,

Fig. 2. Three-dimensional model structure of human G-6-Pase from I-Tasser server.

which transport circulatory glucose into liver cells. Thus chronic therapy accomplished by secondary metabolites from natural sources had fewer side effects and promote glycogen found to be 2.37 fold down-regulated as shown in Fig. 1A. However, the treatment with Leaf extract and GNPs promote the GLUT-2 expression so that the relative expression downregulated only up to 1.9 and 1.8 fold.

Initially, the sustained release of phytocompounds from the GNPs initiates glucose transportation. Later, the transported glucose converted into G-6-P by GKase. In glycolysis, GKase (hexokinase IV) is significant in regulating blood glucose homeostasis, causing high regulatory strength on hepatic glucose discharge and glucose sensor for insulin secretion in pancreatic β-cells (Matschinsky, 1996). The DC group, GKase expression, was downregulated up to 0.2 fold. The high blood glucose levels lower the sensitivity of GKase affinity for glucose, resulting in diabetes. Nevertheless, the treatment with Leaf extract and GNPs upregulate the GKase expression, up to 0.35, and 0.84 fold, respectively as shown in Fig. 1B.

Glucose 6 phosphatases extensively located in the hepatocytes, catalyze the final step in both glycogenolysis and gluconeogenesis by converting Glucose 6 phosphate into glucose and inorganic phosphate (Herling et al., 1998). In DC group, G-6-Pase was found to be increased to 16.9 fold. However, the treatment with Leaf extract and GNPs the G-6-Pase expression was found to be 14.3 and 13.3 fold, respectively (Fig. 1C). Reduction of glucose 6 phosphatases will reduce the oxidative damage because of the reduction in gluconeogenesis and glycogenolysis.

Table 3
Molecular docking results of Human G6Pase with ligand dataset from AutoDockVina.

S. No.	Compound	PubChem CID:	Molecular Formula	Binding affinity (kcal/mol)	Number of Hydrogen bonds
1	Alpha-amyrin	73170	C30H50O	−13.9	2
2	Beta-amyrin	73145	C30H50O	−11.2	1
3	2′,4′-Dihydroxy-6′-methoxy-3′,5′-dimethylchalcone	10424762	C18H18O4	−7.0	2
4	Isatin	7054	C8H5NO2	−5.9	2

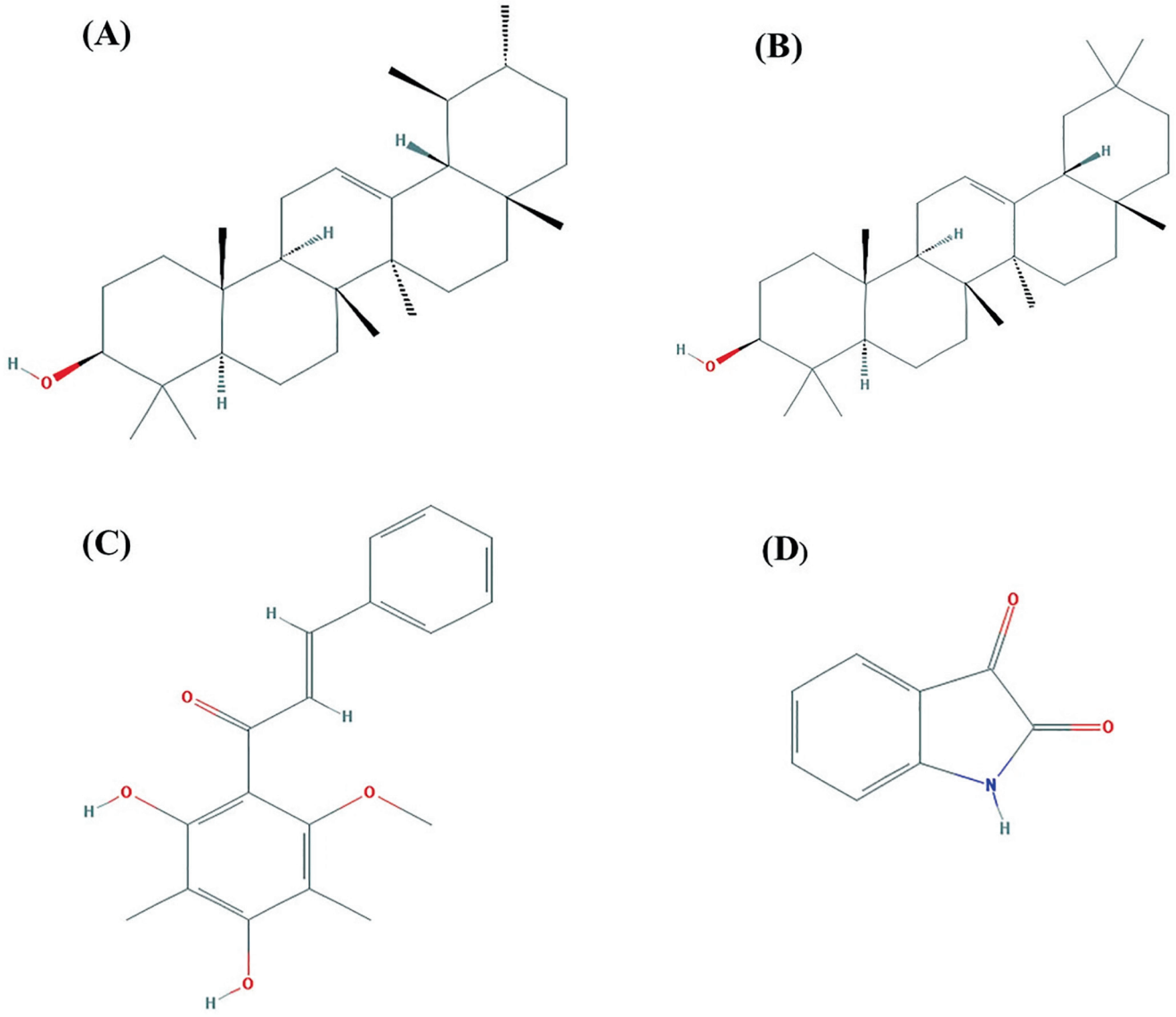

Fig. 3. The two-dimensional structure of the ligand dataset. (A) alpha-amyrin, (B) beta-amyrin, (C) 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone, and (D) isatin.

3.3. Structural prediction of G-6-Pase

G-6-Pase a multicomponent catalytic enzyme comprised of T1, T2 and T3 transporters intact as well scattered in the endoplasmic reticulum (ER) lumen (Burchell et al., 1994). The structure prediction of the G-6-Pase was performed by I-TASSER server and generated 5 best models using the concepts of different templates from ten servers based on the threading method. The critical parameter C-score evaluated the best model of G-6-Pase with a score −1.31. Besides, parameters like TM score 0.55 ± 0.15 and RMSD 9.6 ± 4.6 Å confirmed the overall quality of the model. G-6-Pase model possessed a specific three-dimensional structure with properly

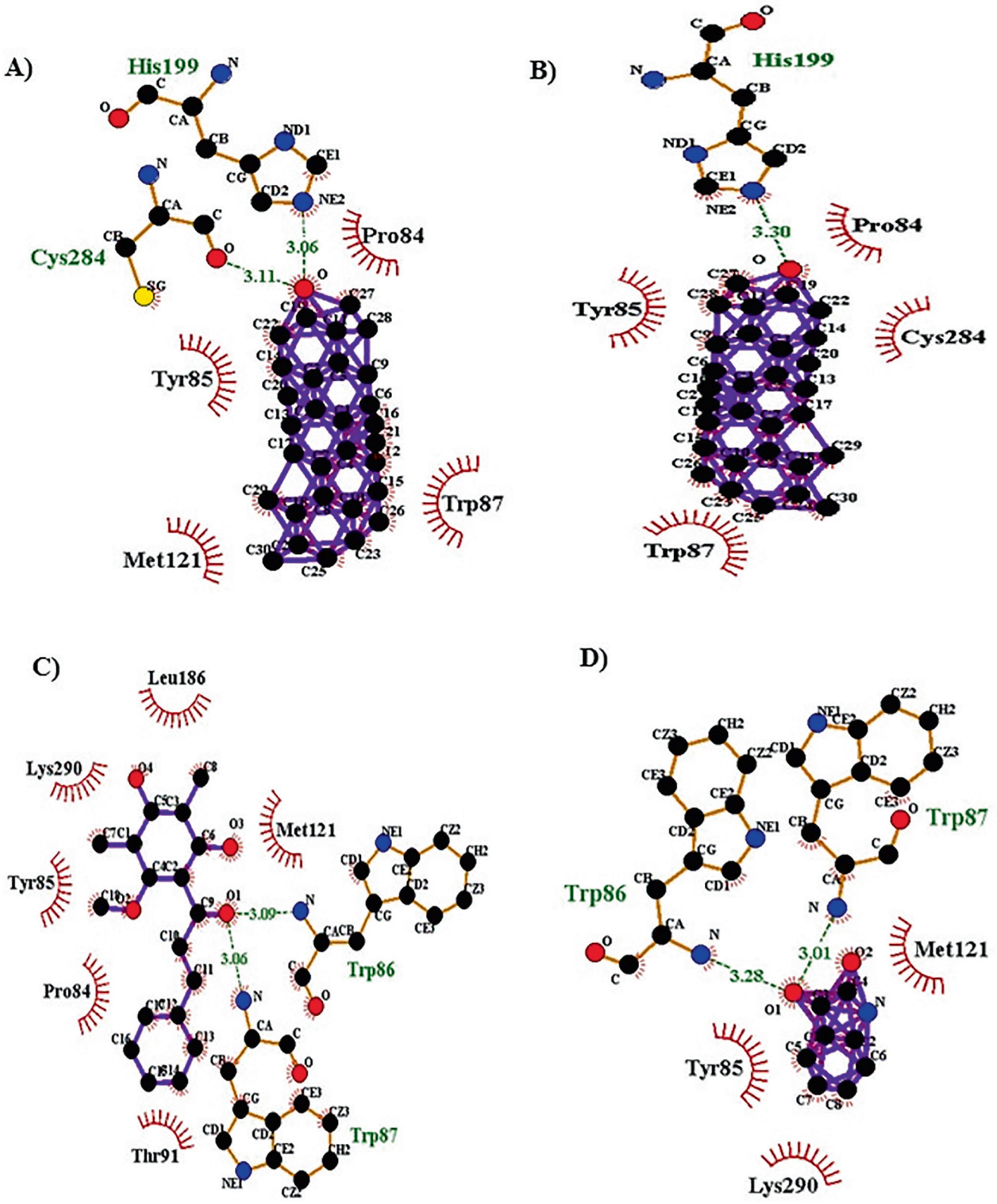

Fig. 4. Molecular docking results of human G-6-Pase with ligands. (A) alpha-amyrin, (B) beta-amyrin, (C) 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone, and (D) Isatin.

folded alpha helix connected with loops and few beta sheets (Fig. 2). Ramachandran plot from PROCHECK server validated the G-6-Pase model with most of the residues in the favored region (82.1%) and remaining residues in the allowed region.

3.4. G-6-Pase inhibition with phytocompounds from Couroupita guianensis: A molecular docking study

The structural investigation of G-6-Pase inhibition was carried out by molecular docking with four ligands in AutoDock Vina. The 2D structure of ligands was retrieved from the PubChem database (Fig. 3). The docking results showed top, binding conformations of ligands with G-6-Pase with an optimal binding affinity (Table 3). The ligand 'alpha-amyrin' showed the best inhibition with two hydrogen bonds involving key residues of G-6-Pase and also formed four hydrophobic interactions with ligand. The next best ligand 'beta-amyrin' showed with one hydrogen bond interaction and four hydrophobic interactions. The ligand '2′,4′-dihy droxy-6′-methoxy-3′,5′-dimethylchalcone' showed with two hydrogen bonds with ligand and six hydrophobic interactions with ligand. The last ligand 'isatin' showed two hydrogen bonds with ligand and also three hydrophobic interactions (Fig. 4). The residues Trp86, Trp87, His199 and Cys284 formed hydrogen bonds with G-6-P ase and confirmed as critical residues if G-6-Pase. The residues Pro84, Tyr85, Trp86, Trp87, Thr91, Met121, Leu186, Cys284 and Lys290 involved in hydrophobic interactions of G-6-Pase ligand complexes predicted using the Ligplot+. The *in-silico* analysis proved the G-6-Pase inhibition with ligands based on weak critical interactions and optimal energy parameters.

Previous studies reported that naturally occurring anthraquinone efficiently improves the postprandial insulin indices (Zhang et al., 2018). The prognostic hydrolysis of T1 -translocase in the G-6-Pase enzyme complex will reduce the excess glucose transportation from the cytoplasm to ER lumen (Barfell et al., 2011). Thus, it favors the inhibition of glucose 6 phosphate entry into ER. Similarly, the isatins from *C. guianensis* derivative of anthraquinones can efficiently involve in glycogen storage under normal conditions (Choi et al., 2005). Also, terpenoids or terpenes found significantly inhibit G-6-Pase, consequently, attenuate insulin resistance in type 2 diabetes. Collectively, nutraceuticals combined with colloids efficiently deprived gluconeogenesis.

4. Conclusion

The experimental findings showed that biogenic gold nanoparticle and leaf extract of *C. guianensis* effectively increase glucose transportation through GLUT 2 marker expression. Also, the transported glucose converted mostly by GK ase enzyme showed upregulation of GK ase expression. Interestingly, the prognostic effect of GNPs reinforces the expression of substrate transport model G-6-Pase suppression. Additionally, docking studies showed the downregulation of G-6-Pase was confirmed by the hydrogen bond interaction profile between G-6-Pase and ligand. Quinones and anthraquinones from the plant leaves of *C. guianensis* showed the antihyperglycemic property. Therefore, these findings prove that G-6-Pase downregulated by GNPs intervention significantly contributes to the regulation of circulatory glucose homeostasis in diabetes.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

We are grateful to the Vellore Institute of Technology (VIT) for providing seed money to carry out our research work. The authors express their sincere appreciation to the Researchers Supporting Project No. RSP-2020-93 the King Saud University, Riyadh, Saudi Arabia.

References

American Diabetes Association, 2017. Microvascular complications and foot care. Diabetes Care 1, 88–98. https://doi.org/10.2337/dc17-S013.

Ashmore, J., Weber, G., 1959. The role of hepatic glucose-6-phosphatase in the regulation of carbohydrate metabolism. Vitam. Horm. 17, 91–130. https://doi.org/10.1016/S0083-6729(08)60269-1.

Bailey, C.J., Day, C., 1989. Traditional plant medicines as treatments for diabetes. Diabetes Care 12, 553–564. https://doi.org/10.2337/diacare.12.8.553.

BarathManiKanth, S., Kalishwaralal, K., Sriram, M., Pandian, S.R.K., Youn, H.S., Eom, S., Gurunathan, S., 2010. Anti-oxidant effect of gold nanoparticles restrains hyperglycemic conditions in diabetic mice. J. Nanobiotechnol. 8, 16. https://doi.org/10.1186/1477-3155-8-16.

Barfell, A., Crumbly, A., Romani, A., 2011. Enhanced glucose 6-phosphatase activity in liver of rats exposed to Mg2+-deficient diet. Arch. Biochem. Biophys. 509, 157–163. https://doi.org/10.1016/j.abb.2011.03.003.

Burchell, A., Allan, B.B., Hume, R., 1994. Glucose-6-phosphatase proteins of the endoplasmic reticulum. Mol. Membr. Biol. 11, 217–227. https://doi.org/10.3109/09687689409160431.

Choi, S.Z., Lee, S.O., Jang, K.U., Chung, S.H., Park, S.H., Kang, H.C., Yang, E.Y., Cho, H.J., Lee, K.R., 2005. Antidiabetic stilbene and anthraquinone derivatives fromRheum undulatum. Arch. Pharm. Res. 28 (9), 1027. https://doi.org/10.1007/BF02977396.

Clarke, P.M., Gray, A.M., Briggs, A., Farmer, A.J., Fenn, P., Stevens, R.J., Matthews, D.R., Stratton, I.M., Holman, R.R., UK Prospective Diabetes Study (UKPDS) Group, 2004. A model to estimate the lifetime health outcomes of patients with type 2 diabetes: the United Kingdom Prospective Diabetes Study (UKPDS) Outcomes Model (UKPDS no. 68). Diabetologia 47, 1747–1759. https://doi.org/10.1007/s00125-004-1527-z.

Davidson, A.L., Arion, W.J., 1987. Factors underlying significant underestimations of glucokinase activity in crude liver extracts: physiological implications of higher cellular activity. Arch. Biochem. Physiol 253, 156–167. https://doi.org/10.1016/0003-9861(87)90648-5.

Glock, G.E., McLean, P., 1953. Further studies on the properties and assay of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase of rat liver. Biochem. J. 55, 400. https://doi.org/10.1042/bj0550400.

Herling, A.W., Burger, H.J., Schwab, D., Hemmerle, H., Below, P., Schubert, G., 1998. Pharmacodynamic profile of a novel inhibitor of the hepatic glucose-6-phosphatase system. Am. J. Physiol. Gastrointest. Liver Physiol. 274, 1087–1093. https://doi.org/10.1152/ajpgi.1998.274.6.G1087.

Hu, Y.C., Luo, Y.D., Li, L., Joshi, M.K., Lu, Y.H., 2012. *In vitro* investigation of 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone for glycemic control. J. Agric. Food Chem. 60, 10683–10688. https://doi.org/10.1021/jf303078r.

Karthick, V., Kumar, V.G., Dhas, T.S., Singaravelu, G., Sadiq, A.M., Govindaraju, K., 2014. Effect of biologically synthesized gold nanoparticles on alloxan-induced diabetic rats- an *in vivo* approach. Colloid Surf. B 122, 505–511. https://doi.org/10.1016/j.colsurfb.2014.07.022.

Kumar, C.S., Naresh, G., Sudheer, V., Veldi, N., Anurag, A.E., 2011. A short review on therapeutic uses of *Couroupita guianensis* Aubl. Int. Res. J. Pharm. App. Sci. 1, 105–108.

Laskowski, R.A., Swindells, M.B., 2011. LigPlot+: multiple ligand–protein interaction diagrams for drug discovery. J. Chem. Inf. Model. 2011, 2778–2786. https://doi.org/10.1021/ci200227u.

Martel, J., Ojcius, D.M., Chang, C.J., Lin, C.S., Lu, C.C., Ko, Y.F., Tseng, S.F., Lai, H.C., Young, J.D., 2016. Anti-obesogenic and antidiabetic effects of plants and mushrooms. Nat. Rev. Endocrinol. 13, 149–160. https://doi.org/10.1038/nrendo.2016.142.

Martínez, A., Conde, E., Moure, A., Domínguez, H., Estévez, R.J., 2012. Protective effect against oxygen reactive species and skin fibroblast stimulation of *Couroupita guianensis* leaf extracts. Nat. Prod. Res. 26, 314–322. https://doi.org/10.1080/14786411003752094.

Matschinsky, F.M., 1996. A lesson in metabolic regulation inspired by the glucokinase glucose sensor paradigm. Diabetes 45, 223–241. https://doi.org/10.2337/diab.45.2.223.

Mosa, R.A., Cele, N.D., Mabhida, S.E., Shabalala, S.C., Penduka, D., Opoku, A.R., 2015. *In vivo* antihyperglycemic activity of a lanosteryl triterpene from *Protorhus longifolia*. Molecules 20, 13374–13383. https://doi.org/10.3390/molecules200713374.

Nair, S.A., Sabulal, B., Radhika, J., Arunkumar, R., Subramoniam, A., 2014. Promising anti-diabetes mellitus activity in rats of β-amyrin palmitate isolated from *Hemidesmus indicus* roots. Eur. J. Pharmacol. 734, 77–82. https://doi.org/10.1016/j.ejphar.2014.03.050.

Peluso, I., Serafini, M., 2017. Antioxidants from black and green tea: from dietary modulation of oxidative stress to pharmacological mechanisms. Br. J. Pharmacol. 174, 1195–1208. https://doi.org/10.1111/bph.13649.

Sengani, M., 2017. Identification of potential antioxidant indices by biogenic gold nanoparticles in hyperglycemic Wistar rats. Environ. Toxicol. Pharmacol. 50, 11–19. https://doi.org/10.1016/j.etap.2017.01.007.

Shekhawat, M.S., Manokari, M., 2016. Impact of auxins on vegetative propagation through stem cuttings of *Couroupita guianensis* Aubl.: A conservation approach. Scientifica 2016, 1–7. https://doi.org/10.1155/2016/6587571.

Sies, H., 1997. Oxidative stress: oxidants and antioxidants. Exp. Physiol. 82, 291–295. https://doi.org/10.1113/expphysiol.1997.sp004024.

Trott, O., Olson, A.J., 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J. Comput. Chem. 31, 455–461. https://doi.org/10.1002/jcc.21334.

Tuan, D.T., Thu, N.B., Nhiem, N.X., Ngoc, T.M., Yim, N., Bae, K., 2009. Palbinone and triterpenes from Moutan Cortex (*Paeonia suffruticosa*, Paeoniaceae) stimulate glucose uptake and glycogen synthesis via activation of AMPK in insulin-resistant human HepG2 Cells. Bioorg. Med. Chem. Lett. 19, 5556–5559. https://doi.org/10.1016/j.bmcl.2009.08.048.

UK Prospective Diabetes Study (UKPDS) Group, 1998. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 352, 837–853. https://doi.org/10.1016/S0140-6736(98)07019-6.

Van der Vies, J., 1954. Two methods for the determination of glycogen in liver. Biochem. J. 57, 410. https://doi.org/10.1042/bj0570410.

Westergaard, N., Madsen, P., 2001. Glucose-6-phosphatase inhibitors for the treatment of Type 2 diabetes. Expert Opin. Ther. Pat. 11, 1429–1441. https://doi.org/10.1517/13543776.11.9.1429.

Yang, C.S., Sang, S., Lambert, J.D., Lee, M.J., ng et al. 2008. Bioavailability issues in studying the health effects of plant polyphenolic compounds. Mol. Nutr. Food Res. 52, 139–151. https://doi.org/10.1002/mnfr.200700234.

Zhang, M., Li, X., Liang, H., Cai, H., Hu, X., Bian, Y., Dong, L., Ding, L., Wang, L., Yu, B., Zhang, Y., 2018. *Semen Cassiae* extract improves glucose metabolism by promoting GLUT4 translocation in the skeletal muscle of diabetic rats. Front. Pharmacol. 9, 235. https://doi.org/10.3389/fphar.2018.00235.

Zhang, Y., 2008. I-TASSER server for protein 3D structure prediction. BMC Bioinf. 9, 40. https://doi.org/10.1186/1471-2105-9-40.