THREE TETRACYCLIC TRITERPENOIDS ISOLATED AND IDENTIFIED FROM THE ETHYL ACETATE EXTRACT OF CASSIA FISTULA STEM BARK WITH HYPOGLYCEMIC POTENTIAL.

Manikkam Rajalakshmi*, Pitchai Daisy

PG & Research Department of Biotechnology & Bioinformatics, Holy Cross College, (Autonomous), Trichy, Tamilnadu.

ABSTRACT

Diabetes is a major disease that affects more than 4% of the global population and management of diabetes without side effects is still a challenge. But, the traditional systems of medicine and medicinal compounds overcome this challenge. Hence, the use of plant derived compounds has become one of the major thirst areas of diabetes research. This work is aimed in establishing the hypoglycemic activity of three novel compounds from ethyl acetate extract of Cassia fistula.

Methods: The novel compounds were isolated and the structures were determined using NMR, Mass and IR spectrum. Toxicity test were carried out at various doses. After the dose determination, 20mg/kg.b.wt of the three compounds were administered to Streptozotocin(STZ)-induced (60mg/kg.b.wt) diabetic male Wistar rats for 6-weeks. Plasma glucose level, body weight, food and water intake, hemoglobin and glycosylated hemoglobin(HbA1C) levels were analyzed using standard methods. The light microscopical analysis of pancreatic sections was done. Results: The compounds were identified as novel tetracyclic triterpenoids. No significant toxic effects were seen in the compound treated rats. Plasma glucose level was significantly reduced and with weight gain after the treatment with compounds and with normal food and water intake. They significantly decreased the HbA1C and increased the hemoglobin content. The light microscopical sections reveal that the compounds did not cause any regeneration of β-cells in the pancreas. Conclusion: The above results confirm that the three novel compounds possess hypoglycemic potential and could be used as potential candidates to develop drugs to manage diabetes mellitus.
Key words: Diabetes mellitus, Cassia fistula, novel tetracyclic triterpenoids, hemoglobin, glycosylated hemoglobin.

1. INTRODUCTION

Diabetes mellitus (DM), considered as a major global health problem, is highly prevalent in its incidence. The number of people affected by DM is increased day by day[1]. Approximately 300,000 deaths each year are attributed to diabetes. Its prevalence increases with age, from about 0.2% in persons less than 17 years of age to about 10% in persons aged 65 years and over[2]. Generally, DM is caused by inherited or acquired deficiency in production of insulin by the pancreas or by the ineffectiveness of the insulin produced. Insulin deficiency results in increased blood glucose concentration, which in turn causes the damage many of the body systems including the blood vessels and nerves. Furthermore, DM causes the glycation of body proteins due to the chronic hyperglycemic condition, which in turn leads to the development of secondary complications affecting eyes, kidneys, nerves and arteries.

Oral hypoglycemic drugs have been recommended to manage the complications of DM over the past few decades. But their use is restricted due to their pharmacokinetic properties, secondary failure rates and the most significant accompanying side effects. Hence, World Health Organization has recommended that traditional methods for the treatment of DM should be further investigated. After that lot of investigations were flinched in exploiting the hypoglycemic properties of medicinal plants[3-4].

Researchers have focused in identifying and validating plant derived compounds for the treatment of DM. As per the data, it is surprise that more than 25% of modern medicines are directly or indirectly derived from plants. In many developing countries, the medicinal plants and their derivatives are commonly used by the indigenous people in rural areas[5].

Cassia fistula(C.fistula)Linn.also known as golden shower, is one such plant widely used in the treatment of DM. It is an Indian medicinal plant that belongs to the Caesalpinaceae. According to the literature, almost all the parts of C.fistula possess many pharmacological activities like antimicrobial, antifungal, antipyretic, analgesic, larvicidal, anti-inflammatory, antioxidant, anti-tumor, hepatoprotective, hypoglycemic activities. Its seeds are recognized as antibilious, aperitif, carminative and laxative agents in Ayurvedic Medicine[6].
With this information in mind, we started investigating the hypoglycemic activity of \textit{C.fistula} stem bark. We have compared the hypoglycemic activity of hexane, ethyl acetate and methanol extracts of the plant and reported\cite{7} that methanol extract was found to possess comparatively better hypoglycemic activity followed by ethyl acetate extract. Furthermore, we have isolated and identified catechin- a flavonoid, from methanol extract using bio-assay guided fractionation and reported its hypoglycemic activity in Streptozotocin (STZ) - induced diabetic male albino Wistar rats\cite{8}.

In the present study, we aimed at the isolation of compounds from the ethyl acetate extract of \textit{C.fistula} using bio-assay guided fractionation and establishing their hypoglycemic activity in STZ-induced diabetic male albino Wistar rats.

2. MATERIALS AND METHODS

2.1. Plant Material
During summer the fresh bark of the \textit{C.fistula} was collected from Kodaikanal, Tamil Nadu, India. The species was identified and authenticated at the Department of Botany, Holy Cross College, Tiruchirapalli, and the voucher specimen is deposited in the herbarium of the Department.

2.2. Isolation and identification of compounds
The shade-dried plants were powdered and 1 kg powder was extracted using hexane, ethyl acetate and methanol in a soxhlet apparatus sequentially and the extracts were evaporated to dryness under reduced pressure in a rotary evaporator. The yield of the hexane extract was 17.8g, ethyl acetate extract was 16.6g and the methanol extract was 20.1 g. The dry residues of the crude extracts obtained were stored at 4°C for further use\cite{8}. The ethyl acetate extract was further chromatographed on a silica gel column (Merck 70-230 mesh, 400 g, 3.5 i.d. x60 cm) and successively eluted with a continuous gradient from 100% hexane, 95% hexane and 5% ethyl acetate till 10% ethyl acetate. The fractions were collected and each fraction was spotted on a precoated Silica gel 60 F254, 0.25mm thick Thin Layer Chromatography (TLC) plate (Merck) and fractions with similar Rf values in TLC pattern were pooled together into 18 fractions. Fractions 2, 8 and 9 gave single spot in TLC. The compounds were subjected to nuclear magnetic resonance (NMR), mass (MS), and infra red (IR) spectral analyses for structural determination.
2.3. Experimental animals

Male albino rats (Wistar strain, weighing 150–220g) bred in the Laboratory of Animal Medicine, Centre for Animal Health Studies, Tamilnadu Veterinary and Animal Sciences Studies, Madhavaram, Chennai, Tamil Nadu, India, were used. All the animals were kept and maintained under laboratory conditions of temperature (22±2° C), humidity (45±5%) and 12 h day:12 h night cycle, and were allowed free access to food (standard pellet diet-(Sai Durga Feeds and Foods, Bangalore, India) and water ad libitum. The experimental protocol has been approved by the Institutional Animals Ethics Committee and by the regulatory body of the government (Reg.No 585/05/A/CPCSEA).

2.4. Induction of experimental diabetes

Diabetes was induced by STZ, Sigma-Aldrich, St.Louis, USA. The animals were fasted overnight and diabetes was induced by a single intra peritoneal injection of a freshly prepared solution of STZ (60 mg/kg b.wt\(^9\), in 0.1 M citrate buffer (pH-4.5). Control rats were injected with citrate buffer alone. On the third day of STZ-injection, the rats were fasted for 6 h and blood was taken by sinocular puncture. Rats with moderate diabetes having hyperglycemia (that is, with blood glucose of 350 – 450 mg/dl) were taken for the experiment. The rats were kept for 15 days to stabilize the diabetic condition\(^{10}\).

2.5. Acute toxicity test and dose determination of compounds

Normal healthy male rats fasted for 12 h were randomly divided into 21 groups of six animals each as given below. Different doses (5, 15, 20mg/kg. b.wt) of the compounds were suspended in vehicle solution (Dimethylsulfoxide [DMSO] 0.5%; 1ml/kg.b.wt) and administered through oral route using an intra-gastric tube for 15 days daily to the respective groups.

Group 1-Normal rats treated with vehicle alone
Group 2-Normal rats + Compound-1 (5mg/kg.b.wt)
Group 3-Normal rats + Compound-1 (15mg/kg.b.wt)
Group 4-Normal rats + Compound-1 (20mg/kg.b.wt)
Group 5-Normal rats + Compound-2 (5mg/kg.b.wt)
Group 6-Normal rats + Compound-2 (15mg/kg.b.wt)
Group 7-Normal rats + Compound-2 (20mg/kg.b.wt)
Group 8-Normal rats + Compound-3 (5mg/kg.b.wt)
Group 9-Normal rats + Compound-3 (15mg/kg.b.wt)
Group 10-Normal rats + Compound-3 (20mg/kg.b.wt)
Group 11-STZ- induced diabetic rats treated with vehicle alone
Group 12-STZ- induced diabetic rats +Compound-1(5mg/kg.b.wt)
Group 13-STZ- induced diabetic rats +Compound-1(15mg/kg.b.wt)
Group 14-STZ- induced diabetic rats + Compound-1(20mg/kg.b.wt)
Group 15-STZ- induced diabetic rats +Compound-2(5mg/kg.b.wt)
Group 16-STZ- induced diabetic rats +Compound-2 (15mg/kg.b.wt)
Group 17-STZ- induced diabetic rats + Compound-2(20mg/kg.b.wt)
Group 18-STZ- induced diabetic rats +Compound-3(5mg/kg.b.wt)
Group 19-STZ- induced diabetic rats +Compound-3(15mg/kg.b.wt)
Group 20-STZ- induced diabetic rats + Compound-3 (20mg/kg.b.wt)
Group 21-STZ- induced diabetic rats + Insulin (3-IU/kg.b.wt)

All doses were given 15 days after injection of STZ. No irritation or restlessness was observed after each drug or vehicle administration. No noticeable adverse effect (i.e., respiratory distress, abnormal locomotion and catalepsy) was observed in any animal after the drug administration. Fasting plasma glucose levels were estimated every week to ascertain the status of diabetes in different groups of rats. Oral administration of 20mg/kg.b.wt dose of all the compounds for 15 days showed significant plasma glucose lowering effect in STZ-induced diabetic rats when compared to other doses (5 and 15mg/kg.b.wt). This dose was fixed as effective dose and was selected for further study.

2.6. Experimental design and treatment schedule

For further study the all groups of animals received 20mg/ kg.b.wt along with normal, STZ-induced diabetic rats and STZ- induced diabetic rats treated with Insulin were continued with the same treatment every day for 6 weeks. Blood glucose levels were estimated during 0, 7, 15,30 and 45th day.

After 6 weeks of treatment, the animals were deprived of food overnight and sacrificed by decapitation. Blood was collected in two different tubes, i.e. one with anticoagulant, potassium oxalate and sodium fluoride for plasma, and another without anticoagulant for serum separation. The blood was then centrifuged at 3000 rpm for 20 min using refrigerated centrifuge at 4°C to separate the plasma and serum. Liver was removed, dried on tissue paper, weighed and stored at −80°C. Pancreas was immediately dissected, washed in ice cold saline, patted dry and weighed. The tissues were fixed in 10% formalin immediately after removal.
from the animal to avoid decomposition. Embedding in paraffin wax was carried out by removal of water using alcohol dehydration and infiltration of chloroform as a solvent for the wax.

2.7. Estimation of glucose
Fasting plasma glucose was estimated using glucose oxidase peroxidase method\(^{[11]}\).

2.8. Determination of hemoglobin and glycosylated hemoglobin (HbA\(_1\)C)
Hemoglobin in the blood was estimated by the method of Drabkin and Austin, (1932)\(^{[12]}\). HbA\(_1\)C was estimated by Diagnostic kit-BioSystems (Costa Brava, Spain).

2.9. Histological studies
Light Microscopic Studies (Paraffin Method\(^{[13]}\))
The pancreas form the untreated and the experimental groups were blotted free of mucus, washed in physiological saline, cut into pieces of desired size and fixed in Bouin-Hollandefixative for 72h. After fixation, the tissues were washed in 70% alcohol for 2 or 3 days to remove the excess picric acid and dehydrated in graded series of alcohol. The tissues were cleared using xylene. The cleared tissues were infiltrated with molten paraffin at 58-60\(^\circ\)C through three changes (20-30 min) and finally embedded in paraffin. 3-5 \(\mu\)m thick sections of all the tissues were obtained using rotary microtome and stained in Ehrlich’s hematoxylin with eosin as the counter stain. The slides were mounted using DPX mountant.

2.10. Statistical analysis
Statistical analysis was performed using SPSS software package Version 17.0. The values were analysed by one-way analysis of variance (ANOVA) followed by Duncan’s multiple range test (DMRT)\(^{[14]}\). All the results were expressed as mean \(\pm\)S.D. for six rats in each group. P-Values <0.05 were considered significant.

3. RESULTS
The spectral data obtained through FT-IR, ESIMS, \(^1\)HNMR and \(^1\)C NMR are provided in figs:1-3 and the structures and molecular weight identified from the spectral data and the molecular formula are presented in figs-4-6.
Figure-1-The a.1H NMR, b.13CNMR , c. IR, d. Mass spectrum of novel compound 2879/CHE/2012

Figure-2-The a.1H NMR, b.13CNMR , c. IR, d. Mass spectrum of novel compound 2880/CHE/2012
Figure-3-The a.1H NMR, b.13CNMR, c. IR, d. Mass spectrum of novel compound 2881/CHE/2012

\[\text{17-[(E)-4-ethyl 1',5' dimethyl 2'-hexenyl]-11 hydroxy-5-(hydroxymethyl)-13,14-dimethyl-5,6,7,11,12,13,14,15,16,17-decahydro-3H-cyclopenta[e]phenanthren-3-one}\]

Molecular formula: \(\text{C}_{30}\text{H}_{46}\text{O}_3\)
Molecular Weight
Experimental: \(m/z\, 453.08\)
Calculated: \(m/z\, 452.67\)

Figure-4- Structure of novel compound 2879/CHE/2012
The compounds were identified as novel tetracyclic triterpenoids. Hence they were filed for Indian Patenting (2879/CHE/2012, 2880/CHE/2012 & 2881/CHE/2012) and were published in The Patent Office Journal 30/11/2012.

The plasma glucose level of normal, diabetic, and compounds treated animals at 0, 15, 30, 45th day are presented in table-1. The table clearly depicts that the plasma glucose in STZ-
diabetic rats was high and was gradually reduced to normal upon the oral administration of the novel compounds. This confirms that the novel compounds possess hypoglycemic potential.

Table 1-Effect of oral administration of novel compounds (Cpd-1: 2879/CHE/2012, Cpd-2: 2880/CHE/2012 & Cpd-3: 2881/CHE/2012) on plasma glucose levels in normal and Streptozotocin-induced diabetic male albino Wistar rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Plasma glucose levels (mg/dl)</th>
<th>0th day</th>
<th>15th day</th>
<th>30th day</th>
<th>45th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>85.2±7.6</td>
<td>84.9±7.7</td>
<td>82.0±8.2</td>
<td>87.5±9.6</td>
<td></td>
</tr>
<tr>
<td>Normal+ Cpd-1 (5mg/kg.b.wt)</td>
<td>83.1±8.2</td>
<td>91.3±9.4</td>
<td>90.1±7.5</td>
<td>86.2±8.2</td>
<td></td>
</tr>
<tr>
<td>Normal+ Cpd-1 (15mg/kg.b.wt)</td>
<td>89.9±7.2</td>
<td>82.4±6.4</td>
<td>87.5±7.5</td>
<td>89.8±8.3</td>
<td></td>
</tr>
<tr>
<td>Normal+ Cpd-1 (20mg/kg.b.wt)</td>
<td>92.5±9.6</td>
<td>88.2±6.4</td>
<td>89.6±8.2</td>
<td>85.4±8.3</td>
<td></td>
</tr>
<tr>
<td>Normal+ Cpd-2 (5mg/kg.b.wt)</td>
<td>86.8±8.1</td>
<td>80.6±8.2</td>
<td>85.3±6.2</td>
<td>87.1±5.9</td>
<td></td>
</tr>
<tr>
<td>Normal+ Cpd-2 (15mg/kg.b.wt)</td>
<td>82.7±8.6</td>
<td>92.7±10.4</td>
<td>85.3±9.5</td>
<td>82.1±9.3</td>
<td></td>
</tr>
<tr>
<td>Normal+ Cpd-2 (20mg/kg.b.wt)</td>
<td>89.3±9.5</td>
<td>93.8±7.3</td>
<td>88.4±6.7</td>
<td>86.4±8.9</td>
<td></td>
</tr>
<tr>
<td>Normal+ Cpd-3 (5mg/kg.b.wt)</td>
<td>84.9±8.2</td>
<td>89.5±9.4</td>
<td>85.3±7.2</td>
<td>91.6±7.4</td>
<td></td>
</tr>
<tr>
<td>Normal+ Cpd-3 (15mg/kg.b.wt)</td>
<td>91.9±8.3</td>
<td>93.6±9.2</td>
<td>79.2±10.5</td>
<td>84.1±6.9</td>
<td></td>
</tr>
<tr>
<td>Normal+ Cpd-3 (20mg/kg.b.wt)</td>
<td>82.5±7.8</td>
<td>86.4±7.7</td>
<td>89.9±8.9</td>
<td>91.5±8.1</td>
<td></td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>420.8±23.4</td>
<td>431.8±30.47</td>
<td>445.7±34.1</td>
<td>451.8±37.3</td>
<td></td>
</tr>
<tr>
<td>Diabetic+ Cpd-1 (5mg/kg.b.wt)</td>
<td>414.9±23.6</td>
<td>382.9±29.5</td>
<td>270.2±25.5</td>
<td>184.4±28.8</td>
<td></td>
</tr>
<tr>
<td>Diabetic + Cpd-1 (15mg/kg.b.wt)</td>
<td>428.7±25.7</td>
<td>368.1±26.4</td>
<td>253.6±28.3</td>
<td>154.7±21.7</td>
<td></td>
</tr>
<tr>
<td>Diabetic + Cpd-1 (20mg/kg.b.wt)</td>
<td>402.4±28.9</td>
<td>308.5±21.8</td>
<td>211.2±23.6</td>
<td>104.2±9.7</td>
<td></td>
</tr>
<tr>
<td>Diabetic + Cpd-2 (5mg/kg.b.wt)</td>
<td>396.3±31.2</td>
<td>292.4±27.2</td>
<td>210.6±15.4</td>
<td>167.5±12.2</td>
<td></td>
</tr>
<tr>
<td>Diabetic + Cpd-2 (15mg/kg.b.wt)</td>
<td>435.8±25.3</td>
<td>338.6±21.8</td>
<td>200.2±21.7</td>
<td>136.1±9.4</td>
<td></td>
</tr>
<tr>
<td>Diabetic + Cpd-2 (20mg/kg.b.wt)</td>
<td>424.1±29.7</td>
<td>315.7±22.2</td>
<td>193.4±19.4</td>
<td>95.7±8.8</td>
<td></td>
</tr>
<tr>
<td>Diabetic + Cpd-3 (5mg/kg.b.wt)</td>
<td>401.9±32.6</td>
<td>301.3±24.5</td>
<td>245.7±26.6</td>
<td>178.3±20.0</td>
<td></td>
</tr>
<tr>
<td>Diabetic + Cpd-3 (15mg/kg.b.wt)</td>
<td>428.3±28.2</td>
<td>299.4±23.7</td>
<td>219.5±12.7</td>
<td>135.8±13.3</td>
<td></td>
</tr>
<tr>
<td>Diabetic + Cpd-3 (20mg/kg.b.wt)</td>
<td>434.9±37.1</td>
<td>253.3±24.4</td>
<td>162.6±14.8</td>
<td>89.9±5.3</td>
<td></td>
</tr>
<tr>
<td>Diabetic+Insulin(3-IU/kg bw)</td>
<td>436.5±36.2</td>
<td>359.5±26.6</td>
<td>226±17.1</td>
<td>103.65±7.2</td>
<td></td>
</tr>
</tbody>
</table>

Each value is mean±S.D. for six rats in each group, , –: no significance.

a -p < 0.05 by comparison with normal rats.
b -p < 0.05 by comparison with Streptozotocin diabetic rats

The food and water intake of the normal diabetic and compounds treated animals are presented in table-2. A significant (p< 0.05) change in the intake of water and food could be seen from the table. The hemoglobin and HbA1C value of the same group of animals are presented in the same table. The table clearly shows a gradual increase in the hemoglobin content with a concomitant reduction in the HbA1C.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight g/day</th>
<th>Hemoglobin mg/dl</th>
<th>Glycosylated Hemoglobin % of total Hb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Induction</td>
<td>After Induction</td>
<td>After treatment</td>
</tr>
<tr>
<td>Normal</td>
<td>221.2±7.6</td>
<td>233.7±9.2</td>
<td>241.6±12.3</td>
</tr>
<tr>
<td>Normal + Cpd 1 (20mg/kg b wt)</td>
<td>196.3±9.2</td>
<td>201.7±12.2</td>
<td>224.8±14b</td>
</tr>
<tr>
<td>Normal + Cpd 2 (20mg/kg b wt)</td>
<td>238.8±7.3</td>
<td>247.1±16.5</td>
<td>265.3±11.7b</td>
</tr>
<tr>
<td>Normal + Cpd 3 (20mg/kg b wt)</td>
<td>211.6±12.7</td>
<td>238.7±13.1</td>
<td>251.4±9.3b</td>
</tr>
<tr>
<td>Diabetic</td>
<td>241.9±14.2</td>
<td>148.3±8.4</td>
<td>113.7±7.3a</td>
</tr>
<tr>
<td>Diabetic + Cpd 1 (20mg/kg b wt)</td>
<td>243.7±9.4</td>
<td>155.6±10.2</td>
<td>229.6±11.1b</td>
</tr>
<tr>
<td>Diabetic + Cpd 2 (20mg/kg b wt)</td>
<td>248.4±15.8</td>
<td>132.8±9.5</td>
<td>235.2±16.9b</td>
</tr>
<tr>
<td>Diabetic + Cpd 3 (20mg/kg b wt)</td>
<td>239.9±7.8</td>
<td>163.3±10.2</td>
<td>242.1±16.2b</td>
</tr>
<tr>
<td>Diabetic+ Insulin(3 I.U/kg b wt)</td>
<td>225.8±14.6</td>
<td>127.4±9.1</td>
<td>224.9±15.2b</td>
</tr>
</tbody>
</table>

Each value is mean±S.D. for six rats in each group, –: no significance.

a -p < 0.05 by comparison with normal rats.
b -p < 0.05 by comparison with Streptozotocin diabetic rats

The hematoxylin and eosin stained sections of the pancreatic islets are given in figure-7. The sections of untreated rats revealed that each islet of Langerhans is formed of numerous compactly arranged cells occurring as dense cords(figure-7a). The islets appeared lightly stained when compared with the surrounding acinar tissue. The Islet cells were round to ovoid with round vesicular nuclei and pale pink cytoplasm. Capillaries were found in between the islet cells. Islets from the pancreas of diabetic control rats showed an entirely different picture in hematoxylin and eosin stained sections (figure-7b). Most of the cells in the islets possessed pyknotic nuclei whereas some cells contained dark nuclei, and few cells at the periphery had round or ovoid nuclei. The novel compounds treated sections showed similar picture like diabetic rats(figure-7c-e).
4. DISCUSSION

The present study was done to evaluate the hypoglycemic activity of the novel compounds, the very new herbal compounds first time identified by us. Acute toxicity studies revealed the non-toxic nature of the novel compounds. Experiment was carried out on normal healthy male rats. No mortality was observed in the novel compounds-treated rats and behavior of the treated rats also appeared normal. There was no lethality or toxic reaction found at any dose selected until the end of the study.

STZ is well known to induce experimental diabetes\textsuperscript{[15]}. Intra-peritoneal administration of 60 mg/kg b.wt of STZ in the present study effectively induced diabetes mellitus in rats. The induction of diabetes mellitus was confirmed by elevated levels of fasting blood glucose. Daily oral administration of novel compounds for 6 weeks resulted in a decrease in blood glucose level in STZ-induced diabetic rats. In addition novel compounds are also found to possess normoglycemic effect as the glucose levels of the normal rats administered with novel compounds were not altered.

The reduced glucose levels in STZ-diabetic animals suggested that novel compounds might increase the insulin secretion from the regenerated pancreas\textsuperscript{[16]}, which in turn might either promote glucose uptake metabolism by inhibiting hepatic gluconeogenesis or by absorption.
of glucose into the muscle and adipose tissues. Interestingly, the hematoxylin and eosin microscopical pancreatic sections of the present study reveal that there is no evidence for the regeneration of β-cells from the destroyed pancreas, even after the treatment with novel compounds. This confirms that the hypoglycemic effectiveness of the novel compounds in diabetic animals is not due to the insulin released from the regenerated pancreatic beta cells. This result goes in accordance with our previous report on the effectiveness of catechin in treating diabetes \[^8\]. Hence, the novel compounds might also have insulin mimetic effect like catechin.

Experimental DM is commonly associated with severe loss of body weight which might be resulted after muscle wastage and loss of tissue protein which in turn caused by insulin deficiency \[^17\]. In the present study, a reduced level of body weight and elevated level of food and water intake were observed in STZ-induced diabetic rats and the administration of novel compounds restored these alterations to near normal. This restoration might be due to an improved glycemic control.

In hyperglycemic condition, the high of circulating blood glucose causes the glycosylation of hemoglobin, resulting in an increase in the HbA\(_1\)C with a concomitant decrease in the hemoglobin level. As it is formed slowly and does not dissociate easily, it reflects the real blood glucose level \[^18\]. Therefore, HbA\(_1\)C can be used as an excellent marker of overall glycemic control. In the present study, a decrease in the HbA\(_1\)C with an increase in hemoglobin levels after the treatment with novel compounds might be due to the improvement of overall blood glucose control. The oxidative stress in rats would have been reduced due to the hypoglycemic condition which in turn would reduce the HbA\(_1\)C. Therefore, at the end of 6 weeks treatment an increase in the hemoglobin and a decrease in the glycosylated hemoglobin levels are seen.

4. **CONCLUSION**

Our results clearly explicate the hypoglycemic activity of the newly identified novel triterpenoids from the ethylacetate extract of *C. fistula*. Hence, they could develop into drugs in treating DM as potential candidates. However, further work needs to be done to substantiate the insulin mimetic activity of the compounds and to elucidate the molecular mechanisms of glucose uptake.
ACKNOWLEDGEMENT
The financial support extended by the University Grants Commission (Project No.32-505/ (SR) is acknowledged.)

Conflict of interest: No conflict to disclose.

REFERENCES
10. Jyoti M, Vihas TV, Ravikumar A, Sarita G. Glucose lowering effect of aqueous extract of
Ethnopharmacol, 2002; 81: 317-20.

11. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative

12. Drabkin DL, Austin JM. Spectrophotometric constants for common hemoglobin


14. Duncan BD. Multiple range tests for correlated and heteroscedastic means. Biometrics,

of osteopenia in streptozotocin-induced diabetic mice. A possible role of oxidative stress.

16. Bolkent S, Yamardag R, Tabakogluoguz A, Ozsoy–sacaon O. Effects of chord (Beta
vulgaris L. Var. cicla) extract on pancreatic a cells in Streptozotocin-diabetic rats: a

17. Vats V, Yadav, SP, Grover JK. Ethanolic extract of Ocimum sanctum leaves partially
attenuates streptozotocin induced alterations in glycogen content and carbohydrate

18. Guoyan J. Practical diabetes mellitus. The People’s Medical Publishing House, Beijing,