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Research Article

Flavones as Potential Anti-Diabetics: Design, Synthesis and Biological Evaluation

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Abstract

Aim: The aim of the study was to develop new anti-diabetic agents from synthetic route.

Materials and Methods: An attempt was made to synthesize various flavones. The structures of the compounds were elucidated by UV, IR, ^{1}H-NMR, and mass spectrometry. Furthermore, an *in silico* library of synthesized compounds were designed. Aldose reductase, protein tyrosine phosphate and alpha amylase were selected as targets to conducting *in-vivo* study to afford potential inhibition of those enzymes. Designed library of flavones were docked into the active site of all three enzymes and the most active 10 flavones (-9.71 to -6.72 kcal/mole) were selected for synthesis. Finally, selected compounds were evaluated for their *in-vivo* anti-diabetic activity by streptozotocin induced model.

Results: Docking study revealed that flavones such as F1, F2, F3, F5 and F8 were potentially considered for *in-vivo* anti-diabetic activity by streptozotocin induced model. Fasting blood glucose and biochemical parameters like total protein, urea, creatinine, SGOT, SALP and SGPT were performed for the biological evaluation and compared with that of standard glibenclamide (5 mg/kg). Among the five consolidated flavones, F8 possess high significant ($p < 0.01$) results and restores the blood glucose level, liver enzymes and renal parameters. Based on these results, a promising potent drug would be developed in the management of diabetes mellitus.

Conclusion: *In-vivo* evaluations of selected compounds were carried out for its anti-diabetic activity using three enzymes as a target. All the selected flavones showed excellent interactions with targeted enzymes and established a noticeable correlation between *in silico* score and *in-vivo* anti-diabetic activity.

Keywords: *Anti-Diabetic Activity; Aldose Reductase (AR); Protein Tyrosine Phosphatase 1B; Alpha Amylase; Streptozotocin; In Silico Score; Docking Study*

Abbreviations

DM: Diabetic Mellitus; WHO: World Health Organization; PTP1B: Protein Tyrosine Phosphatase 1 B; CPCSEA: Committee for the Purpose of Control and Supervision of Experiments of Animals; STZ: Streptozotocin; SGOT: Serum Glutamic Oxaloacetic Transaminase SGPT: Serum Glutamic Pyruvic Transaminase

Introduction

The major cause of Diabetic Mellitus (DM) is characterized by the imbalance secretion or inappropriate utilization of insulin, leads to the development of hyperglycaemia. As per WHO The diabetic mellitus may be the one of the major causes of death in 2030 [1] and it is estimated that 1.7 - 5.3 million people were death from 2015 - 2018. In spite of the development of many oral hypoglycaemic drugs, the management of DM is still a challenging task in the present day. There must be a simultaneous management of proper diet regimen, day to day habits, physical exercises and the pharmacological treatments of diabetic mellitus [2]. Now a days, the prevention and treatment of diabetic mellitus is a big challenge for clinicians as there is a lot of side effects and adverse effects are observed. To overcome such problem natural products may be the potential target of the development of new candidate for the prevention and treatment of complication of diabetic mellitus. The naturally occurring flavonoids have been documented as the potential pharmacological properties such as anti-diabetic, anti-microbial, anti-oxidant, cytotoxic agent, hepatoprotective agent and anti-osteoporotic agent [3]. The pancreatic enzymes such as alpha-glucosidase and alpha-amylase are significant intestinal enzymes meant for the digestion of starch producing glucose and maltose leads to the enhancement of postprandial glucose level. To inhibit these enzymes is the prime objective and management of development of diabetic conditions, the inhibition of the enzymes such as alpha-glucosidase and alpha-amylase lead to the reduction of starch digestion [4].

Oral anti-diabetic drugs along with glucosidase inhibitors should be used to achieve better diabetic control [5]. Many natural products with flavonoids as major constituents have been reported to have excellent anti-diabetic activity [6]. These phytochemicals such as polyphenols, flavonoids and their related natural compounds are also have shown promising anti-diabetic activity through *in vivo* and *in vitro* model [7].

In present study, we have selected flavonoids nucleus for synthesis as flavones having basic ring, 2-phenyl-4H-chromen-4-one [3]. The flavonoids have been classified into many groups with various biological activities such as anti-inflammatory, hepatoprotective, antimutagenic effects, antioxidant, antimicrobial activity and anti-allergic activities. The flavones also inhibit various enzymatic pathways [8-11] which is one of the prime targets in the treatment of DM. A class of synthetic flavonoid may have shown promising alpha-glucosidase and alpha-amylase inhibitory properties [12,13]. One such method to reduce type II diabetes mellitus is by inhibiting digestion and absorption of dietary carbohydrates.

In current study a series of flavones analogues are designed, synthesized for inhibition of the diabetic enzymes such as alpha-amylase, Protein tyrosine phosphatase 1 B (PTP1B) and aldose reductase using molecular docking study, and they have been screened before for being considered for preclinical and clinical evaluation of anti-diabetic property. The selected synthesized compounds are evaluated for *in-vivo* anti-diabetic activity by inducing streptozotocin in Wister rats. The blood, liver and renal parameters are also screened and compared with standard drugs on treated Wister rats. Hence the present study mainly deals with the development of potential candidate in the management, prevention and treatment of Diabetes Miletus.

Materials and Methods

Chemical and reagents

All the related chemical and reagents used in the study were purchased from reputed company such as Sigma Aldrich, USA, Hi-media Pvt. Ltd, Mumbai, SRL Pvt. Ltd, Mumbai and Loba chemicals Cochin. Reagents, solvent and various kits were of analytical grade

Scheme of synthesis [14-16]

Algar-Flynn-Oyamada method is used to synthesized all the flavones, illustrated in the figure 1 (F1-F10).

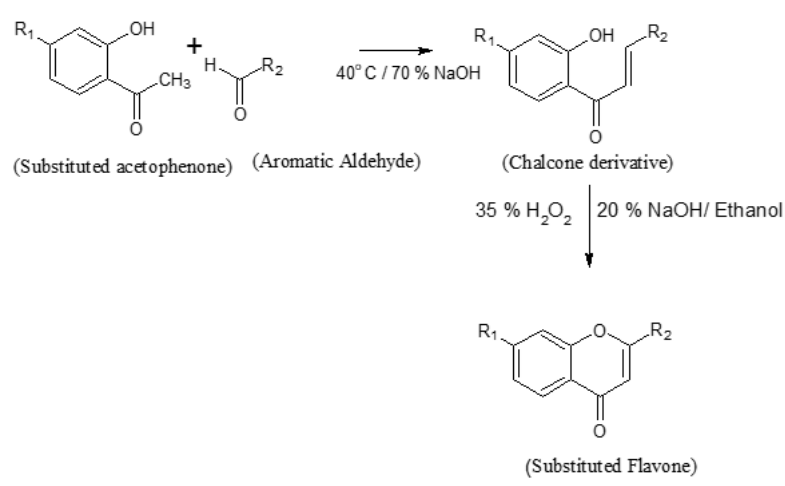

Figure 1: *Scheme of synthesis of flavones.*

Compound code	R1	R2
F1	-H	$-C_6H_5$
F2	-H	$-C_6H_4$-2 (Cl)
F3	-H	$-C_6H_4$-4 (Cl)
F4	-H	$-C_6H_4$-4 (F)
F5	-H	$-C_6H_4$-2 (NO_2)
F6	-H	$-C_6H_4$-2 (OH)
F7	-H	$-C_6H_4$-4 (OH)
F8	-H	$-C_6H_4$-4 (OCH_3)
F9	-H	$-C_6H_4$-2,4 (OCH_3)
F10	-H	$-C_6H_4$-N $(CH_3)_2$

Table 1: *Different substitution patterns on basic nucleus of flavones.*

Molecular docking studies [17]

Methodology

The free binding energies of all the compounds were calculated using Autodock 4.0 software. Aldose reductase (PDB: 3m4h), PTP1b (PDB: 1een) and α-amylase (PDB: 3ole). were obtained from Protein Data Bank (http://www. rcsb.org/pdb) place at Brookhaven National Laboratory in 1971 for the study.

Docking analysis

The prepared crystal structures of ligand and active site such as alpha amylase, aldose reductase and protein tyrosine phosphate were subjected to Auto dock 4.2.6 for measuring their binding energies.

Evaluation of anti-diabetic activity (in-vivo)

Experimental animals

The male albino Wister rats weighing 150 - 200g used for the study. The animals were procured from College of Veterinary and Animal Sciences, Thrissur, India and housed in the animal house maintained under standard hygienic conditions, at 25 ± 2°C, humidity (60 ± 10%) with 12 hr day and night cycle, with food and water. The study was carried out as per CPCSEA norms after obtaining approval (CPCSEA/58/2020), Katihar Medical College and Hospitals. Throughout the experimental period, all four groups of animals were fed with a normal laboratory chow standard pellet diet (Sai feeds, Bangalore, India) and water ad libitum throughout the experimental period.

Induction of diabetes

A freshly prepared streptozotocin (STZ) (Himedia) at the concentration of 60 mg/kg bodyweight [18] i.p. in 0.1 mol/L cold citrate buffer, pH 4.5 is used to induce diabetic condition in animals which were already kept for 12h fasting. To maintain drug-induced hypoglycaemia the STZ-treated animals were allowed to drink 5% glucose solution to overnight. Rats showing constantly hyperglycaemia and glycosuria together with fasting glucose more than 250 mg/dL on the 3rd day after STZ injection were taken into consideration as diabetic induced animal and hence, used for experimental purpose.

Experimental design

Animals were divided into 8 groups, consisting of a minimum of four animals each as follows:

- Group I= Vehicle control rats received 0.1 mol/L citrate buffer (pH 4.5).
- Group II= Diabetic control.
- Group III, IV, V, VI and VII diabetic rats were administered with synthesized compounds F1, F2, F3, F5 and F8 for 21 days.
- Group VIII, diabetic rats were administered 5 mg/kg glibenclamide solution orally per day for 21 days.

The fasting blood glucose level were determined after the induction of diabetes in Wistar rats the experimental animals having blood glucose level more than 250 mg/d L were considered as diabetic and utilised for further study. The measured doses of the synthesized compounds were administered every day till completion of the experiments (i.e., 21 days). The untreated and diabetic control groups were treated as per the protocol of the study. At the end of the experiment, the blood samples were collected for biochemical studies. The serum was separated by centrifugation and subjected for assay immediately or the serum may be stored at -20°C for future study.

Biochemical estimations

The rats were kept for overnight fasting and the blood was collected from the tail vein at 0th (before the start of the experiments), 4th day, 7th day, 14th day and 21st day. The blood glucose level was determined by glucometer. The individual animal's weight was recoded gravimetrically on 0th and 21st days of the study. After the completion of protocol regimen, the blood was collected through retro-orbital puncture of the eye of animals. The serum was separated by centrifugation at 3000 rpm for 15 minutes. The biochemical parameters of liver such as SALP, SGOT, SGPT and the serum bilirubin were determined by usual kits [19]. The renal parameters such as serum urea, creatinine and proteins were determined using Auto analyser [20-22].

Statistical analysis

Data obtained from pharmacological experiments, are expressed as mean ± SEM. Differences between control and treated groups were tested for significance using ANNOVA followed by Dunnett's t-test, with $P < 0.05$ were considered as significant.

Results and Discussion

Synthesis of flavone

All the compounds were synthesized on the basis of Claisen Schmidt reaction. All the compounds were characterized by IR, UV, ^{1}H-NMR spectroscopy and mass spectrometry. The purity of synthesized compounds was checked by determining thin layer chromatography. A single spot was observed indicating their purity and the melting points were also determined which was found to be incorrect.

Spectral study

All the synthesized compounds were characterized by various spectroscopic techniques such as UV, IR, ^{1}H-NMR and mass spectrometry.

F1: 2-phenyl-4H-chromen-4-one

MP: 131-132ºC; R_f = 0.55; % yield = 64.2% w/w; UV λmax: $CHCl_3$, nm: 298; IR (KBr cm^{-1}): 1738 (lactone), 1644 (CO str), 1584, 1552 (C=C Arom.str), 1135, 1089 (COC str), 780 (C-C bending); ^{1}H-NMR (500 MHZ, DMSO): δ 7.5 - 7.8 (m, 8H, ArH), 6.8 (m, 1H, ArH); m/z: 221 (m+1), 121.7 $(C_7H_6O_2)^+$, 106.2 $(C_7H_8)^+$, 91.9 $(C_6H_6O)^+$, 78.0 $(C_6H_5)^+$.

F2: 3-(2-chlorophenyl)-4H-1-benzopyran-4-one

MP: 148-165ºC; R_f = 0.45; % yield = 54.4% w/w; UV λmax: $CHCl_3$, nm: 239; IR (KBr cm^{-1}): 1760 (lactone), 1690 (CO str), 1580, 1570 (C=C Arom.str), 1102, 1145, 1041 (COC str), 760 (C-C bending); ^{1}H NMR (500 MHZ, DMSO): δ; 6.3 (m, 1H, ArH), 7.2 - 7.9 (m, 8H, ArH); m/z: 255 (m+1), 139.0 $(C_8H_7Cl)^+$, 121.1 $(C_7H_6O_2)^+$, 91.9 (C_6H_5O), 77 $(C_6H_5)^+$.

F3: 3-(4-chlorophenyl)-4H-1-benzopyran-4-one

MP: 156-171ºC; R_f = 0.76; % yield = 62.4% w/w; UV λmax: $CHCl_3$, nm: 271; IR (KBr cm^{-1}) 1740 (lactone), 1679 (CO str), 1589, 1581 (C=C Arom.str), 1141, 1089 (COC str), 772 (C-C bending); ^{1}H NMR (500 MHZ, DMSO): δ 7.3 - 7.8 (m, 8H, ArH), 6.8 (m, 1H, ArH); m/z: 256 (m+1), 120.6 $(C_7H_6O_2)^+$, 140.5 $(C_8H_7Cl)^+$, 75.6 $(C_6H_5)^+$.

F4: 2-(4-fluorophenyl)-4H-chromen-4-one

MP: 253-258ºC; R_f = 0.72; % yield = 67.1% w/w; UV λmax: $CHCl_3$, nm: 272; IR (KBr cm^{-1}): 1782 (lactone), 1665 (CO str), 1568, 1523 (C=C Arom.str), 1133, 1115, 1041 (COC str), 761 (C-C bending); ^{1}H NMR (500 MHZ, DMSO): δ 6.7 (m, 1H, ArH), 7.4 - 8.4 (m, 8H, ArH); m/z: 241 (m+1), 121 $(C_7H_6O_2)^+$, 120 $(C_8H_6F)^+$, 76.6 $(C_6H_3)^+$.

F5: 3-(2-nitrophenyl)-4H-1-benzopyran-4-one

MP: 139-141ºC; R_f = 0.42; % yield = 82.6% w/w; UV λmax: $CHCl_3$, nm: 289; IR (KBr cm^{-1}): 1791 (lactone), 1667 (CO str), 1573, 1590 (C=C Arom.str), 1129, 1112 (COC str), 773 (C-C bending); ^{1}H NMR (500 MHZ, DMSO): δ 6.9 - 6.56 (m, 8H, ArH), 6.7 (m, 1H, ArH); m/z: 266 (m+1), 123.0 $(C_7H_6O_2)^+$, 150.0 $(C_8H_7NO_2)^+$, 78.2 $(C_6H_5)^+$.

F6: 2-(2-hydroxyphenyl)-4H-chromen-4-one

MP: 181-184ºC; R_f = 0.46; % yield = 72.2% w/w; UV λmax: $CHCl_3$, nm: 251; IR (KBr cm^{-1}): 1741 (lactone), 1692 (CO str), 1549, 1539 (C=C Arom.str), 1140, 1134 (COC str), 752 (C-C bending) 3416, 3651 (OH str); ^{1}H NMR (500 MHZ, DMSO): δ 6.7 (m, 1H, ArH), 7.2 - 7.8 (m, 8H, ArH); m/z: 238 (m+1), 122 $(C_7H_6O_2)^+$, 107 $(C_7H_6O)^+$, 105 $(C_8H_8)^+$, 79 $(C_6H_6)^+$.

F7: 2-(4-hydroxyphenyl)-4H-chromen-4-one

MP: 171-173ºC; R_f = 0.52; % yield = 71.5% w/w; UV λmax: $CHCl_3$, nm: 279; IR (KBr cm^{-1}): 1781 (lactone), 1689 (CO str), 1559, 1553, 1521 (C=C Arom.str), 1052, 1149 (COC str), 734 (C-C bending) 3412, 3537 (OH str); ^{1}H NMR (500 MHZ, DMSO): δ 6.7 (m, 1H, ArH), 7.3 -7.5 (m, 8H, ArH); m/z: 238 (m+1), 121 $(C_7H_6O_2)^+$, 118 $(C_8H_7O)^+$, 92 $(C_6H_6O)^+$, 76.9 $(C_6H_6)^+$.

F8: 3-(4-methoxyphenyl)-4H-1-benzopyran-4-one

MP: 172-173ºC; R_f = 0.57; % yield = 69.2% w/w; UV λmax: $CHCl_3$, nm: 271; IR (KBr cm^{-1}): 1667 (CO str), 1589, 1543 (C=C Arom.str), 1163, 1048 (COC str), 739 (C-C bending); ^{1}H NMR (500 MHZ, DMSO): δ 3.1 (s, OCH_3, ArH), 6.2 (m, 1H, ArH), 7.3, 7.4 (m, 8H, ArH); m/z: 252 (m+1), 135.9 $(C_9H_{10}O)^+$, 106 $(C_7H_6O)^+$, 121.0 $(C_7H_5O_2)^+$, 78.1 $(C_6H_5)^+$.

F9: 2-(2,4-dimethoxyphenyl)-4H-chromen-4-one

MP: 165-168ºC; R_f = 0.57; % yield = 63.4% w/w; UV λmax: $CHCl_3$, nm: 261; IR (KBr cm^{-1}): 1658 (CO str), 1595, 1561 (C=C Arom.str), 1132, 1045 (COC str), 761 (C-C bending); ^{1}H NMR (500 MHZ, DMSO): δ 3.7 (s, OCH_3, ArH), 6.8 (m, 1H, ArH), 6.5 - 6.7 (m, 7H, ArH); m/z: 283 (m+1), 121 $(C_7H_6O_2)^+$, 92.7 $(C_6H_6O)^+$, 164 $(C_{10}H_{11}O_2)^+$, 137.5 $(C_8H_{10}O_2)^+$, 76.7 $(C_6H_5)^+$.

F10: 2-[4-(dimethylamino)phenyl]-4H-chromen-4-one

MP: 164-167ºC; R_f = 0.69; % yield = 58.4% w/w; UV λmax: $CHCl_3$, nm: 289; IR (KBr cm^{-1}): 1787 (lactone), 1667 (CO str), 1551, 1540 (C=C Arom.str), 1132, 1054 (COC str), 721 (C-C bending); ^{1}H NMR (500 MHZ, DMSO): δ 3.1 (s, 6H, N $(CH_3)_2$), 6.1 - 6.4 (m, 1H, ArH), 7.2 - 7.8 (m, 8H, ArH); m/z: 265 (m+1), 222 $(C_{15}H_{10}O_2)^+$, 122 $(C_7H_6O_2)^+$, 105.0 $(C_8H_8)^+$, 78.1 $(C_6H_5)^+$.

Molecular docking studies

The docking process was done as per their parameters, the synthesized compounds were docked into the active site of the receptors or proteins. The standard inhibitors were Fidarestat, Ertiprotafib and Acarbose against aldose reductase, PTP1B and α-amylase enzymes respectively. The free kinetic energy was calculated and interpreted for all the synthesized compounds as presented in table 2.

Docking analysis of aldose reductase inhibitor (3m4h)

The docking results of synthesized flavones with the aldose reductase inhibitor were given good information about the nature of the binding mode. From the conformation of compound F3 (docking score= -9.71 kcal/mole**,** Table 2) observed that formed conventional hydrogen with His 306 established interactions with oxygen atom of carbonyl center, carbon hydrogen bond with Ser 305 and π- donor hydrogen bond with Thr 304 respectively of the compound F3 as shown in figure 2. Whereas, the docking results of synthesized flavones F1 and F5 with the aldose reductase inhibitor were shown the information about the nature of the binding mode. From the conformation of compound F1 and F5 (docking score= **-**9.13 kcal/mole and -8.68 kcal/mole respectively**,** Table 2). The compound F1 showed the conventional hydrogen bond with Thr 304, established interactions with oxygen atom of carbonyl center while, the F5 compound showed the conventional hydrogen bond with His 305 and Thr 304, recognized the interactions with oxygen atoms, carbon hydrogen bond with Lys 307, π- σ interaction with Thr 309 and π- lone pair bond with Thr 304 as shown in figure 2.

Docking analysis of Protein tyrosine phosphatase 1 B (PTP1B) inhibitor (1een)

The docking results of the synthesized flavones with PTP1B inhibitor enzyme were given good information about the nature of the binding mode. From the conformation of compound F2 (docking score= -8.90 kcal/mole**,** Table 2) and the interactions results showed the conventional hydrogen bond with Asp 48, Ala 217, Gly 220, Gln 262, Leu 219, Ser 215, Gly 218 and Ser 216, carbon hydrogen bond with Apg 47, π- σ interaction with Tyr 46 and Ala 217, π- alkyl interaction with Apg 47, covalent interaction with Asp 3 and attractive charges Apg 221 respectively of the compound F2 as shown in figure 3.

Whereas, the docking results of synthesized flavones F3 and F5 with the PTP1B inhibitor were shown the information about the nature of the binding mode. From the conformation of compound F3 and F5 (docking score= -8.20 kcal/mole and -8.42 kcal/mole respectively, Table 2) it was observed that, both the F3 and F5 compounds showed interactions of conventional hydrogen bond with Asp 48, Ala 217, Gly 220, Gln 262, Ile 219, Ser 215, Gly 218 and Ser 216, carbon hydrogen bond with Apg 47, π- σ interaction with Tyr 46 and Ala 217, π-alkyl interaction with Apg 47, covalent interaction with Asp 2 and attractive charges Apg 221 respectively of the compound F3 and F5 as shown in figure 3.

Docking analysis of alpha-amylase inhibitor (3ole)

The docking results of synthesised flavones with the alpha-Amylase inhibitor were posses good information about the nature of the binding mode. The docking results revealed the best accommodation of **F5** and **F8** (docking score = - 8.62 kcal/mol and -7.56 kcal/mol) compounds respectively. The compound **F5** showed their conventional bond with ser 219, carbon hydrogen bond with Asn 216, π- donor hydrogen bond with Asn 218, π- σ bond with Asn 218, Amide - π Stacked with Leu 217 and alkyl interaction with Ala 224 of the target enzyme (docking score= **-**7.69 kcal/mole, showed in the table 2 and figure 4).

Interaction study of few compounds with different receptors showing comparatively higher docking score

Docked pose of aldose reductase enzyme inhibitor (3m4h) with the compound F1 F2 and F3

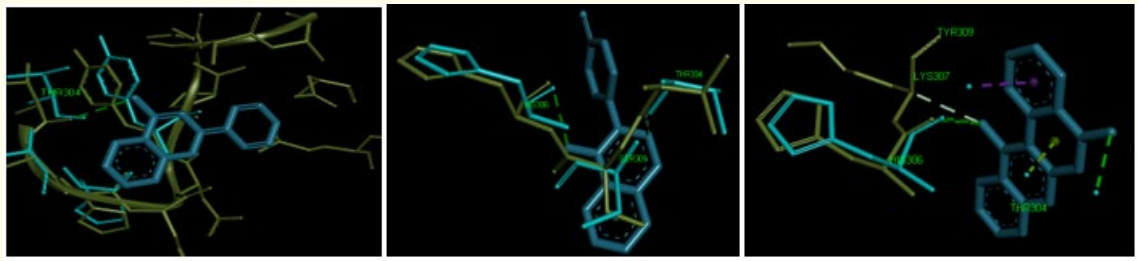

Figure 2: *Visualization of synthesized ligands (F1, F3 and F5) interaction with aldose reductase inhibitor.*

Docked pose of PTP1B (1een) with the compound F2, F3 and F5

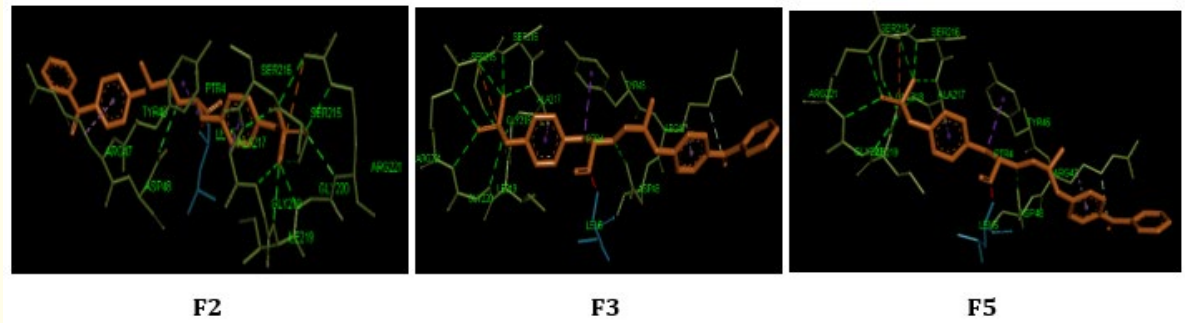

Figure 3: *Visualization of synthesized ligands (F2, F3 and F5) interaction with PTP 1B inhibitor.*

Docked pose of alpha amylase with the compound F5 and F8

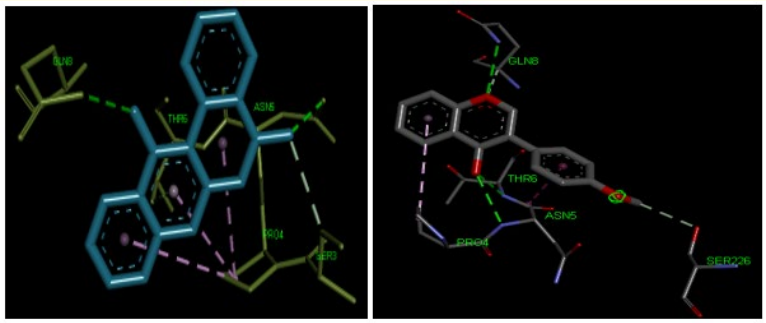

Figure 4: *Visualization of synthesized ligands (F5 and F8) interaction with alpha amylase inhibitor.*

Docking score

Docking score of all synthesized compounds were done against three receptors namely aldol reductase, PTP1B and α amylase. On the basis of docking score, *in vivo* anti-diabetic activity of the selected flavones were evaluated.

Docking score of all synthesized componds revealed a kind of significant potential towards the all three enzymes with different flavones.

Anti-diabetic activity by (STZ induced model)

The selected compounds such as F1, F2, F3, F5 and F8 were used for the evaluation of anti-diabetic activity by STZ (Streptozotocin) induced rat model. The most prime parameter, i.e. blood glucose level was observed a significant value ($p < 0.01$) compared to normal rat, as shown in table 3. The blood glucose level was found to be very low or reduced after oral administration of synthesized compounds for 21 days as compared to the diabetic control rat. The significant reduction ($p < 0.01$) in blood glucose level was found on 14th and 21st day when synthesized compounds such as F1 F2, F3, F5 and F8 were orally administered, hence the synthesized flavones were shown to improve the metabolic activity significantly as compared with untreated rats as represented in figure 5.

Groups	Fasting Blood Glucose Level (mg/dl)			
	1st day	7th day	14th day	21st day
Normal Control	94.23 ± 2.03	97 ± 2.65	96 ± 2.65	96.13 ± 1.16
Diabetic Control	267.43 ± 3.53**	283.35 ± 2.40**	313.22 ± 2.91**	318.46 ± 4.33**
Standard	253.66 ± 3.18*	191.66 ± 2.03**	158 ± 3.46**	122.66 ± 4.98**
F1	255.66 ± 2.33*	203.67 ± 4.96**	167.33 ± 4.09**	124.33 ± 2.90**
F2	259.33 ± 2.60^{ns}	276.58 ± 2.85^{ns}	302.62 ± 3.48*	311.37 ± 2.73*
F3	257.23 ± 3.53^{ns}	276.27 ± 3.48^{ns}	305.46 ± 4.33*	309.18 ± 3.57*
F5	268.39 ± 5.17^{ns}	275.67 ± 4.82*	301.69 ± 4.27*	313.13 ± 3.74^{ns}
F8	253.24 ± 3.46*	199.24 ± 2.31**	171.33 ± 3.45**	127.33 ± 4.72**

Table 3: *Effect of synthesized flavones on blood glucose level on STZ induced diabetic rats.*

*Values are mean ± SEM for n = 4; *P < 0.05 = Significant; **P < 0.01 = more significant and ns = nonsignificant as compared with diabetic control.*

Effect of synthesized flavones on blood glucose level on STZ induced diabetic rats

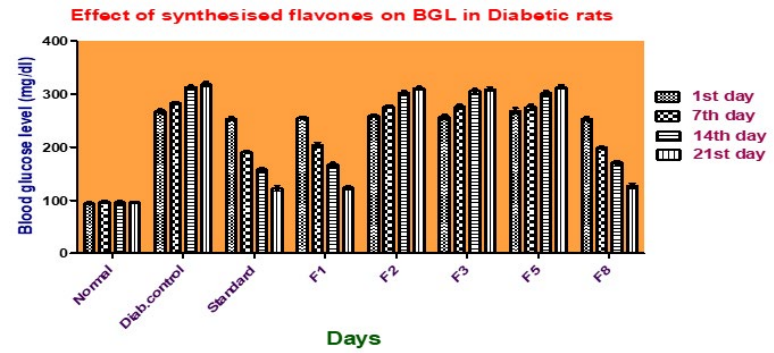

Figure 5: *Effect of synthesized flavones on blood glucose level on STZ induced diabetic rats.*

Biochemical parameters

The liver parameters such as SGOT, SGPT, SALP and total bilirubin levels were increases in diabetic rats, which significantly ($p < 0.01$) restores the liver biomarker enzymes after treatment of synthesized flavones. The total protein level was decreases in diabetic rats and significantly increases after treatment of 21 days with synthesized flavones (Table 4).

Liver biomarker enzymes and total protein on STZ induced diabetic rats

Groups	SGOT (IU/l)	SGPT (IU/l)	SALP (IU/l)	Total bilirubin (mg/dl)	Total protein (g/dl)
Normal control	54.33 ± 1.20	25.20 ± 1.73	102.33 ± 5.18	0.49 ± 0.02	6.93 ± 0.02
Diabetic control	126.15 ± 5.16**	54.38 ± 1.53**	228.31 ± 9.46**	3.94 ± 0.23**	4.87 ± 0.07**
Standard	52.54 ± 1.21**	29.81 ± 2.43**	118.13 ± 6.52**	0.94 ± 0.02**	6.51 ± 0.03**
F1	77.36 ± 4.74**	33.25 ± 2.08**	124.52 ± 8.13**	1.43 ± 0.08**	6.12 ± 0.21**
F2	122.14 ± 5.34^{ns}	48.66 ± 3.78*	221.33 ± 9.51^{ns}	3.88 ± 0.26^{ns}	4.73 ± 0.15^{ns}
F3	121.56 ± 4.16*	52.23 ± 3.17^{ns}	209.66 ± 8.82*	3.50 ± 0.28^{ns}	4.80 ± 0.31^{ns}
F5	123.34 ± 2.64^{ns}	50.56 ± 4.18^{ns}	216.33 ± 9.38^{ns}	3.68 ± 0.28^{ns}	4.77 ± 0.28^{ns}
F8	56.33 ± 3.87**	31.51 ± 2.58**	125.58 ± 8.12**	2.34 ± 0.14**	6.12 ± 0.04**

Table 4: *Effect of synthesized flavones in Liver biomarker enzymes and total protein on STZ induced diabetic rats.*

*Values are mean ± SEM for n = 4; *P < 0.05 = Significant; **P < 0.01 = more significant and ns = nonsignificant as compared with diabetic control.*

Liver parameters

Effects of liver parameter were illustrated in the figure 6.

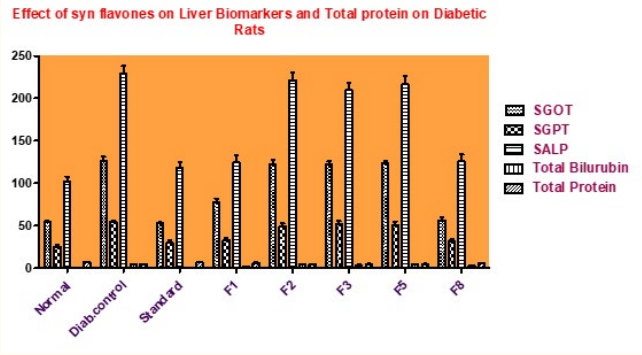

Figure 6: *Effect of synthesized flavones on Liver biomarker enzymes and total protein on STZ induced diabetic rats.*

Renal parameters

The renal parameters such as blood urea and serum creatinine were shown to be in the normal range on STZ induced diabetic rats after administration of synthesized flavones as mentioned in table 5 and figure 7.

Groups	Blood urea (mg/dl)	Serum creatinine (mg/dl)
Normal control	16.32 ± 0.53	0.68 ± 0.03
Diabetic control	31.72 ± 2.01**	0.98 ± 0.08**
Standard	19.36 ± 1.45**	0.72 ± 0.05**
F1	25.33 ± 1.67*	0.74 ± 0.05**
F2	27.14 ± 2.35^{ns}	0.96 ± 0.02^{ns}
F3	28.43 ± 1.86^{ns}	0.92 ± 0.06^{ns}
F5	29.57 ± 1.55^{ns}	0.91 ± 0.04^{ns}
F8	21.78 ± 1.13**	0.74 ± 0.06**

Table 5: *Effect of synthesised flavones in renal parameters on STZ induced diabetic rats.*

*Values are mean ± SEM for n = 4; *P < 0.05 = Significant; **P < 0.01 = more significant; ns = nonsignificant.*

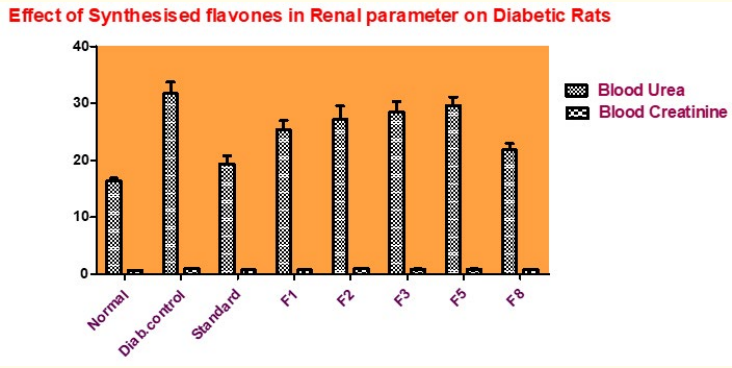

Figure 7: *Effect of synthesised flavones in renal parameters on STZ induced diabetic rats.*

Discussion

All the synthesized compounds (F1- F10) were purified by determining melting points and these were characterized by various spectroscopic techniques such as UV, IR, ^{1}H NMR and mass spectrometry.

An *in-silico* library of synthesized flavones was designed. Aldose reductase, PTP1B and alpha amylase were selected as a drug target and is considered as known target for natural flavones against anti-diabetic activity. Designed library of 100 flavones were docked into the active site of all three enzymes and the most active 10 flavones were synthesized. Furthermore, synthesized flavones (F1-F10) were subjected for molecular docking studies on the diabetic enzymes with inhibitory activity against aldose reductase, PTP1B and alpha amylase [23,24].

To inhibit aldose reductase process might be the important approach to reduce diabetic complication on long term therapy [25]. The compound F3 shown excellent aldol reductase inhibitory activity might be due to the presence of electron withdrawing groups (carbonyl oxygen) and (-Cl group). Among these ten synthesized flavones F1-F10, F3 showed the best binding score value, which is compared to standard fidarestat (binding score is -10.35 kcal/mole), a known aldose reductase enzyme inhibitor. On docking study, in case of PTB 1B inhibitor, the compounds F2, F3 (with - Cl group on the position 2 and 4 respectively) and also the compound F5 (with -NO_2 group) possess the high potent inhibitory activity. Among these ten synthesized flavone derivatives F1-F10, F2 showed the best binding score value, which is compared to standard ertiprotafib (binding score is -7.72 kcal/mole), a known PTP1B enzyme inhibitor.

The docking study of a-amylase inhibitor on the synthesised flavones reveals that compound F5 (-NO_2 group) and F8 (-OCH_3 group) showed equal potency as compared with the standard acarbose (binding score is **-**7.61 kcal/mole), a known alpha amylase enzyme inhibitor. This proves that the effective binding sites are present in the selected flavones and are potential when compared with the standard inhibitors respectively [26].

The blood glucose level was found normal after oral administration of test compounds for 21 days as compared to diabetic rats. The test compounds F1 and F8 were shown to decrease ($p < 0.01$) the blood glucose level compared with diabetic control.

The diabetic rats had significantly ($p < 0.01$) increased in transaminase and decreased in protein content than normal rats. After oral administration of test compounds had moderate decrease in liver enzymes, blood urea and creatinine as compared to diabetic rats. The synthesized flavones F1 (benzo pyrone ring) and F8 (-OCH_3 electron donating group) were significantly ($p < 0.01$) restores the liver and renal parameters compared with the diabetic rats [27,28].

Conclusion

Virtual screening of 100 (One Hundred) flavones library resulted in the identification of 10 compounds. These 10 compounds with the highest estimated free energy of binding -6.65 to -9.71 kcal/mole against aldose reductase, PTP1B and a-amylasewere selected for synthesis. Top ranked compounds F1, F2, F3, F5 and F8 with highest estimated free energy mentioned in table 2 had promising anti-diabetic activity was in tune with wet lab experiments.

The excellent interactions of all three enzymes with all top ranked compounds indicated and established a high degree co-relationship between *in silico* approach and *in vivo* studies.

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Conflicts of Interest

There are no conflicts of interest.

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Volume 13 Issue 2 February 2025