D.Sivaraman /J. Pharm. Sci. & Res. Vol. 11(8), 2019, 2844-2849

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

In Silico Investigation of Safety and Efficacy profile of Phytotherapeutics targeting Dipeptidyl-peptidase-4 Enzyme: Lead Identification approach

D.Sivaraman*

**Centre for Laboratory Animal Technology and Research, Col.Dr.Jeppiaar Research Park, Sathyabama Institute of Science and Technology , Jeppiaar Nagar, Rajiv Gandhi road, Chennai - 600 119, Tamil Nadu, India.*

Abstract

The dipeptidyl-peptidase-4 (DPP-4) belongs to serine exopeptidase family have been explored for its wide range of molecular activity interlinked in type II diabetes, neurodegeneration, Inflammation, liver fibrosis, cardiovascular, renal failure, cardiovascular, atherosclerosis etc. Inhibition of this bioactive enzyme render beneficial effect in aforementioned disease. There is an acute need of alternate source of DPP4 inhibitors preferably from herbal origin which could be safe and effective as well. Present study was aimed at evaluating safety and efficacy of phytotherapeutics (ascorbic acid, linoleic acid, oleanolic acid, salacinol) against DDP-4 enzyme along with standard sitagliptine by using ADMET, tox predictor and AutoDock 4 analytical tools. Safety predictions strongly suggested that lethal dose (LD50) value of the selected lead molecules ranges from 2,000 mg/kg to 10,000 mg/kg. Results of study clearly emphasize that the lead molecules oleanolic acid, ascorbic acid and salacinol possess significant DDP-4 inhibition activity by having potential interaction with bioactive amino acid residues (205 GLU, 206 GLU, 209 SER,547 TYR, 357 PHE ,358 ARG, 630 SER, 710 ASN) on DPP-4 enzyme similar to that of the standard drug sitagliptine. Similarly, highest docking score ranked by oleanolic acid (-7.06 Kcal/mol), followed by salacinol (-5.35 Kcal/mol), linoleic acid (-5.26 Kcal/mol) when compared to sitagliptine (-3.66 Kcal/mol). It was concluded from the results of the present investigation that plant derived bioactive phytotherapeutics like oleanolic acid, ascorbic acid, linoleic acid and salacinol have wide safety margin with less chances of causing adverse event upon clinical application. Further with proper preclinical investigations these lead compounds may have higher translational values as new generation peptidase inhibitors in halting the progression of DPP-4 enzyme in most of the inflammatory and degenerative disorders.

Keywords: Dipeptidyl-peptidase-4 (DPP-4), ascorbic acid, linoleic acid, oleanolic acid, salacinol, inflammatory, degenerative disorders.

1.INTRODUCTION

Dipeptidyl-peptidase-4 (DPP-4) belongs to the category of surface peptidase which has spectrum of biologically activity and primarily mediated cell signaling pathway. Further its chemistry and functionality is preserved in the sequence of evolution in both prokaryotes and eukaryotes organisms [1]. It was evident through research that inhibition of DPP4 activity renders some beneficial activity in halting endothelial dysfunction, atherogenesis and also limiting the cytokine production [2].

DPP4 potentially breaks the biologically significant gastrointestinal hormones like glucagon like peptides (GLP) and other gastric inhibitory polypeptides (GIP). These hormones are known to induce the secretion of insulin mediated by meal signaling. Increased expression of DDP4 in diabetic patients tends to exerts its out breaking action against GIP and GLP directly restricts the secretion of insulin and further leads to hyperglycemia. DPP4 inhibitors occupy considerable market in treating T2DM the know inhibitors include sitagliptin, saxagliptin, vildagliptin, alogliptin and linagliptin [3]. Recent clinical evidences suggested that DPP-4 inhibitors reveal absolute safety and kinetics in pediatrics patients similar to that of the adults.

Recent preclinical and clinical investigation emphasize extra pharmacological activity of DPP4 inhibitors. Immunomodulatory activity of some DPP4 inhibitors reported with reduction in activity of nuclear factor-κB binding (NF-Kβ) which is considered to be a rate limiting factor in controlling the expression of some inflammatory cytokines like interleukin (IL-1,IL-6) and tumor necrosis factor (TNF-α) [4].

Diabetic rats fed with DPP4 inhibitors reduces hepatic steatosis and fibrosis and decreases hepatic inflammation. Similarly, in high fat fed rats these agents exhibited significant reduction in both plasma and hepatic triglyceride and lower the levels of inflammatory mediators [5]. Similarly, sitagliptin improved the renal blood flow and in rats with spontaneous hypertension by molecular inhibition of cAMP. Hence it was advocated that DPP4 inhibitors exerts high level of clinical benefits in renal protection of diabetic patients with kidney complications [6].

DPP4 enzyme express more on endothelial and epithelial kidney tissues and render protective action on kidney tissues by reducing inflammation and fibrosis and improving overall function [7-8].

Usage of DPP4 inhibitors either as monotherapy or in combination with sulfonylurea derivatives attributes some potential side effects such as acute kidney injury, respiratory tract infections, and acute pancreatitis [9], hypoglycemia, headache, tremor, dizziness, asthenia, and nausea [10]. Hence the need of alternate source is of highly clinical importance

DDP4 not only mediates the gastro intestinal hormones several other mediators acts as a substrate for this enzyme the list enumerated as follows GRF: growth hormone-releasing factor; GRP: gastrin-releasing peptide; IGF-1:

insulin-like growth factor 1; IL-1β: interleukin-1β; IL-2: interleukin-2; GCP-2: granulocyte chemotactic protein 2; IP-10: interferon γ-inducible protein 10; I-TAC: interferon γ-inducible T cell alpha chemoattractant; SDF-1α: stromal cell-derived factor 1α; SDF-1β: stromal cell-derived factor 1β; LD78β: isoform of macrophage inflammatory protein-1α (MIP-1); MCP: monocyte chemotactic protein; VIP: vasoactive intestinal peptide [11-13].

Linoleic acid is an octadecadienoic acid in which the two double bonds are at positions 9 and 12 (polyunsaturated omega-6 fatty acid). Research focus on linoleic acid attains greater importance as it becomes a potential drug of choice in lowering the risk associated with coronary heart disease [14]. Salacinol (thiosugar sulfonium sulfate) a potential anti-diabetic compound known to possess excellent α-glucosidase enzyme inhibition activity has tremendous beneficial activity in treating diabetes mellitus [15]. Oleanolic acid belongs to the category of pentacyclic triterpenoid exerts hepatoprotective activity [16]. Study also revealed the anti- cancer potential of oleanolic acid against human colon carcinoma cell line HCT15 [17]. Oleanolic acid also possess significant anti-oxidant and inhibits the expression of inflammatory cytokine in silicotic rat rodent model [18]. Ascorbic acid is well known water soluble micro nutrient. It has numerous pharmacological activity such as antioxidant, anti-cancer, anti-inflammatory and cardiovascular diseases prevention [19]. Plant phytocomponents have a proven track record of becoming an ailment for several infective and degenerative disorders. Traditional herbal supplement's believed to have possess high therapeutic efficacy with low or no side effects. To counteract the potential adverse effect caused by conventional DPP-4 inhibitors an attempt of exploring an alternate drug candidate have me made. Hence present investigation aimed at evaluating the efficacy of novel lead moieties (ascorbic acid, linoleic acid, oleanolic acid, salacinol) against target dipeptidyl peptidase IV.

2.MATERIALS AND METHODS

2.1. Protein-ligand docking

In silico molecular docking analysis were performed by using AutoDock version 4 analytical program. (https://www.dockingserver.com), which exactly predicts the interactions between selective lead compounds with that of the enzyme target dipeptidyl-peptidase-4 (DPP-4).

2.2.Protein and Ligand preparation

Three dimensional structure of DPP-4 with PDB code (2P8S) retrieved from the RCSB source. Structure were cleaned by defined standard optimized procedure using Auto Dock 4 [21]. 2D to3D structures of lead compound's (ascorbic acid, linoleic acid, oleanolic acid, salacinol along with standard sitagliptine prepared using Chem Draw software.

2.3.ADME and Toxicity profile prediction

Swiss ADME and tox prediction tools were utilized for accessing the lethal dose and organ related toxicity nature of all the compounds. Further kinetic profiling (Absorption, distribution, metabolism and elimination) properties of all the selected compounds [22].

2.4.Docking simulations

Docking calculations were carried out using Auto Dock 4. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Test compounds ascorbic acid, linoleic acid, oleanolic acid, salacinol along with standard sitagliptine docked against the taget DPP-4 (PDB 2P8S). Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools. Affinity (grid) maps of ×× Å grid points and 0.375 Å spacing were generated using the Autogrid program. 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) [23] and the Solis & Wets local search method [24]. 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.

3.RESULTS AND DISCUSSION

3.1.ADMET and safety prediction Analysis

In silico predication improves the specificity of drug binding thereby it greatly minimizes the adverse events and also aids in selection of accurate lead. Advancement in the field of drug discovery offers researcher a wider platform in experimenting their novelty at less time even more economical that conventional methods [25]. Demand on docking rises constantly as it is considered as ideal alternate to lab animal model, improves focus of research, reduce the failure rate, saves more time, opportunity to explore the alternate therapeutics and translate new drug entities.

Physicochemical nature of the drug determines the behavior of the compound on the biological system. Data's on molecular weight and functional group suggestively helps in predicting the barrier crossing potential of the compound's (Table 1). Results of ADMET predication analysis shown that that all lead molecules such as ascorbic acid, linoleic acid, oleanolic acid and salacinol exerts good absorption through gastro intestinal route and has no interaction with the cytochrome group of enzymes. This prediction concludes the safety nature of the leads and also non interactive nature with cytochrome inhibitors may also reduce the chance of interaction. Average LD 50 value of the compound's ranges from 2000 to 10000 mg/kg further shows the wide safety margin of the selected molecules (Table 2). Safety prediction scoring of all the leads seems less than one which ensures nontoxic nature of the compounds with respect to the cytotoxicity, hepatotoxicity, carcinogenicity, immunotoxicity and mutagenicity (Table 3).

Table 1: Physicochemical properties of Selected Lead compounds along with standard (Ascorbic Acid, Linoleic acid, Oleanolic acid, Salacinol and standard Sitagliptine)

Compound	Molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds	Log P
Ascorbic Acid	176.124 g/mol	C6H8O6	4	6	2	-1.6
Linoleic acid	280.452 g/mol	C18H32O2	1	2	14	6.8
Oleanolic acid	456.711 g/mol	C30H48O3	2	3	1	7.5
Salacinol	334.354 g/mol	C9H18O9S2	5	9	6	-3
Sitagliptine	407.32 g/mol	C16H15F6N5O	1	10	4	0.7

Table 2: Pharmacokinetic profile of Lead compounds (Ascorbic Acid, Linoleic acid, Oleanolic acid, Salacinol and standard Sitagliptine)

Pharmacokinetic Property	Ascorbic acid	Linoleic acid	Oleanolic acid	Salacinol	Sitagliptine
GI absorption	High	High	Low	Low	High
BBB permeant	No	Yes	No	No	Yes
P-gp substrate	No	No	No	Yes	Yes
CYP1A2 inhibitor	No	Yes	No	No	No
CYP2C19 inhibitor	No	No	No	No	No
CYP2C9 inhibitor	No	Yes	No	No	No
CYP2D6 inhibitor	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	No
Log K_p (skin permeation)	-8.54 cm/s	-3.05 cm/s	-3.77 cm/s	-10.43 cm/s	-8.29 cm/s
LD 50 in mg/kg	3367mg/kg	10000 mg/kg	2000 mg/kg	5000 mg/kg	2500 mg/kg

Abbreviations: GI – Gastro Intestinal, BBB- Blood brain barrier, P-gp- P-glycoprotein, CYP- Cytochrome, LD- Lethal dose.

Table 3: Toxicity Prediction Analysis of Lead compounds (Ascorbic Acid, Linoleic acid, Oleanolic acid, Salacinol and standard Sitagliptine)

Target	Ascorbic acid	Linoleic acid	Oleanolic acid	Salacinol	Sitagliptine
Hepatotoxicity	0.86	0.55	0.52	0.82	0.60
Carcinogenicity	0.92	0.64	0.57	0.79	0.50
Immunotoxicity	0.99	0.96	0.79	0.99	0.82
Mutagenicity	0.87	1.0	0.85	0.55	0.55
Cytotoxicity	0.65	0.71	0.99	0.73	0.73

3.2.In silico molecular docking analysis

Virtual analytical tools play a phenomenal role in the journey of new drug discovery. It greatly reduced the time and showcase high level of prediction accuracy. Information regarding absorption, distribution, metabolism and elimination are utilized for ensuring the bio-availability and kinetic behavior of the study molecule [26]. Identification of the active site reveals the functionality of the amino acid and their role in mediating the enzymatic reactions [27]. This would be useful for the synthetic chemist to focus on the functional group and side chain moieties that are capable of forming interactions with this core active site of the receptors [28,29].

Docking score essentially helps the researcher in identifying the hit out of other leads. As per the results of the present study oleanolic acid ranked first with the -7.06 Kcal/mol. Followed by this salacinol with -5.35 Kcal/mol and linoleic acid with -5.26 Kcal/mol when compared with standard sitagliptine (-3.66 Kcal/mol). Total interactive surface occupied by oleanolic acid was 844.98, next to this linoleic acid with 742.58, salacinol with 585.73 when compared with sitagliptine 451.64 (Table 4).

Research outcomes strongly recommended that catalytic activity of the enzyme Dipeptidyl peptidase IV majorly mediated by amino acids such as 205 GLU, 206 GLU, 209 SER,547 TYR, 357 PHE ,358 ARG, 630 SER, 710 ASN. It was observed from the study that therapeutic leads such as oleanolic acid, ascorbic acid and salacinol possess significant DDP-4 inhibition activity by having potential interaction with bioactive amino acid residues (205 GLU, 206 GLU, 209 SER,547 TYR, 357 PHE ,358 ARG, 630 SER, 710 ASN) on the enzyme (Table 5, Fig.1and 2).

Table 4: Summary of the molecular docking studies of the lead compounds (Ascorbic Acid, Linoleic acid, Oleanolic acid, Salacinol and standard Sitagliptine) against Dipeptidyl peptidase IV (2P8S)

Phytocompounds	Binding Free energy Kcal/mol	Inhibition constant Ki µM (*mM)(**nM)	Intermolecular energy Kcal/mol	Total Interaction Surface
Ascorbic Acid	-3.52	2.61*	-3.64	411.81
Linoleic acid	-5.26	139.45	-8.94	742.58
Oleanolic acid	-7.06	6.65	-7.72	844.98
Salacinol	-5.35	118.9	-7.25	585.73
Sitagliptine	-3.66	2.08*	-3.67	451.64

Table 5: Interaction of lead compounds Ascorbic Acid, Linoleic acid, Oleanolic acid, Salacinol and standard Sitagliptine) with active site amino acid residue of Dipeptidyl peptidase IV (2P8S)

Compounds/ Standard	Amino Acid Interactions									
Sitagliptine	125 ARG	**205 GLU**	**206 GLU**	**209 SER**	356 ARG	**357 PHE**	**358 ARG**	**547 TYR**	662 TYR	666 TYR
Ascorbic Acid	125 ARG	**205 GLU**	**206 GLU**	**209 SER**	**357 PHE**	**358 ARG**	666 TYR	669 ARG		
Linoleic acid	**357 PHE**	**547 TYR**	552 SER	554 LYS	**630 SER**	631 TYR	656 VAL	659 TRP	662 TYR	666 TYR
Oleanolic acid	125 ARG	**205 GLU**	**206 GLU**	**357 PHE**	**547 TYR**	552 SER	**630 SER**	662 TYR	666 TYR	**710 ASN**
Salacinol	125 ARG	**205 GLU**	**206 GLU**	**209 SER**	**357 PHE**	**547 TYR**	662 TYR	666 TYR	669 ARG	

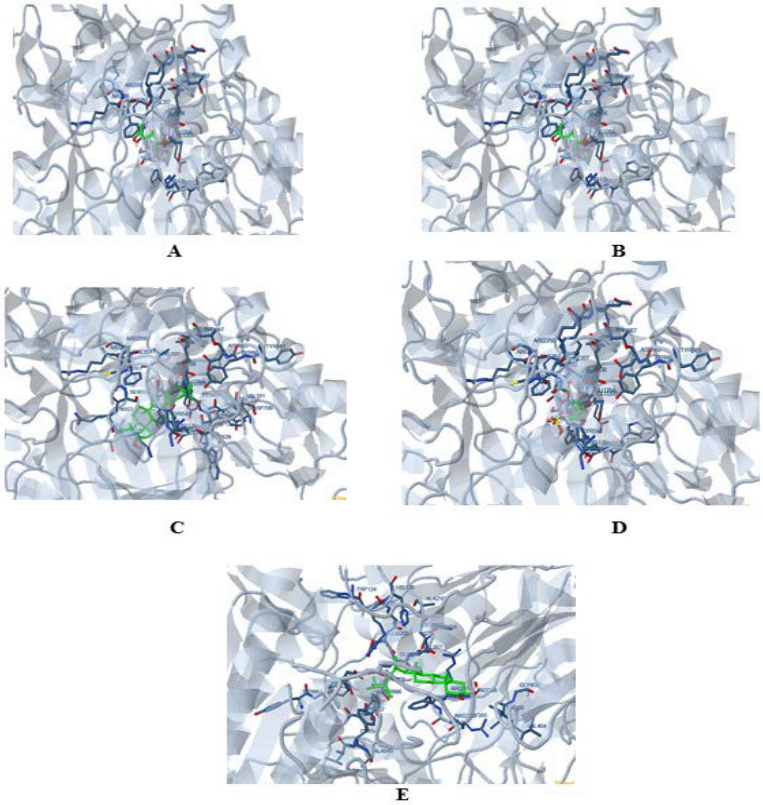

Fig 1. The 3D docking pose showing the interactions between ligand molecules (A) Ascorbic acid, (B) Linoleic acid, (C) Oleanolic acid, (D) Salacinol, (E) Sitagliptine and DPP-4 (2P8S) enzyme

Abbreviations:3D – Three dimensional, DPP-4- Dipeptidyl-peptidase-4

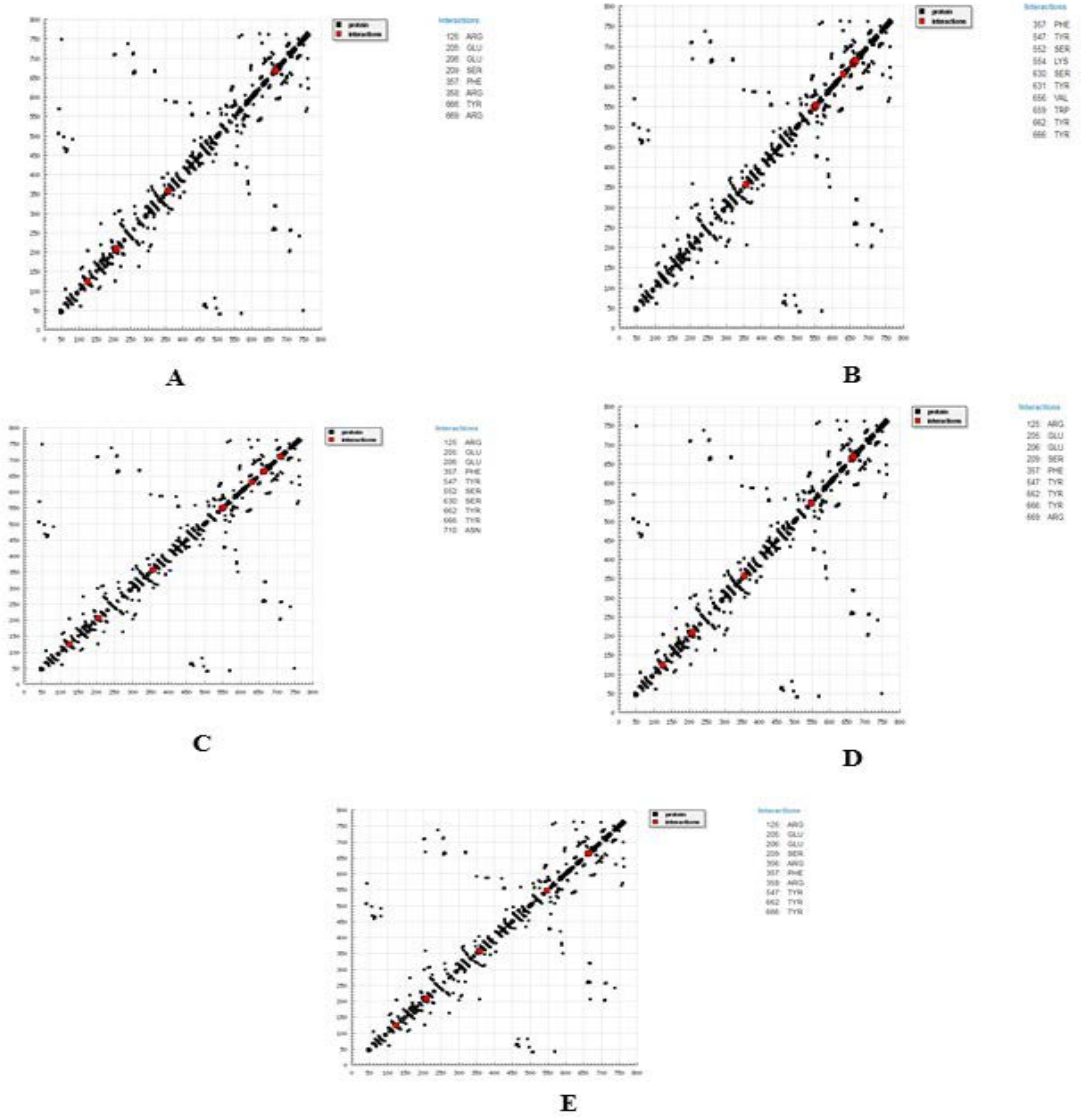

Fig. 2. HB plotting analysis on hydrogen bond formation between ligand molecules (A) Ascorbic acid, (B) Linoleic acid, (C) Oleanolic acid, (D) Salacinol ,(E) Sitagliptine and DPP-4 (2P8S) enzyme

4.CONCLUSION

DPP-4 is a versatile enzyme that influence several biological activities with numerous substrates to act on. Inhibiting the enzyme physiologically renders beneficial activity in particular to diabetes, cardiovascular, inflammation and neurodegeneration. Considering the adverse effects of the conventional DPP-4 inhibitors shift of focus towards herbal components are now becomes alternate drug of choice. Results of the study indicates that phytocomponents such as oleanolic acid, ascorbic acid and salacinol possess significant DDP-4 inhibition property with wide margin of safety. Hence it was concluded that these novel moieties may have a greater translational value as an alternate drug of choice in the management of inflammatory and degenerative disorders.

Acknowledgments

We express our sincere thanks to the management of Sathyabama institute of science and technology, Chennai, Tamil Nadu, India for providing necessary infrastructure in completing this research work.

REFERENCES

1. Matteucci E and Giampietro O. Dipeptidyl peptidase-4 (CD26): knowing the function before inhibiting the enzyme. *Current Medicinal Chemistry*.2009; 16:2943–2951.
2. Hu Y, Liu H, Simpson RW, Dear AE. GLP-1-dependent and independent effects and molecular mechanisms of a dipeptidyl peptidase 4 inhibitor in vascular endothelial cells. *Molecular Biology Reports*.2013;40:2273–2279.
3. Michelle A. Van Name MD. Medications for the Treatment of Type II Diabetes in Pediatric Type II Diabetes. *Pediatric Type II Diabetes*.2019;1:101-106.
4. Makdissi A, Ghanim H, Vora M. Sitagliptin exerts an anti-inflammatory action. *J Clin Endocrinol Metab*. 2012; 97:3333–3341.
5. Shirakawa J, Fujii H, Ohnuma K. Diet-induced adipose tissue inflammation and liver steatosis are prevented by DPP-4 inhibition in diabetic mice. *Diabetes*. 2011;60:1246–1257.
6. Von WK, Reichetzeder C, Hocher B. Physiology and pathophysiology of incretins in the kidney. *Curr Opin Nephrol Hypertens*. 2014;23:54–60.

7. Haluzik Martin, Frolik Jan, Rychlik Ivan. Renal Effects of DPP-4 Inhibitors: A Focus on Microalbuminuria. *International Journal of Endocrinology*. 2013;2013:1–7.
8. Marques C. Sitagliptin Prevents Inflammation and Apoptotic Cell Death in the Kidney of Type 2 Diabetic Animals. *Mediators Inflamm*. 2014;2014:1–15.
9. Hampp C, Borders-Hemphill V, Moeny DG, Wysowski DK. Use of Antidiabetic Drugs in the U.S., 2003–2012. *Diabetes Care*. 2014;37:1367–1374.
10. Mario Miguel Rosa, Teresa Dias. Neurologic Aspects of Systemic Disease Part II. *Handbook of Clinical Neurology*.2014;120: 809-824.
11. Lambeir AM, Durinx C, Scharpe S, Meester I. Dipeptidyl-peptidase IV from bench to bedside: an update on structural properties, functions, and clinical aspects of the enzyme DPP IV. *Critical Reviews in Clinical Laboratory Sciences*.2003; 40:209–294.
12. Drucker DJ. Dipeptidyl peptidase-4 inhibition and the treatment of type 2 diabetes: preclinical biology and mechanisms of action. *Diabetes Care*.2007; 30:1335–1343.
13. Vanderheyden M, Bartunek J, Goethals M. Dipeptidyl-peptidase IV and B-type natriuretic peptide. from bench to bedside. *Clinical Chemistry and Laboratory Medicine*.2009;47:248–252.
14. Ronald J. Jandacek. Linoleic Acid: A Nutritional Quandary. *Healthcare (Basel)*. 2017; 5: 20-25.
15. Masayuki Y,Toshiyuki M, Hiromi S, Hisashi M. Salacinol, potent antidiabetic principle with unique thiosugar sulfonium sulfate structure from the Ayurvedic traditional medicine Salacia reticulata in Sri Lanka and India. *Tetrahedron Letters*.1997;48:8367-8370.
16. Jacob P, Alain G. Molecules of Interest Oleanolic acid. *Phytochemistry*.2012;77:10-15
17. Jie L, Wei JG, Qing YY. Effects of ursolic acid and oleanolic acid on human colon carcinoma cell line HCT15. *World J Gastroenterol*. 2002; 8: 493–495.
18. Peng HB, Wang RX, Deng HJ, Wang YH, Tang JD. Protective effects of oleanolic acid on oxidative stress and the expression of cytokines and collagen by the AKT/NF‑κB pathway in silicotic rats. *Mol Med Rep*. 2017; 15:3121-3128.
19. Grosso G, Bei R, Mistretta A, Marventano S, Calabrese G, Masuelli L, Giganti MG, Modesti A, Galvano F, Gazzolo D. Effects of vitamin C on health: a review of evidence. *Front Biosci*. 2013; 18:1017-1029.
20. Huey R, Morris GM, Olson AJ, Goodsell DS. A semiempirical free energy force field with charge-based desolvation. *J Comput Chem*. 2007; 28:1145–1152.
21. Bikadi Z, Hazai E. Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock. *J. Cheminf*.2009;1:1- 15.
22. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep*. 2017;7:42717.
23. Morris GM, Goodsell DS. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *Journal of Computational Chemistry*.1998;19:1639-1662.
24. Solis FJ, Wets RJ. Minimization by random search techniques. *Mathematics of Operations Research*.1981; 6: 19–30.
25. Waring MJ, Arrowsmith J, Leach AR, Leeson PD, Mandrell S, Owen RM, Pairaudeau G, Pennie WD, Pickett SD, Wang J. An analysis of the attrition of drug candidates from four major pharmaceutical companies. *Nat. Rev. Drug Discov*. 2015; 14:475-486.
26. Louis L, Harvey W. Predicting Oral Drug Absorption: Mini Review on Physiologically-Based Pharmacokinetic Models. Pharmaceutics. 2017 ; 9(4): 1-14.
27. Chu X, Bleasby K, Evers R. Species differences in drug transporters and implications for translating preclinical findings to humans. *Expert Opin. Drug Metab. Toxicol*. 2013; 9:237–252.
28. Chanteux H, Staelens L, Mancel V, Gerin B, Boucaut D, Prakash C, Nicolas JM. Cross-species differences in the preclinical pharmacokinetics of CT7758, an α4β1/α4β7 integrin antagonist. *Drug Metab. Dispos. Biol. Fate Chem*. 2015; 43:1381–1391.
29. Tian S, Wang J, Li Y, Li D, Xu L, Hou T. The application of in silico drug-likeness predictions in pharmaceutical research. *Adv Drug Deliv Rev*. 2015; 86:2-10.