EVALUATION OF ANTIDIABETIC EFFECT OF CITRULLUS LANATUS (THUNB) SEEDS AND SARACA ASOCA (ROXB.) DE.WILD. FLOWERS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Objective: To evaluate the in-vivo antidiabetic potential of the methanolic extracts of Citrullus lanatus seeds (CLS) and Saraca asoca flowers (SAF) in the streptozotocin (STZ) -induced rat model.

Methods: Acute oral toxicity study was performed according to the OECD test guidelines and antidiabetic activity was carried out using STZ-induced diabetes in the male Sprague Dawley rats. The rats were treated with methanolic extracts of CLS and SAF at the dose of 100, 200 and 400 mg/kg of b.wt. administered orally for 28 days. Metformin was used as a standard drug at a dose of 100 mg/kg, b.wt. The antioxidant and histopathological studies were carried out using standard protocols. Results: The extracts did not show any sign of toxicity at a dose of 2000 mg/kg body weight. Oral administration of both the extracts at a dosage of 400 mg/kg body weight for 28 days resulted in a significant reduction in blood glucose, lipid profile (cholesterol, triglyceride), hepatic and renal markers (SGOT, SGPT, ALP, creatinine, urea and protein) when compared with the diabetic control. In in-vivo antioxidant enzymes study on both the extracts showed a significant (p<0.05) decrease in LPO level and increase in SOD and CAT levels as compared to the diabetic control. Histopathological studies of the liver, kidney and pancreas revealed normal histological pattern in the normal and treated groups. Conclusion: The experimental data revealed that both the extracts, CLS and SAF have significant antihyperglycemic and antioxidant activity in streptozotocin-induced rats compared to the standard drug.

KEYWORDS: Acute oral toxicity, Antidiabetic, Citrullus lanatus seeds, Saraca asoca flowers, Histopathology, pancreas.
INTRODUCTION
Diabetes mellitus is the condition arising due to metabolic disorder characterized by a high blood glucose concentration (hyperglycemia) caused by insulin deficiency, often combined with insulin resistance. It is caused by altering the metabolism of carbohydrates, proteins and fat; and an increased risk of complications from vascular disease.\(^1\) According to an estimation of the International Diabetes Federation, diabetes has now reached epidemic proportion with a current global prevalence of about 382 million, expected to rise to 592 million by the year 2035.\(^2\) The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. It may present classical characteristic features such as blurring of vision, excessive thirst, excessive feeding, excessive urination, and weight loss. In its most severe forms, ketoacidosis may develop leading to stupor, coma and in the absence of effective treatment, death ensues.\(^3\) Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations. The drugs include taking the medicines throughout life time, high cost therapy, side effects such as hypoglycemia, weight gain, gastrointestinal disturbances, liver toxicity etc. Herbal drugs with antidiabetic activity are yet to be commercially formulated as modern medicines, even though they have been acclaimed for their therapeutic properties in the traditional systems of medicine.\(^4,5\) Traditional medicine is used for treatment of diabetes in developing countries where the cost of conventional medicines is a burden to the population.\(^6\) Many indigenous Indian medicinal plants have been found to be useful to successfully manage diabetes. One of the greatest advantages of medicinal plants is that these are readily available and have very low side effects.

*Citrullus lanatus* (family – Cucurbitaceae) is commonly called as the watermelon and locally as “Tharpooshini”. The seeds are reported to contain phenols, flavones, tannins, saponins and alkaloids.\(^7\) The Ayurvedic pharmacopoeia of India indicate the seeds to possess a high lipase activity, in addition to high lipoxygenase, urease and trypsin inhibitory activities. The ripe fruits are edible and largely used for making confectionery. Its nutritive values are also useful to human health. The seed is demulcent, diuretic, pectoral and tonic. It is sometimes used in the treatment of the urinary passages bed wetting. The seed is also a good vermifuge and has a hypotensive action. Fatty oil in the seed, as well as aqueous or alcoholic extracts, paralyzes tapeworms and roundworms.\(^8\) Methanol extract of the seed has shown to exhibit α-glucosidase inhibitory activity\(^9\) and antimicrobial activity.\(^10\)
Saraca asoca (Roxb.) de Wilde is one of the most ancient medicinal plants known in India. It is commonly known as Asoka. It belongs to the family Caesalpiniaceae. All the plant parts are considered to contain medicinal properties. Flowers pounded in water are used in haemorrhagic dysentery, diabetes, uterine infections, biliousness and syphilis. Dried flower buds are reported to have antibacterial activity.\textsuperscript{[11]} Aqueous suspension of Saraca indica flower has antiulcer activity in albino rats.\textsuperscript{[12]} Saraca asoca bark and flowers exhibit antitumour activity\textsuperscript{[13]}, Larvicidal activity has also been reported.\textsuperscript{[14]}

Hence, the present study was undertaken to investigate the anti-diabetic potential of methanol extracts of Citrullus lanatus seeds (CLS) and Saraca asoca flowers (SAF) in streptozotocin induced diabetic rats so as to provide a scientific proof for the traditionally acclaimed activity.

**MATERIALS AND METHODS**

*Plant collection and authentication*

Citrullus lanatus seeds were collected from Vellore district, Tamilnadu, India. Saraca asoca flowers were collected from Alappuzha district, Kerala, India. Both the plants were identified and authenticated by Prof. Dr. Jayaraman, Director, Plant Anatomy Research Center (PARC), Chennai and a voucher specimen was deposited at the herbarium of PARC for future reference (No: PARC/2012/1195, PARC/2014/2275).

*Chemicals*

Streptozotocin was purchased from Sigma chemicals, Bangalore, India. The biochemical parameters was carried out using kits to estimate glucose, triglyceride (TG), total cholesterol (TC), serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), creatinine, urea and protein were purchased from Accurex Biomedical Pvt.Ltd., Mumbai. All other chemicals used were of analytical grade.

*Extraction*

The collected seeds and flowers were shade-dried at room temperature and coarsely-powdered and subjected to soxhlet extraction with methanol for 24 hours. The extracts were filtered and solvent was completely removed by using rotary evaporator. The concentrated extracts were stored at 4°C in glass container for future use.
Experimental animals
Male Sprague Dawley rats (160-200 g) were used for the study. Animals were divided into 9 groups (6 animals/cage) and housed in polypropylene cages in a well-ventilated room (air cycles: 15/min; recycle ratio: 70:30) under an ambient temperature of 22 ± 3°C and 40 – 65% relative humidity, with a 12-h light/dark artificial light cycle. They were provided with rodent feed (M/s. Provimi Animal Nutrition India Pvt. Ltd, India) and purified water ad libitum.

Ethical considerations
The Institutional Animal Ethics Committee (IAEC), Sri Ramachandra University, Chennai, India (Approval no: IAEC/XLIII/SRU/425/2015) approved the study.

Acute oral toxicity study
Acute oral toxicity study was performed according to the OECD test guideline 423- Acute toxic class method.[15] 12 young healthy adult Sprague Dawley female rats (130-180g, b. wt.) were divided into 4 groups of 3 animals each. The CLS and SAF extracts were administered once orally with the maximum dose of 2000 mg/kg b. wt. Body weight was recorded before dosing and thereafter once a week till completion of the experiment. Lethality and abnormal clinical signs were observed on the day of dosing and thereafter for 13 days. Gross pathological changes were also observed at the end of experiment.

Induction of diabetes mellitus
Experimental diabetes was induced in rats by single intraperitoneal injection of STZ dissolved in citrate buffer (pH 4.5) at a dose of 40 mg/kg, b. wt. The development of diabetes was confirmed after 72 h of the streptozotocin injection. The animals having fasting blood glucose levels greater than 250 mg/dl were considered as diabetic rats and selected for the experiment.

Experimental design
For antidiabetic study, 54 rats were randomly divided into 9 groups of 6 animals each. The different doses of both the extracts were administered orally to the STZ induced diabetic rats. The extracts were suspended in 0.5% CMC suspension and administered orally for 28 days.

Group 1 Normal control group
Group 2 STZ induced group
Group 3 Diabetic + Metformin (100 mg/kg b.wt.)
Group 4 Diabetic + CLS extract (100 mg/kg b.wt.)
Group 5  Diabetic +CLS extract (200 mg/kg b.wt.)
Group 6  Diabetic + CLS extract (400 mg/kg b.wt.)
Group 7  Diabetic + SAF extract (100 mg/kg b.wt.)
Group 8  Diabetic + SAF extract (200 mg/kg b.wt.)
Group 9  Diabetic + SAF extract (400 mg/kg b.wt.)

**Biochemical analysis**

Blood samples were withdrawn by retro-orbital puncture under mild ether anesthesia on the 0th, 7th, 14th, 21st and 28th day of the study for estimating blood glucose, total cholesterol and total triglyceride. At the end of 28 days treatment, all the animals were euthanized and the levels of SGOT, SGPT, ALP, creatinine, urea and protein were determined using commercial kits following the manufacturer standard protocols in a semi-auto analyzer.

**In vivo antioxidant studies**

Liver tissues were collected from each animal to determine the antioxidant activity. The levels of Lipid peroxidation (LPO), Superoxide dismutase (SOD) and Catalase were estimated using standard protocols.\[16,18\]

**Histopathological examination**

At the end of the study, all the rats were euthanized, organs like pancreas, liver and kidney were collected from each animal and washed in normal saline and fixed in 10% formalin, embedded in paraffin and sections of 3-5μm thickness were cut and routinely stained with basic dye haematoxylin and acidic dye eosin for histopathology estimation. The sections were examined using a light microscopy (Motic DMB1-2MP, China).

**Statistical analysis**

The results were expressed as Mean ± SEM of six replicates. Statistical significance between the groups were evaluated by one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison tests using Graph Pad Prism 5.03 (Graph Pad Software, San Diego, CA, USA). p< 0.05, p<0.01 and p<0.001 value was considered as statistically significant.

**RESULTS**

**Acute oral toxicity**

No treatment related deaths, abnormal clinical signs or remarkable body weight changes were observed in all the experimental animals. No gross pathological observation was recorded in
major organs of all the experimental animals. LD$_{50}$ of both the test drugs were found to be greater than 2000 mg/kg b. wt, and was found to be safe when administered once orally to fasted female Sprague Dawley rats.

**Antidiabetic study**

The baseline body weight of all the experimental group of rats initially was similar. At the end of the experimental period, the body weight of diabetic control rats were significantly (p<0.001) reduced in contrast to the control group of rats (Table 1). While, oral administration of CLS and SAF extracts (100, 200 and 400 mg/kg b. wt.) to the diabetic rats significantly increased the body weight during the course of the experiment. Metformin exhibited increase in the body weight after the treatment. Among the doses of the extracts used, 400 mg/kg of the CLS extract showed greater increase in the body weight of the animal.

**Table 1: Effect of CLS and SAF extract on the body weight of STZ-induced diabetic rats for 28 days**

<table>
<thead>
<tr>
<th>Group</th>
<th>0 Day</th>
<th>7 day</th>
<th>14 Day</th>
<th>21 Day</th>
<th>28 Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>170.0±8.00</td>
<td>179.0±10.69</td>
<td>185.3±4.79</td>
<td>190±3.99</td>
<td>198.3±5.75</td>
</tr>
<tr>
<td>STZ</td>
<td>164.1±8.48</td>
<td>148.8±8.73</td>
<td>136.2±7.40</td>
<td>126.3±7.25</td>
<td>114±6.66</td>
</tr>
<tr>
<td>STZ+MET</td>
<td>162.8±3.91</td>
<td>173.4±2.96</td>
<td>177.8±3.11</td>
<td>180.8±2.98</td>
<td>188.4±2.61</td>
</tr>
<tr>
<td>STZ+CLS 100mg</td>
<td>163.4±1.18</td>
<td>168.5±0.74</td>
<td>171.3±1.13</td>
<td>175.3±6.01</td>
<td>179.1±5.74</td>
</tr>
<tr>
<td>STZ+CLS 200mg</td>
<td>161.7±4.68</td>
<td>169.5±5.92</td>
<td>171.6±5.66</td>
<td>172±5.56</td>
<td>177.6±5.27</td>
</tr>
<tr>
<td>STZ+CLS 400mg</td>
<td>162.9±5.56</td>
<td>169.5±9.95</td>
<td>171.7±5.73</td>
<td>174±5.77</td>
<td>181.1±6.12</td>
</tr>
<tr>
<td>STZ+SAF 100mg</td>
<td>164.9±3.66</td>
<td>169.3±9.28</td>
<td>172±4.75</td>
<td>175.8±5.17</td>
<td>179.1±4.32</td>
</tr>
<tr>
<td>STZ+SAF 200mg</td>
<td>162.2±5.37</td>
<td>170.3±4.53</td>
<td>174.8±5.69</td>
<td>176.9±5.33</td>
<td>181.1±4.70</td>
</tr>
<tr>
<td>STZ+SAF 400mg</td>
<td>162.6±4.26</td>
<td>171.3±8.4</td>
<td>175.2±4.35</td>
<td>178.7±5.15</td>
<td>183.3±7.15</td>
</tr>
</tbody>
</table>

All values are Mean ±SEM for six rats. #, ##, ### indicates p<0.05, p<0.01, p<0.001 respectively vs. normal control; *, **, *** indicates p<0.05, p<0.01, p<0.001 respectively vs. diabetic control.

**Table 2: Effect of CLS and SAF extract on blood glucose levels in STZ-induced diabetic rats for 28 days**

<table>
<thead>
<tr>
<th>Group</th>
<th>0 Day</th>
<th>7 Day</th>
<th>14 Day</th>
<th>21 Day</th>
<th>28 Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>81.79±4.53</td>
<td>83.72±2.06</td>
<td>82.58±4.85</td>
<td>83.26±3.69</td>
<td>82.92±4.68</td>
</tr>
<tr>
<td>STZ</td>
<td>262.2±8.25</td>
<td>264.4±1.22</td>
<td>265.8±4.43</td>
<td>267.3±1.17</td>
<td>269.5±6.06</td>
</tr>
<tr>
<td>STZ+MET</td>
<td>259.1±3.31</td>
<td>211.7±7.42</td>
<td>171.5±1.71</td>
<td>140.9±4.68</td>
<td>96.85±2.28</td>
</tr>
<tr>
<td>STZ+CLS 100mg</td>
<td>260.3±2.87</td>
<td>241.1±6.72</td>
<td>221.1±3.94</td>
<td>203.7±2.22</td>
<td>188.5±5.35</td>
</tr>
<tr>
<td>STZ+CLS 200mg</td>
<td>262±4.05</td>
<td>235.9±3.52</td>
<td>208.9±7.18</td>
<td>189.9±1.89</td>
<td>162.7±3.67</td>
</tr>
<tr>
<td>STZ+CLS 400mg</td>
<td>261.5±8.1</td>
<td>223.8±3.85</td>
<td>186.3±1.84</td>
<td>153.9±7.57</td>
<td>123.9±2.89</td>
</tr>
<tr>
<td>STZ+SAF 100mg</td>
<td>269±2.37</td>
<td>243±4.21</td>
<td>226.6±2.55</td>
<td>210.3±1.77</td>
<td>190±2.30</td>
</tr>
<tr>
<td>STZ+SAF 200mg</td>
<td>262.1±6.66</td>
<td>240±7.43</td>
<td>218.2±1.79</td>
<td>198.8±2.46</td>
<td>171.3±0.49</td>
</tr>
<tr>
<td>STZ+SAF 400mg</td>
<td>260.5±5.42</td>
<td>228±4.59</td>
<td>191.3±3.10</td>
<td>161.6±2.16</td>
<td>130.3±1.32</td>
</tr>
</tbody>
</table>
All values are Mean ±SEM for six rats. #, ##, ### indicates p<0.05, p<0.01, p<0.001 respectively vs. normal control; *, **, *** indicates p<0.05, p<0.01, p<0.001 respectively vs. diabetic control.

**Table 3: Effect of CLS and SAF extract on Lipid profile in STZ-induced diabetic rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>58.88±11.87</td>
<td>68.37±7.2</td>
</tr>
<tr>
<td>STZ</td>
<td>121.70±8.70###</td>
<td>130.63±5.20###</td>
</tr>
<tr>
<td>STZ+MET 100mg</td>
<td>64.87±8.33***</td>
<td>73.46±2.33***</td>
</tr>
<tr>
<td>STZ+CLS 100mg</td>
<td>102.18±4.72</td>
<td>119.12±1.4</td>
</tr>
<tr>
<td>STZ+CLS 200mg</td>
<td>94.47±10.72</td>
<td>98.26±5.15***</td>
</tr>
<tr>
<td>STZ+CLS 400mg</td>
<td>80.16±5.23**</td>
<td>81.65±2.44***</td>
</tr>
<tr>
<td>STZ+SAF 100mg</td>
<td>101.95±16.0</td>
<td>120.4±4.6</td>
</tr>
<tr>
<td>STZ+SAF 200mg</td>
<td>99.09±5.51</td>
<td>96.82±9.1***</td>
</tr>
<tr>
<td>STZ+SAF 400mg</td>
<td>83.92±3.07*</td>
<td>83.14±7.57***</td>
</tr>
</tbody>
</table>

**Table 4: Effect of CLS and SAF extract on hepatic and renal markers in STZ-induced diabetic rats on 28th day**

<table>
<thead>
<tr>
<th>Group</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>Creatinine (mg/dl)</th>
<th>Urea (mg/dl)</th>
<th>Protein (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>25.14±2.49</td>
<td>21.38±2.1</td>
<td>93.13±8.86</td>
<td>1.52±0.09</td>
<td>30.11±2.9</td>
<td>8.32±0.79</td>
</tr>
<tr>
<td>STZ</td>
<td>145.43±13.86##</td>
<td>115.4±11.26##</td>
<td>145.23±13.38</td>
<td>2.85±0.18###</td>
<td>60.17±3.84###</td>
<td>4.25±0.38##</td>
</tr>
<tr>
<td>STZ+MET 100mg</td>
<td>36.24±2.94##</td>
<td>32.45±3.15##</td>
<td>92.36±8.93</td>
<td>1.35±0.09###</td>
<td>28.4±2.41***</td>
<td>8.32±0.8***</td>
</tr>
<tr>
<td>STZ+CLS 100mg</td>
<td>130.14±12.54</td>
<td>103.42±10.08</td>
<td>140.02±13.75</td>
<td>2.5±0.2</td>
<td>41.75±4.1</td>
<td>5.741±0.49</td>
</tr>
<tr>
<td>STZ+CLS 200mg</td>
<td>96.38±8.67*</td>
<td>92.86±8.79</td>
<td>130.47±12.54</td>
<td>2.7±0.23</td>
<td>39.67±3.81**</td>
<td>6.98±0.56</td>
</tr>
<tr>
<td>STZ+CLS 400mg</td>
<td>49.41±4.51***</td>
<td>46.8±4.51***</td>
<td>116.54±11.03</td>
<td>1.97±0.16*</td>
<td>33.85±3.35***</td>
<td>8.125±0.74**</td>
</tr>
<tr>
<td>STZ+SAF 100mg</td>
<td>140.11±13.55</td>
<td>110.2±11.03</td>
<td>140.11±13.56</td>
<td>2.55±0.19</td>
<td>42.51±3.8</td>
<td>4.96±0.39</td>
</tr>
<tr>
<td>STZ+SAF 200mg</td>
<td>105.42±10.63</td>
<td>101.52±10.03</td>
<td>135.33±13.14</td>
<td>2.3±0.2</td>
<td>39.47±3.76**</td>
<td>5.34±0.48</td>
</tr>
<tr>
<td>STZ+SAF 400mg</td>
<td>66.71±5.89*</td>
<td>78.36±6.69</td>
<td>123.71±11.86</td>
<td>1.92±0.1**</td>
<td>38.12±3.8**</td>
<td>7.21±0.71*</td>
</tr>
</tbody>
</table>

All values are Mean ±SEM for six rats. #, ##, ### indicates p<0.05, p<0.01, p<0.001 respectively vs. normal control; *, **, *** indicates p<0.05, p<0.01, p<0.001 respectively vs. diabetic control.

**Table 5: Effect of CLS and SAF extract on Liver antioxidant assay in STZ-induced diabetic rats on 28th day**

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD U/mg of protein</th>
<th>LPO mmoles/mg of protein</th>
<th>CAT U/mg of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>12.01±1.2</td>
<td>1.36±0.12</td>
<td>210±21</td>
</tr>
<tr>
<td>STZ</td>
<td>6.42±1.6#</td>
<td>2.98±0.22###</td>
<td>100.2±10###</td>
</tr>
<tr>
<td>STZ+MET 100mg</td>
<td>10.3±1.6</td>
<td>1.61±0.16**</td>
<td>198.3±19***</td>
</tr>
</tbody>
</table>
The effect of both the extracts on the blood glucose of STZ-induced diabetic rats was evaluated. Diabetic rats treated with CLS and SAF extracts at doses of (100, 200 and 400 mg/kg b. wt) for 4 weeks produced a significant (p<0.001) blood glucose reduction compared to diabetic control (Table 2). Treatment with CLS and SAF extracts exhibited results comparable to that of the standard metformin. The levels of total cholesterol and triglycerides were significantly (p<0.05, p<0.001) normal levels of both the extracts (Table 3).

The efficacy of both the extracts on hepatic and renal markers was analyzed on the 28th day. Administration of CLS extract at dose of 400 mg/kg showed significant (p<0.001) reduction in SGPT, SGOT, ALP and urea levels when compared with the diabetic control group (Table 4). The levels of creatinine and protein showed significant (p<0.01, p<0.05) reduction in the treated groups of SAF extracts as compared with the diabetic control.

The effect on liver antioxidant enzymes of both the extracts on streptozotocin induced diabetic rats showed a significant (p<0.05) decrease in LPO level and increase in superoxide dismutase and catalase levels as compared to the diabetic control (Table 5). Histological examination of liver, kidney and pancreas showed normal histology in normal rats. But the pancreatic sections of diabetic control showed atrophy and destruction of β cells and shrinkage of islets cells. The photomicrograph changes of liver and kidney were restored near to normal after treatment with 400 mg/kg extract. Pancreas in diabetic rats showed shrinkage of islets and the presence of pancreatic acini. This abnormal histological signs dramatically decreased in both the extract-treated groups (Fig.1).

**DISCUSSION**

Recently diabetes is one of the leading diseases around the globe. Management of diabetes is being a tough task with the synthetic medicines as they have many side effects. The interest has been shifted towards the medicinal plants used as a remedy for reducing the risk of
diseases. Medicinal plants, the potential sources of bioactive agents are gaining adequacy worldwide.\textsuperscript{[19]} In the present scenario, the scientists have emphasized the potential herbal extracts and initiated extensive research to observe their effective and protective role in the diseased animal models.\textsuperscript{[20]}

In the present study represents the assessment of anti-hyperglycemic effects of the methanolic extracts from CLS and SAF in streptozotocin induced diabetic rats. Pancreas is the primary organ involved in sensing the organism’s dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose, insulin will be secreted.\textsuperscript{[21]} However, streptozotocin is a naturally occurring nitrosourea product and it is widely used to induce diabetes in experimental animals. Usually, the intraperitoneal injection of a single dose (40 mg/kg, b. wt) exerts damage of pancreatic β-cells resulting in necrosis within 48-72 h and causes a permanent hyperglycemia.\textsuperscript{[22]} When there are not enough available β-cells to supply sufficient insulin to meet the needs of the body, insulin-dependent diabetes results.\textsuperscript{[23]} The CLS and SAF methanol extracts at the tested doses of 100, 200 and 400 mg/kg showed comparable activity with the standard. Metformin decreases hyperglycaemia primarily by suppressing glucose production by the liver.\textsuperscript{[24]}

The streptozotocin induced diabetes is characterized by a severe loss in body weight.\textsuperscript{[25]} The decrease in the body weight of diabetic rats in this study was due to the loss or degradation of structural proteins. Insulin plays an important role in the regulation of protein synthesis and proteolysis in skeletal muscle. In insulin resistance or deficiency state, muscle wasting and weight loss in diabetic rats results from the excessive catabolism of protein which provides amino acids for gluconeogenesis.\textsuperscript{[26,27]} In the present study, diabetic control rats showed marked reduction in their body weight when compared to normal rats. The weight loss was reverted by administration of both the extracts to the diabetic rats for a period of 28 days. The ability of the extracts to protect body weight loss in diabetic rats seems to be the result of their ability to reduce hyperglycemia.

During the study, it was found that both the extracts significantly (p<0.001) controlled the blood glucose level in STZ-induced diabetic rats. The CLS and SAF of methanol extracts showed reduction in blood glucose level in STZ-induced diabetic rats when compared to the diabetic control group. However, at a dose of 400 mg/kg, CLS extract showed more significant antidiabetic activity as compared to SAF extract. The possible mechanism of anti-hyperglycemic action in diabetic rats might be by potentiating the insulin effect of plasma, by
either increasing the pancreatic secretion of insulin from the existing \( \beta \)-cells or by its release from the bound form.

Elevated lipid profile, hepatic and renal marker enzymes are well-known manifestations indicating the progression of diabetic state. Four weeks treatment of diabetic rats with CLS and SAF methanolic extracts showed a significant decrease in serum cholesterol and triglyceride levels and thus could be beneficial in preventing diabetic complications as well as improving lipid metabolism in diabetics. Elevation of serum biomarker enzymes such as SGOT, SGPT and ALP were observed in diabetic rats indicating impaired liver function, which is obviously due to hepatocellular necrosis. In STZ-induced diabetic rats, elevated levels of SGOT, SGPT and ALP were observed and it may be due to STZ mediated liver damages which may cause leakage of the above enzymes into blood. Restoration of these biomarker enzymes towards normal level indicates decreased diabetic complications in methanolic extracts of CLS and SAF treated groups. The diabetic hyperglycemia induces elevation of the serum levels of urea and creatinine, which were considered as significant markers of renal function. The diabetic hyperglycemia induces elevation of the plasma levels of urea and creatinine which are significant markers of renal dysfunction and reflecting a decline in the glomerular filtration rate. These were recovered by treated groups. Increased level of serum protein in STZ induced diabetic rats as compared to diabetic control rats are presumed to be due to increased protein catabolism and gluconeogenesis during diabetes.

The \textit{in vivo} antioxidant activity displayed by enhanced levels of LPO was observed in the liver of diabetic rats which indicates excessive formation of free radicals and activation of lipid peroxidative system. In the present study administration of both the extracts and standard inhibits production of lipid peroxides. The antioxidant enzymes SOD and CAT play an important role in protecting cells from oxidative damage. SOD scavenges the superoxide radical by converting it to hydrogen peroxide and molecular oxygen while CAT brings about the reduction of hydrogen peroxides and protects liver tissues from the highly reactive hydroxyl radicals. The activities of both SOD and CAT were augmented in diabetic rats which could be attributed to their strong antioxidative properties.

The histopathology reveals that the site of action may be pancreatic or extrapancreatic. The decrease in blood glucose may be attributed to the stimulation of glucose uptake by peripheral tissues and decrease in the gluconeogenesis. Hence, the antihyperglycemic effect may be probably brought about by an extrapancreatic mechanism. The histopathological
observation however, supports pancreatic mechanism wherein both the number and structural integrity of islets of Langerhans were restored towards normalization. This phenomenon could lead to an increase in insulin synthesis and secretion thereby correcting the diabetic state.\(^{[36]}\)

In the present study, shows that the histopathological examination of pancreas of STZ induced diabetic rats revealed destruction of pancreatic β–cells and reticular changes of islets as evidenced by fibrosis. After 28 days of treatment with CLS and SAF extract at higher dose (400 mg/kg), islets of Langerhans showed improvement and there was restoration of normal cellular population size of islets as well as those that were atrophied.

**Figure 1**

A – Pancreas; B – Kidney; C – Liver; Group 1 - Photomicrograph of normoglycaemic rats; Group 2 - Photomicrograph of hyperglycaemic rats; Group 3 - Photomicrograph of CLS extract (400 mg/kg) treated rats; Group 4 - Photomicrograph of SAF extract (400 mg/kg) treated rats.
Numerous epidemiological studies suggest that herbs/diets rich in phytochemicals and antioxidants execute a protective role in health and disease. Flavonoids, sterols, triterpenoids, alkaloids, saponins and phenolics are reported as bioactive antidiabetic principles.\cite{37} The present results demonstrated that the methanol extracts of CLS and SAF have been proved to possess potential antidiabetic action in STZ-induced diabetic rats and the effect was found to be more similar to the reference drug metformin. The active compounds tannins and phenols in the methanolic extracts could have ameliorated the diabetic condition by acting synergistically.

CONCLUSION

Based on our results obtained in the present study we can conclude that the methanol extracts of CLS and SAF possesses antihyperglycaemic properties. Therefore, these medicinal plants could be considered as a potential and alternative approach for the treatment of diabetes. However, further investigations are needed to identify the lead molecule and to elucidate absolute mechanism of action for antidiabetic effect. In this study, investigation has also opened avenues for further research to the development of potent phytomedicine for diabetes mellitus from the CLS and SAF.

CONFLICT OF INTERESTS

We declare that we have no conflict of interest.

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