Antidiabetic Potential of Flavones on Streptozotocin-induced Diabetes Mellitus in Rat

Binish Inam¹, Adil Inam¹, Monika Verma², Kamlesh Kumar Naik³, Afroze Alam⁴,⁵*
¹Department of Community Medicine, Narayan Medical College and Hospital, Jamuhar, Sasaram-Rohtas, Bihar, INDIA.
²Department of Paediatrics, Advanced Paediatrics Centre - PGIMER, Chandigarh, Punjab, INDIA.
³Department of Pharmaceutical Chemistry, Nandha College of Pharmacy, Perundurai, Main Road, Erode, Tamil Nadu, INDIA.
⁴Narayan Institute of Pharmacy, Gopal Narayan Singh University, Jamuhar, Rohtas (Sasaram), Bihar, INDIA.
⁵School of Pharmacy, Al-Karim University, Kathihar-Purnia Road, Sirsa, Karim Bagh, Kathihar, Bihar, INDIA.

ABSTRACT

Objectives: The objective of the study was to develop new antidiabetic agents from synthetic route. Methods: 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 in vivo antidiabetic activity study was carried out by streptozotocin induced model. Biochemical parameters were extensively studied to support anti-diabetic potential of synthesized flavones. Results: The study reveals that flavones such as F1, F2, F3, F5 and F8 were potentially considered for in-vivo anti-diabetic activity. Fasting blood glucose and biochemical parameters like total protein, urea and 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 considering different biochemical parameters. Some of the selected flavones showed excellent and noticeable antidiabetic activity.

Key words: Antidiabetic activity, Creatinine, Flavones, Total Protein, SGOT, SALP, SGPT, Streptozotocin.

Correspondence

Dr. Afroze Alam, Narayan Institute of Pharmacy, Jamuhar-821305, (Rohtas) Sasaram, Bihar, INDIA.
Phone no: +91-7018196843
Email: afrozepharma@gmail.com
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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 and it clearly assessed that 1.5–4.9 million people were death from 2012 to 2014. According to the current scenario, the development of hypoglycaemic drugs in the management of diabetes mellitus, as well as in the prevention of diabetic complication should be a challenging one in clinical importance.

The naturally available flavonoid plays vital role in treating so many major diseases, in that one of the chronic disease is diabetes mellitus. 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.²⁻⁹

There are many herbal extracts having reported anti-diabetic potentials.¹⁰ Among these phytochemicals, flavonoids and its 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.¹²⁻¹⁰ 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 antidiabetic agents. Based on the result flavones were evaluated for in vivo antidiabetic activity by inducing streptozotocin in Wister rats. Streptozotocin is selectively used as a toxicant compared with alloxan to produce diabetes by destruction of β cells on islet of Langerhans.¹⁴ By which blood glucose level, liver and renal parameters 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.

MATERIALS 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.

Scheme of Synthesis

The scheme of this synthesis is based on Algar-Flynn-Oyamada method for the synthesis of flavones (F1-F10).¹⁵⁻¹⁷

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Inam, et al.: Anti-diabetic Potential of Flavone Analogues

Blood were collected from the tail vein of the overnight fasting rat at 0\textsuperscript{th} (before the start of the experiment), 4\textsuperscript{th} day, 7\textsuperscript{th} day, 14\textsuperscript{th} day and 21\textsuperscript{st} day. The glucose levels were estimated by using Accu-Check Active glucometer. Weight of individual animals was measured gravimetrically on 0\textsuperscript{th} and 21\textsuperscript{st} 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 Eppendorf's tube (1 ml). Containing 50 µl of anticoagulant (10 % trisodium citrate) and serum were 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\textsuperscript{19} (Ecoline, manufactured by Merck specialties, private Limited, Ambarnath) and renal parameters such as Protein,\textsuperscript{20} creatinine\textsuperscript{21} and serum urea,\textsuperscript{22} 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, ¹H-NMR and mass spectrometry.

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

MP: 130-132°C; R\textsubscript{f} = 0.56; % yield = 65.3 % w/w; UV \(\lambda_{max}\): CHCl\textsubscript{3}, nm: 297; IR (KBr cm\textsuperscript{-1}): 1739 (lactone), 1643 (CO str), 1585, 1550 (C=C Arom. str), 1134, 1093 (COC str), 771 (C-C bending); ¹H NMR (500 MHZ, DMSO): δ 7.4 - 7.9 (m, 8H, ArH), 7.6 - 7.8 (m, 8H, ArH); m/z: 222(m+1), 120.7(C\textsubscript{6}H\textsubscript{4}O\textsubscript{2}), 105.8 (C\textsubscript{6}H\textsubscript{4}O), 92.8 (C\textsubscript{6}H\textsubscript{4}), 77.9 (C\textsubscript{6}H\textsubscript{4}).

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

MP: 157-160°C; R\textsubscript{f} = 0.3; % yield = 42.4 % w/w; UV \(\lambda_{max}\): CHCl\textsubscript{3}, nm: 241; IR (KBr cm\textsuperscript{-1}): 1797 (lactone), 1687 (CO str), 1593, 1564 (C=C Arom. str), 1124, 1103, 1037 (COC str), 754 (C=C bending); ¹H NMR (500 MHZ, DMSO): δ 7.4 - 7.9 (m, 8H, ArH), 7.4 - 7.8 (m, 8H, ArH); m/z: 256 (m+1), 138.9 (C\textsubscript{6}H\textsubscript{4}Cl), 120.9 (C\textsubscript{6}H\textsubscript{4}O), 91.9 (C\textsubscript{6}H\textsubscript{4}O), 77 (C\textsubscript{6}H\textsubscript{4}).

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

MP: 167-170°C; R\textsubscript{f} = 0.84; % yield = 40.4 % w/w; UV \(\lambda_{max}\): CHCl\textsubscript{3}, nm: 261; IR (KBr cm\textsuperscript{-1}): 1735 (lactone), 1685 (CO str), 1593, 1573 (C=C Arom. str), 1130, 1091 (COC str), 761 (C=C bending); ¹H NMR (500 MHZ, DMSO): δ 7.4 - 7.9 (m, 8H, ArH), 6.7 (m, 1H, ArH); m/z: 256 (m+1), 121.6(C\textsubscript{6}H\textsubscript{4}O\textsubscript{2}), 139.5 (C\textsubscript{6}H\textsubscript{4}Cl), 76.6 (C\textsubscript{6}H\textsubscript{4}).

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

MP: 241-243°C; R\textsubscript{f} = 0.85; % yield = 59.3 % w/w; UV \(\lambda_{max}\): CHCl\textsubscript{3}, nm: 250; IR (KBr cm\textsuperscript{-1}): 1772 (lactone), 1685 (CO str), 1577, 1514 (C=C Arom. str), 1126, 1107, 1024 (COC str), 773 (C=C bending); ¹H NMR (500 MHZ, DMSO): δ 6.9 - 7.6 (m, 8H, ArH), 7.6 - 8.6 (m, 8H, ArH); m/z: 240 (m+1), 122 (C\textsubscript{6}H\textsubscript{4}O\textsubscript{2}), 119.5 (C\textsubscript{6}F\textsubscript{3}H), 75.5 (C\textsubscript{6}H\textsubscript{4}).

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

MP: 145-148°C; R\textsubscript{f} = 0.43; % yield = 51.6 % w/w; UV \(\lambda_{max}\): CHCl\textsubscript{3}, nm: 306; IR (KBr cm\textsuperscript{-1}): 1797 (lactone), 1681 (CO str), 1593, 1573 (C=C Arom. str), 1128, 1091 (COC str), 761 (C=C bending); ¹H NMR (500 MHZ, DMSO): δ 7.4 - 7.6 (m, 8H, ArH), 6.2 (m, 1H, ArH); m/z: 267 (m+1), 121.1(C\textsubscript{6}H\textsubscript{4}O\textsubscript{2}), 148.9 (C\textsubscript{6}F\textsubscript{4}NO\textsubscript{2}), 76.9 (C\textsubscript{6}H\textsubscript{4}).

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

MP: 185-188°C; R\textsubscript{f} = 0.58; % yield = 47.2 % w/w; UV \(\lambda_{max}\): CHCl\textsubscript{3}, nm: 248; IR (KBr cm\textsuperscript{-1}): 1734 (lactone), 1683 (CO str), 1558, 1541 (C=C Arom. str), 1139, 1093 (COC str), 765 (C=C bending) 3566, 3547 (OH str); ¹H NMR(500 MHZ, DMSO): δ 6.5 (m, 1H, ArH), 7.1 - 7.9 (m, 8H, ArH); m/z: 238 (m+1), 121(C\textsubscript{6}H\textsubscript{4}O\textsubscript{2}), 106 (C\textsubscript{6}H\textsubscript{4}O), 104 (C\textsubscript{6}H\textsubscript{4}) - 78 (C\textsubscript{6}H\textsubscript{4}).

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

MP: 181-183°C; R\textsubscript{f} = 0.41; % yield = 47.5 % w/w; UV \(\lambda_{max}\): CHCl\textsubscript{3}, nm: 287; IR (KBr cm\textsuperscript{-1}): 1772 (lactone), 1691 (CO str), 1560, 1543, 1516 (C=C Arom. str), 1047, 1139 (COC str), 748 (C=C bending) 3300, 3545 (OH str); ¹H NMR(500 MHZ, DMSO): δ 6.8 (m, 1H, ArH), 7.7 - 7.4 (m, 8H, ArH); m/z: 238 (m+1), 121(C\textsubscript{6}H\textsubscript{4}O\textsubscript{2}), 118 (C\textsubscript{6}H\textsubscript{4}O), 92 (C\textsubscript{6}H\textsubscript{4}O), 76.9 (C\textsubscript{6}H\textsubscript{4}).
Table 1: Effect of synthesized flavones on blood glucose level on STZ induced diabetic rats.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>1st day</th>
<th>7th day</th>
<th>14th day</th>
<th>21st day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>94.2±2.03</td>
<td>97.2±2.65</td>
<td>96.2±2.65</td>
<td>96.13±1.16</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>267.43±3.53</td>
<td>283.35±2.40</td>
<td>313.22±2.91</td>
<td>318.46±4.33</td>
</tr>
<tr>
<td>Standard</td>
<td>253.66±3.18</td>
<td>191.66±2.03</td>
<td>158.34±3.46</td>
<td>122.66±4.98</td>
</tr>
<tr>
<td>F1</td>
<td>255.66±2.33</td>
<td>203.67±4.96</td>
<td>167.33±4.09</td>
<td>124.33±2.90</td>
</tr>
<tr>
<td>F2</td>
<td>259.33±2.60</td>
<td>276.58±2.85</td>
<td>302.62±3.48</td>
<td>311.37±2.73</td>
</tr>
<tr>
<td>F3</td>
<td>257.23±3.53</td>
<td>276.27±3.48</td>
<td>305.46±3.33</td>
<td>309.18±3.57</td>
</tr>
<tr>
<td>F5</td>
<td>268.39±5.17</td>
<td>275.67±4.82</td>
<td>301.69±4.27</td>
<td>313.13±3.74</td>
</tr>
<tr>
<td>F8</td>
<td>253.24±3.46</td>
<td>199.24±2.31</td>
<td>171.33±3.45</td>
<td>127.33±4.72</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n=4); *P < 0.05 as compared with normal control; **P < 0.01 as compared with diabetic control.

Table 2: Effect of synthesized flavones in Liver biomarker enzymes and total protein on STZ induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGOT (IU/l)</th>
<th>SGPT (IU/l)</th>
<th>SALP (IU/l)</th>
<th>Total bilirubin (mg/dl)</th>
<th>Total Protein (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>54.33±1.20</td>
<td>25.20±1.73</td>
<td>102.33±5.18</td>
<td>0.49±0.02</td>
<td>6.93±0.20</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>126.15±5.16</td>
<td>54.38±1.53</td>
<td>228.31±9.46</td>
<td>3.94±0.23</td>
<td>4.87±0.07</td>
</tr>
<tr>
<td>Standard</td>
<td>52.54±1.21</td>
<td>29.81±2.43</td>
<td>118.13±6.52</td>
<td>0.94±0.02</td>
<td>6.51±0.03</td>
</tr>
<tr>
<td>F1</td>
<td>77.36±4.74</td>
<td>33.25±2.08</td>
<td>124.52±8.13</td>
<td>1.43±0.08</td>
<td>6.12±0.21</td>
</tr>
<tr>
<td>F2</td>
<td>122.14±5.34</td>
<td>48.66±3.78</td>
<td>221.33±9.51</td>
<td>3.88±0.26</td>
<td>4.73±0.15</td>
</tr>
<tr>
<td>F3</td>
<td>121.56±4.16</td>
<td>52.23±3.17</td>
<td>209.66±8.82</td>
<td>3.50±0.28</td>
<td>4.80±0.31</td>
</tr>
<tr>
<td>F5</td>
<td>123.34±2.64</td>
<td>50.56±4.18</td>
<td>216.33±9.38</td>
<td>3.68±0.28</td>
<td>4.77±0.28</td>
</tr>
<tr>
<td>F8</td>
<td>56.33±3.87</td>
<td>31.51±2.58</td>
<td>125.58±8.12</td>
<td>2.34±0.14</td>
<td>6.12±0.04</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n=4); *P < 0.05 as compared with normal control; **P < 0.01 as compared with diabetic control.

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

MP: 175-177°C; Rf = 0.43; % yield = 49.2 % w/w; UV λmax (CHCl3): 265; IR (KBr cm−1): 1658 (CO str), 1597, 1550 (C=C Arom.str), 1126, 1064 (COC str), 727 (C-C bending); 1H NMR (500 MHZ, DMSO): δ 3.8 (s, OCH3, ArH), 6.4 (m, 1H, ArH), 7.0, 7.8 (m, 8H, ArH); m/z: 252 (m+1), 134.9(C6H3O), 107 (C6H5O)2, 126.15±5.16; 152 (C6H3O), 77.0 (C6H3).

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

MP: 178-180°C; Rf = 0.6; % yield = 46.4 % w/w; UV λmax (CHCl3): 250; IR (KBr cm−1): 1660 (CO str), 1597, 1550 (C=C Arom.str), 1124, 1066 (COC str), 752 (C-C bending); 1H NMR(500 MHZ, DMSO): δ 3.8 (s, OCH3, ArH), 6.9 (m, 1H, ArH), 7.0 - 7.7 (m, 7H, ArH); m/z: 283 (m+1), 121(C6H3O), 92.7 (C6H3O)2, 164 (C10H11O)2, 137.5 (C8H10O)2, 87.7 (C6H3).

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

MP: 169-171°C; Rf = 0.71; % yield = 48.4 % w/w; UV λmax (CHCl3): 295; IR (KBr cm−1): 1795 (lactone), 1658 (CO str), 1548, 1537 (C=C Arom.str), 1124, 1064 (COC str), 727 (C-C bending); 1H NMR(500 MHZ, DMSO): δ 2.4 (s, 6H, N(CH3)2), 6.3 - 6.5 (m, 1H, ArH), 7.5 - 7.9 (m, 8H, ArH); m/z: 265 (m+1), 222(C15H10O)2, 121 (C6H3O)2, 104.9 (C6H3), 77 (C6H3).

Table 3: Effect of synthesized flavones in renal parameters on STZ induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood urea (mg/dl)</th>
<th>Serum Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>16.32±0.53</td>
<td>0.68±0.03</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>31.72±2.01**</td>
<td>0.98±0.08**</td>
</tr>
<tr>
<td>Standard</td>
<td>19.36±1.45**</td>
<td>0.72±0.05**</td>
</tr>
<tr>
<td>F1</td>
<td>25.33±1.67*</td>
<td>0.74±0.05**</td>
</tr>
<tr>
<td>F2</td>
<td>27.14±2.35</td>
<td>0.96±0.02</td>
</tr>
<tr>
<td>F3</td>
<td>28.43±1.86</td>
<td>0.92±0.06</td>
</tr>
<tr>
<td>F5</td>
<td>29.57±1.55</td>
<td>0.91±0.04</td>
</tr>
<tr>
<td>F8</td>
<td>21.78±1.13**</td>
<td>0.74±0.06**</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n = 4); *P < 0.01 as compared with normal control; **P < 0.05 as compared with diabetic control.

Biological Activity

Antidiabetic Activity

The synthesised flavones (F1, F2, F3, F5 and 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 1. 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 dia-

Diabetes Mellitus is a metabolic disorder and one of the most common chronic diseases. As per WHO report, it is the 5th leading cause of death after twenty years. It is also have the chance of developing associated diseases such as hypertension, hyperlipidemia and obesity which may leads to metabolic complications of both clinical and experimental diabetes. The present study was carried out with novel synthesis of flavones and evaluated for antidiabetic 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 synthesized flavones for 21 days, which significantly act as a hypoglycemic drug.

Diabetes mellitus is a multifactorial disease, there is a risk in development of acute metabolic complication due to hyperglycemia results in ketoacidosis, hypoglycemia, hyperglycemic hyperosmolar nonketotic coma. Along with this, the development of chronic complications may occur results in hypertension, cardiac disorders, retinopathy, neuropathy, nephropathy and ulceration of foot. These metabolic fluctuations may also existed in the animals such as elevation of blood glucose level, total bilirubin and liver enzymes (SGOT, SGPT and SALP). In renal parameter, the total protein decreases which exist in elevation of blood urea level.

The study was carried out with consolidated synthesised flavones (F1, F2, F3, F5 and 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. On 14th and 21st day the F1 and F8 were significantly decreases (p<0.01) in liver enzyme activities and blood urea nitrogen as compared with diabetic control rats. On 14th and 21st day the compounds, such as F1 F2, F3, F5 and 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 see Figure 1, 2.

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 2. The renal parameters were showed in the Table 3, which reveals that synthesized flavones restore the renal parameters such as blood urea and serum creatinine on STZ induced diabetic rats Table 3.

**DISCUSSION**

The focussed flavones were synthesized according to the protocol reported in the general scheme of synthesis. The percentage yields of the synthesized flavones were obtained moderately and melting point of those compounds were also recorded and presented incorrectly. The purity of the each synthesized compounds were determined by thin layer chromatography using silica gel G plate as an stationary phase and hexane, ethyl acetate as mobile phase. The single spot was obtained, which indicate the pure state of the compound. The physico chemical parameters of all synthesized compounds were presented in the Table 1. Further, the focussed compounds were characterized by UV, IR, GCMS and 1H NMR spectroscopy. Based on the results of above, the structure of synthesized compounds were proved and free from impurities.

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 as...
well as serum creatinine were significantly increases by compared with diabetic rats (Figure 3). The synthesized flavones F1 (benzo pyrone ring) and F8 (-OCH3, 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 antidiabetic activity on selected synthesised flavones which possess good anti-diabetic activity. The selected synthesised flavones were F1, F2, F3, F5 and 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 (-OCH3) substitution on benzopyrone ring were reduces the blood glucose level and also restores the liver and renal parameters for the treated rats significantly. These results suggested this study have not fulfil in establishing the precise mechanism and action of this molecule, hence this study should be proceed through molecular pharmacology and toxicological studies along with screening of therapeutic application of this drug. By which we can develop the potent drug in the management of diabetes mellitus.

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CONFLICTS OF INTEREST

There authors declare no conflict of interest.

REFERENCES