ORIGINAL RESEARCH PAPER
Volume-8 | Issue-9 | September - 2019 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

IN-VIVO ANTI-DIABETIC ACTIVITY OF FLAVONE ANALOGUES

Pharmacy

Nitesh Kumar	Narayan Nursing College, Jamuhar, Sasaram, (Rohtas)-821305
Monika Verma	Department of Padiatrics, Advance Padiatrics Centre PGIMER, Chandigarh, Panjab-160012- India
Adil Inam	Narayan Medical College and Hospital, Jamuhar, Sasaram-Rohtas, Bihar-821305
Kamlesh Kumar Naik	Nandha College of Pharmacy,Perundurai Main Road,Erode, Tamil Nadu-638052, India
Afroze Alam*	M.Pharm, PhD Narayan Institute of Pharmacy, Jamuhar, Rohtas (Sasaram)-821305, Bihar –India *Corresponding Author

ABSTRACT

An attempt was made to synthesize various flavones. The structures of the compounds were elucidated by UV, IR, 1H-NMR, and mass spectrometry. Furthermore, an -vivo anti-diabetic activity study was carried out. The study reveals 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.

KEYWORDS

Anti-diabetic Activity; Creatinine; Flavones; Total Protein; SGOT; SALP; SGPT; Streptozotocin

INTRODUCTION

Diabetic mellitus is a metabolic disorder, which is characterised by improper secretion or utilisation of insulin, results in hyperglycaemia [1]. As per WHO report, diabetic mellitus is an one of the leading cause of death in 2030 [2] and it clearly assessed that 1.5 – 4.9 million people were death from 2012 to 2014.

The naturally available flavonoid plays vital role in treating so many major disease, in that one of the chronic disease is diabetes mellitus. In the present study the selected flavonoid analogue is flavones; its basic ring is as 2-phenyl-4H-chromen-4-one[3]. Basically flavonoids and its classes bears low molecular weight, which exists various biodynamic properties such as antioxidant, antimicrobial activity, anti-allergic, anti-inflammatory, hepatoprotective, antimutagenic effects and also inhibit various enzymes [4-7].

For achieving better glycemic control, oral antidiabetic agents including glucosidase inhibitors are used for therapeutic activity [8,9]. There are many herbal extracts having reported anti-diabetic potentials [10]. Among these phytochemicals, flavonoids and their related natural compounds are known to possess anti-diabetic activity, established in various animal models [11]. Flavonoids are the most common polyphenolic compounds used as medicaments for diabetes mellitus since ancient times[12,13]. One of the ways to reduce type II diabetes mellitus is by suppressing absorption and digestion of dietary carbohydrates.

The current study deals with the evaluation of the inhibitory activity of flavone as potential anti-diabetic agents [14, 15]. Based on the result flavones were evaluated for *in-vivo* anti-diabetic activity by inducing streptozotocin in wister rats [16].The study significantly explains about blood glucose level, liver and renal parameters which were evaluated and compared with standard drug on treated rats. Hence the present study deals on development of potent drug in the management of diabetes mellitus.

MATERIAL AND METHODS

Chemical and Reagents

Substituted acetophenones, aromatic aldehydes and streptozotocin (STZ) were purchased from SRL Pvt. Ltd, Mumbai, Hi-media Pvt. Ltd, Mumbai and Loba chemicals, Cochin. The solvents and other reagents and kits were purchased commercially and were of analytical grade.

Experimental Methods

Scheme of Synthesis[17-19]

The scheme of this synthesis is based on Algar-Flynn-Oyamada method illustrated in the Fig. 1 for the synthesis of flavones (F1-F10).

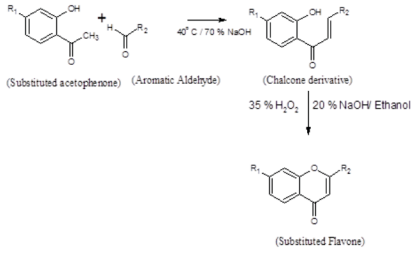

Fig. No. 1 General synthetic scheme of flavones

Table 1 Different substitution patterns on basic nucleus (γ-benzopyrone ring)

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(NO2)
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)$_2$

Determination on Anti-Diabetic Activity By in-vivo Method

Induction of Diabetes

Animals were allowed fasting for 12 h and administered freshly prepared streptozotocin (STZ) (Himedia) at the concentration of 60 mg/kg bodyweight [20], i.p. in 0.1 mol/L cold citrate buffer, pH 4.5. The STZ-treated animals were allowed to drink 5 % glucose solution overnight to overcome drug-induced hypoglycemia. Rats having persistent glycosuria and hyperglycaemia with a fasting blood glucose >250 mg/dL on the third day after the STZ injection were considered as diabetic and use for further experimentation.

Experimental design

Animals were divided into 8 groups, consisting of a minimum of four animals each as follows: Group I, control rats received 0.1 mol/L citrate buffer (pH 4.5); Group II, diabetic control; Group III, IV, V, VI & VII diabetic rats were administered with synthesized compounds F1, F2, F3, F5 & F8 for 21 days. Group VIII, diabetic rats were administered 5 mg/kg glibenclamide solution orally per day for 21 days.

After one week the induction of diabetes in Wistar rats, the fasting blood glucose levels of fasted rats were measured. Rats with blood glucose > 250 mg/dl were considered as diabetes in this study. The different doses of synthesized flavonoids were administered every day till the completion of the experiment (i.e., 21 days), whereas untreated and diabetic control groups were treated with 0.1 mol/L citrate buffer every day orally.

At the end of the experiment, the blood samples were collected for biochemical studies. The serum were separated by centrifugation and subjected for assay immediately or stored at −20 °C.

Biochemical Estimations

Blood were collected from the tail vein of the overnight fasting rat at 0th (before the start of the experiment), 4th day, 7th day, 14th day and 21st day. The glucose levels were estimated by using Accu-Check Active glucometer. Weight of individual animals was measured gravimetrically on 0th and 21st days of the experiment.

After the experimental regimen, the blood were collected through the retro-orbital puncture of eye of animals under mild diethyl ether anaesthesia in Eppendorff's tube (1 ml) Containing 50 µl of anticoagulant (10 % trisodium citrate) and serum was separated by Centrifugation at 3000 rpm for 15 min. The biochemical parameters of liver such as SGPT, SGOT, SALP and Serum bilirubin were determined by using the Commercial kit available [21] (Ecoline, manufactured by Merck specialities, private Limited, Ambernath) and renal parameters such as Protein [22], creatinine [23] and serum urea [24]. Measure the values using Auto Analyzer.

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

Spectral Analysis

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

Biological Activity

Anti-Diabetic Activity by (STZ induced Model)

The synthesised flavones (F1, F2, F3, F5 & F8) were subjected for pharmacological evaluation of anti-diabetic activity by streptozotocin induced rat model. The blood glucose level in rats was showed in the Table.3. The blood glucose level was highly significant ($p < 0.01$) compared to normal rats. After oral administration of synthesised flavones for 21 days were significantly reduced the blood glucose level compared with diabetic control rats. On 14th and 21st day the compounds, such as F1 F2, F3, F5 & F8 were significantly decreases ($p < 0.01$) the blood glucose level compared with diabetic control. It was evident from the table that diabetic control rats had elevated blood glucose level and the synthesised flavones were able to improve the metabolism significantly by comparing with the untreated rats which can be represents in the (Fig 2).

Table 2 Effect of synthesized flavones on blood glucose level on STZ induced diabetic rats.

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±2.60ns	276.58±2.85ns	302.62±3.48*	311.37±2.73*
F3	257.23±3.53ns	276.27±3.48ns	305.46±4.33*	309.18±3.57*
F5	268.39±5.17ns	275.67±4.82*	301.69±4.27*	313.13±3.74ns
F8	253.24±3.46*	199.24±2.31**	171.33±3.45**	127.33±4.72**

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

Effect of Synthesized flavones on blood glucose level on STZ induced diabetic rats.

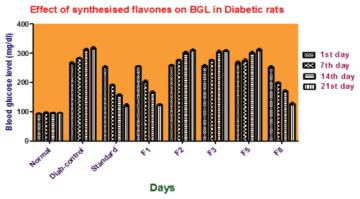

Fig.2 Effect of synthesized flavones on BGL in Diabetic rats

Biochemical Parameters

The liver parameters such as SGOT, SGPT, SALP and total bilurubin 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.3).

Liver biomarker enzymes and total protein on STZ induced diabetic rats.

Table.3. Effect of synthesized flavones in 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±5.34ns	48.66±3.78*	221.33±9.51ns	3.88±0.26ns	4.73±0.15ns
F3	121.56±4.16*	52.23±3.17ns	209.66±8.82*	3.50±0.28ns	4.80±0.31ns
F5	123.34±2.64ns	50.56±4.18ns	216.33±9.38ns	3.68±0.28ns	4.77±0.28ns
F8	56.33±3.87**	31.51±2.58**	125.58±8.12**	2.34±0.14**	6.12±0.04**

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***

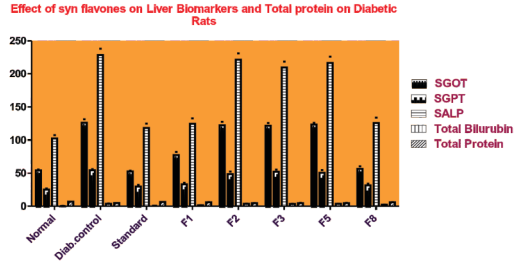

Fig.3. Effect of synthesized flavones on Liver biomarker enzymes and total protein on STZ induced diabetic rats.

Renal parameters

The renal parameters were showed in the (Table 4), which reveals that synthesized flavones restore the renal parameters such as blood urea and serum creatinine on STZ induced diabetic rats. The effects of synthesised flavones on renal parameters were showed on the (Fig.4).

Table.4. Effect of synthesised flavones in renal parameters on STZ induced diabetic rats

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±2.35ns	0.96±0.02ns
F3	28.43±1.86ns	0.92±0.06ns
F5	29.57±1.55ns	0.91±0.04ns
F8	21.78±1.13**	0.74±0.06**

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

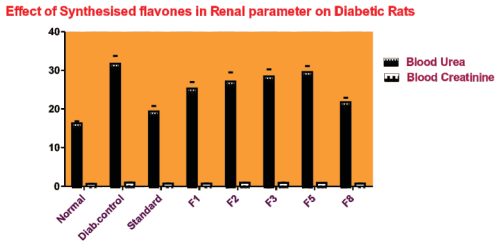

Fig.4. Effect of Synthesised flavones in Renal Parameters on STZ induced diabetic rats.

4. DISCUSSION

All synthesised flavones (F1-F10) were analysed for physicochemical parameters and characterised by various spectroscopic techniques such as UV, IR, GCMS and ^{1}H NMR. Based on that, the structures of synthesized compounds were proved and free from impurities. Further the study was carried out with consolidated synthesised flavones (F1, F2, F3, F5 & F8) were subjected for anti-diabetic activity in rats by inducing streptozotocin. The selection of STZ based on its action by inhibiting the secretion of pancreatic insulin due to damaging the β cells which leads to develop Type 2 diabetes mellitus in rats. Those rats were treated with the related synthesized flavones for 21 days, which significantly act as a hypoglycemic drug. The blood glucose level was observed in normal rats and it is compared with diabetic and treated rats. After oral administration of synthesized flavones for 21 days were significantly reduced the blood glucose level compared with diabetic control rats (Fig. 2). On 14th and 21st day the F1 and F8 were significantly decreases ($p < 0.01$) the blood glucose level compared with diabetic control. This indicates that which improve the metabolism significantly by comparing with the untreated rats. The liver and kidney plays an important role in elimination of metabolite and some toxic moieties. Liver and kidney dysfunction may lead to increase the biochemical substances in the blood stream due to administration of certain drugs. In diabetic condition, the level of transaminase in liver such as SGOT, SGPT and SALP were increases, which are highly active in absence of insulin (Fig.3). This is because of availability of amino acids in diabetic blood, which increases gluconeogenesis and ketogenesis those were observed in diabetes. As per the above phenomena, the diabetic rats had significantly ($p < 0.01$) increased in transaminase and decreased in protein content than normal rats. After treatment with synthesised flavones had moderate significant decreases ($p < 0.01$) in liver enzyme activities and blood urea nitrogen aswell as serum creatinine were significantly increases by compared with diabetic rats (Fig.4). 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.

CONCLUSION

Based on the result, the study should focus on the *in-vivo* anti-diabetic activity on selected synthesised flavones which possess good anti-diabetic activity. The selected synthesised flavones were F1, F2, F3, F5 & F8 were subjected for STZ induced antidiabetic activity. By which, the blood glucose level, liver and renal parameters were observed. The results showed that the compound F1 with basic ring structure of bezopyran ring and F8 with electron donating group (-OCH_3) substitution on benzopyrone ring were reduces the blood glucose level and also restores the liver and renal parameters for the treated rats significantly.

REFERENCES

1. Mealey, B.L.; Ocampo, G.L.. Diabetes mellitus and periodontal disease. Periodontology., 2007, 44(1), 127-153.
2. Helaine, E.; Resnick, M. In This Issue of Diabetes Care. Diabetes Care, 2013, 36(6), 1431-1432.
3. Das, S.; Mitra, I.; Batuta, S.; Alam, M.N.; Roy, K.; Begum, N.A.. Design, synthesis and exploring the quantitative structure–activity relationship of some antioxidant flavonoid analogues. Bioorg. Med. Chem. Lett. 2014, 24(21), 5050-5054.
4. Cushnie, T.P.; Lamb, A.J. Recent advances in understanding the antibacterial properties of flavonoids. Int. J. of Antimicrob. Agents., 2011, 38(2), 99-107.
5. Liu, H.B.; Wang, Z.L.; Qiao, Y.X.; Zhou, J.J. Flavonoids with aldose reductase inhibiting activity: Pharmacophore modelling and implications for mechanism. Act. Phys. Chim. Sini., 2007, 23, 25-35.
6. Gonzalez, R.; Ballester, I.; Efects of flavonoids and other polyphenols on inflammation. Crit. Rev. Food. Sci. Nutr., 2011, 51(4), 331-362.
7. Nishiumi, S.; Miyamoto, S. Dietary flavonoids as cancer preventive and therapeutic biofactors. Front. Biosci. (Schol Ed)., 2011, 3, 1332-1362.
8. Unnikrishnan, P.S.; Suthindhiran, K.; Jayasri, M.A. Alpha-amylase inhibition and antioxidant activity of marine green algae and its possible role in diabetes management. Pharmacogn Mag, 2015, 11, 511-515.
9. Moller, D.E. New drug targets for type 2 diabetes and the metabolic syndrome. Nature, 2001, 414, 821-827.
10. Balaji, R.M.; Jeyaram, C.; Sundaram, K.M.; Ramasamy, M.S. Studies on Antidiabetic Activity of Indian Medicinal Plants Using α-Amylase and α-Glucosidase Inhibitory Activity-A Pathway to Antidiabetic Drugs. World. J. Med. Sci., 2015, 12, 207-212.
11. Mohan, S.; Nandhakumar, L. Role of various flavonoids: Hypotheses on novel approach to treat diabetes. J. Med. Hypo. Id., 2014, 8, 1-6.
12. Garcia, L.A.; Guillamon, E.; Villares, A.; Rostagno, M.A.; Martinez, J.A. Flavonoids as anti-inflammatory agents: implications in cancer and cardiovascular disease. Inflamm Res, 2009, 58(9), 537-552.
13. Liu, S.; Li, D.; Huang , B.; Chen, Y.; Lu, X.; Wang, Y. Inhibition of pancreatic lipase, α-glucosidase, α-amylase, and hypolipidemic effects of the total flavonoids from Nelumbo nucifera leaves. J. Ethnopharmacol., 2013, 149(1), 263-269.
14. Zhang, S.; Kumar, K.; Jiang, X. DOVIS: an implementation for high through put virtual screening using Autodock. BMC Bioinform, 2008, 9, 126-128.
15. Cosconati, S.; Forli, S.; Perryman, AL.; Harris, R.; Goodsell, D.S.; Olson, A.J. Virtual screening with Autodock: Theory and Practice. Expert. Opin. Drug. Discov., 2010, 5(6), 597-607.
16. Eleazu, C.O.; Eleazu, K.C.; Chukwuma, S.; Essien, U.N. Review of the mechanism of cell death resulting from streptozotocin challenge in experimental animals, its practical use and potential risk to humans, J. Diab. Metab. Disord., 2013, 12, 1-7.
17. Wagner, H.; Farkas, L. Synthesis of flavanoids, The flavanoids. Springer US, 1975; 127-213.
18. Core, A.L.; Hossain, S.; Cole, A.M.; Phansteil, O. Synthesis and bioevaluation of substituted chalcones, coumaranones and other flavonoids as anti-HIV agents, Bioorg. Med. Chem., 2016, 24, 2768-2776.
19. Pramod, S.; Dasharath, D.; Ravi, V.; Pudukulathan, K. Cyclization of 2′-hydroxychalcones to flavones using ammonium iodide as an iodine source – an eco-friendly approach, J. Serb. Chem. Soc., 2013, 78 (7), 909 – 916.
20. Ghasemi, A.; Khalifi, S.; Jedi, S. Review - Streptozotocin-nicotinamide-induced rat model of type 2 diabetes, Act. Physio. Hung., 2014, 101(4), 408-420.
21. Dhanabal, S.P.; Koate, C.K.; Ramanathan, M.; Elango, K.; Suresh, B. The hypoglycemic activity of Coccinia indica Wight and its influence on certain biochemical parameters, Indian. J. Pharmacol., 2004, 3(4), 244-250.
22. Lowry, O.H.; Rosebrough, N.J.; Farr, A.L.; Randall, R.J. The original method, J. Biol. Chem., 1951, 193, 265-275.
23. Folin, O.; Wu, H. A system of blood analysis, J. Bio. Chem., 1919, 38, 81 -110.
24. Talke, H.; Schubert, G.E. Enzymatic Determination of Urea Using the Coupled Urease-GLDH Enzyme System. Mediat. Inflamm., 1965, 43, 174-176.