Antihyperglycemic and antioxidative attribute of hydroethanolic extract of 
_Butea monosperma_ (Lam.) seeds and its active constituents

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Treatment of diabetic mice with glibenclamide and crude extract (BE) significantly declined the FBG content. However, amongst the 6 isolated compounds, 3 compounds (C1, C4 and C6) appreciably subsided the exaggerated level of FBG. Simultaneously, glibenclamide, BE, C4 and C6 treatment markedly enhanced the hepatic glycogen content as compared to diabetic control group. Administration of crude extract, C4, C5 and C6 also exerted a protective effect on the declined activity of SOD, CAT and GSH-Px in the three tissues. However, all the herbal treatments produced a pronounced escalation in GSH content. Contrarily the elevated level of hepatic, pancreatic and renal TBARS monitored in diabetic animals was significantly diminished in treated groups of animals. Alloxan administration severely deteriorated the structure of liver and pancreas of diabetic mice, which was found to be restored to a certain extent in glibenclamide, BE and C6 treated animals. Identification of the most potent antihyperglycemic compound C6 by HPLC confirmed its triterpene nature. C6 was then further characterized via various spectroscopic methods (IR, NMR and Mass) that revealed its similarity with laccijalaric ester-I, a triterpene present in soft resin of _B. monosperma_ seeds.

**Keywords**: Antihyperglycemic, Antioxidation, _Butea monosperma_, Laccijalaric ester-I, Triterpene

Diabetes mellitus (DM) is a complex and multifactorial group of disorders which has reached epidemic proportions in the present century. Various drugs presently available to reduce diabetes associated hyperglycemia are associated with several side-effects. Hence, in the recent years, there is growing interest in herbal medicine all over the world, as they have little or no side effects. Ethnopharmacological surveys indicate that more than 1200 plants are used in traditional medicine for antihyperglycemic activity.

*Butea monosperma* (Lam.) commonly known as ‘flame of the forest’ belongs to family Fabaceae and is common throughout India, Burma and Ceylon except in very arid parts. The plant is known to possess numerous medicinal properties and almost all parts of the plants are being used since decades in medicine and for other purposes. But till date the antihyperglycemic and antioxidative attributes of active constituents of its seeds have not been reported. In view of this, the present study was undertaken to investigate the antihyperglycemic and antioxidant potential of hydroethanolic extract of _B. monosperma_ seeds and its active constituents. Besides, its cytoprotective effect in situations of oxidative injury caused by persistent hyperglycemia in liver and pancreas of diabetic mice model was also studied. Further, the active constituents present in this extract were isolated and their antihyperglycemic and antioxidant potential was also evaluated followed by identification and characterization of the most potent proved antihyperglycemic constituent.

**Materials and Methods**

*Reagents*—Alloxan monohydrate was purchased from SD Fine Chemicals, Mumbai, India. All other chemicals used were of analytical grade and obtained from HIMEDIA (India) and Qualigens (India/Germany). TLC plates were purchased from Merck.

*Extraction and isolation procedure*—Seeds of _Butea monosperma_ were collected from Sanjay Van, Newai (Rajasthan, India) during May, 2007 and were taxonomically identified by Dr. G.S. Shekhawat (Botanist, Dept. of Bioscience & Biotechnology, Banasthali University, Rajasthan, India). Crude extract of _B. monosperma_ seeds was prepared by the

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method as described earlier. The active constituents were isolated from it by the method as described elsewhere. For this, the ethanolic extract was developed on pre-coated TLC plates (Merck) with benzene and acetic acid (45:5) as the developing solvent (mobile phase). Six distinct spots were observed on the TLC plates after placing them in the iodine chamber for 30-45 min. The calculated $R_F$ value of the six compounds (corresponding to six spots) was 0.187, 0.253, 0.533, 0.719, 0.827 and 0.907, respectively. For obtaining the six compounds in large amount, preparative TLC was carried out. The clearly separated dark yellow bands (Fig. 1) were scratched and suspended in the mobile phase separately for 3-4 days (Purity of the isolated compounds was checked by obtaining a single spot in TLC). Subsequently, the mobile phase was sucked out, vacuum evaporated and the residue left was collected till sufficient amount for dosing was obtained. The isolated compounds were assessed for their antihyperglycemic and antioxidant potential.

**Experimental animals and alloxan-induction of experimental diabetes**—Healthy adult male Swiss albino mice (Mus musculus) weighing 25-30 g were procured from C.C.S. Haryana Agricultural University, Hissar (Haryana, India). Animals were housed in polypropylene cages at 23° ± 2°C and RH 55 ± 5% under 12 h light and dark cycle. Water and standard pellet diet was provided *ad libitum* throughout the experimental period. The schedules and procedures performed on experimental animals were in compliance with the principles of Institutional Animal Ethics Committee constituted as per the directions of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

After one week of acclimatization, mice were randomly divided into 10 experimental groups viz. I (normal control, NC), II (diabetic control, DC), III (alloxan-diabetic + glibenclamide treated, GT), IV (alloxan-diabetic + BE treated), V (alloxan-diabetic + C1 treated), VI (alloxan-diabetic + C2 treated), VII (alloxan-diabetic + C3 treated), VIII (alloxan-diabetic + C4 treated), IX (alloxan-diabetic + C5 treated) and X (alloxan-diabetic + C6 treated). Each group contained 7 mice. After overnight fasting, mice of groups II-X were injected intraperitoneally with alloxan monohydrate dissolved in ice-cold normal saline at a dose of 150 mg kg$^{-1}$ body weight (bw)$^7$. One week after alloxan treatment, mice with fasting blood glucose (FBG) level greater than 140 mg dl$^{-1}$ were considered diabetic and included for further study.$^8$ Glibenclamide (10 mg kg$^{-1}$) and BE (300 mg kg$^{-1}$) was administered to animals of group III and IV respectively. However, animals of group V to X were administered with the compound C1, C2, C3, C4, C5 and C6, respectively (50 mg kg$^{-1}$ bw). The dose of crude extract (BE) was selected by toxicity evaluation in mice and the dose of 6 isolated compounds was one-sixth that of the crude extract.

**Estimation of FBG level**—FBG concentration was determined by using one-touch ultra glucometer (Johnson & Johnson Co. USA) and compatible blood glucose strips$^9$ at regular time intervals i.e. before alloxan injection, after confirming diabetes and after providing various herbal treatments for 45 days.

**Identification and characterization**—Among the 6 isolated compounds, C6 with $R_F$ value 0.907 revealed maximum anti-hyperglycemic potential and therefore, this compound was chosen for identification and further characterization via HPLC and various spectroscopic methods.

HPLC of the isolated compound C6 was carried out to confirm its nature by comparing it against standard ($\alpha$-amyrin). For this an isocratic HPLC (Shimadzu HPLC class VP series) with two LC – 10 AT VP pumps (Shimadzu), variable wavelength programmable photodiode array detector SPD MIOA.
VP (Shimadzu), CTO-IOAS VP column oven (Shimadzu), SCL-10A VP system controller (Shimadzu) and a reverse phase Luna 5 µC18 Phenomenex column (250 mm × 4.6 mm) was used. The HPLC system was equipped with software class VP series version 6.1 (Shimadzu). The mobile phase components methanol:water (80:20) were pumped from the solvent reservoir to the column at a flow rate of 1 ml min⁻¹ which yielded a column backpressure of 16-165 Kgf cm⁻². The column temperature was maintained at 27°C. 20 µl of sample was injected using Rheodyne syringe (Model 7202, Hamilton).

C6 was then characterized after analyzing the spectra obtained from IR spectroscopy (recorded on RKEN ELMER spectrophotometer), proton nuclear magnetic resonance (¹H NMR) spectroscopy (recorded at 400 Hz on BRUKER AVANCE II 400 NMR spectrophotometer) and Mass spectroscopy (recorded on TOF MS-spectrophotometer).

IR spectra was recorded as % T on KBr pellet; for ¹H NMR spectra chemical shifts were reported in ppm (δ) using CDCl₃ as solvent and coupling constant were expressed in hertz.

Preparation of tissue homogenate and assay of oxidative metabolism—Tissue homogenate supernatant of experimental mice was used to evaluate antioxidant properties of BE and six compounds isolated from it. For this, liver, pancreas and kidney were removed, freed from adhering tissues, washed with ice-cold normal saline solution (0.9%), blotted dry and weighed. After mincing into small pieces, tissues were homogenized in ten times its volume of 0.2 M tris HCl with the help of homogenizer. The homogenate was centrifuged at 10,000 rpm for 20 min at 4°C and the supernatant was used for estimation of total protein (TP), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), reduced glutathione (GSH) and lipid peroxidation products (TBARS). Hepatic glycogen was measured according to the anthrone-H₂SO₄ method using glucose as standard.

Histopathological examination—Freshly dissected liver and pancreas of crude extract and C6 treated animals were fixed in 10% formalin, embedded in paraffin wax, serial sections of 5 micrometer were cut, stained with hematoxylin-eosin, mounted on glass slides and photomicrographed. The observations made were compared with normal control, diabetic control and glibenclamide treated groups.

Statistical analysis—Results are expressed as mean ± S.D., analyzed by one-way ANOVA, followed by Tukey’s post-hoc multiple comparison test using SPSS (version 16.0). P < 0.05 was the established level of significance. Whenever sphericity was significant, degrees of freedom and F values were corrected by Huynh-Feldt epsilon.

Results

Effect on FBG level—Fasting blood glucose (FBG) level in normal control group (I) remained unchanged throughout the experimental period. Whereas, in diabetic control group (II) increase in FBG level by 67.8% was persistently observed subsequent to alloxan administration (Fig. 2). Likewise, in groups III to X the escalation discovered following alloxan imposition was 73.4, 65.0, 61.5, 62.9, 63.8, 62.6, 67.4 and 67.0%, respectively. However, treatment of group III with reference drug glibenclamide significantly declined the FBG content by 49.3%. BE interposition reduced the FBG level by 70.9%. But amongst the
6 isolated compounds only 3 i.e., C1, C4 and C6 were capable in subsiding the exaggerated level of FBG appreciably by 20.6, 41.2 and 54.9% in the respective treated groups. Still FBG level of C1 treated group lies in moderately diabetic range and that of C4 lies in marginally diabetic range. Only C6 treatment proved potent enough in restoring the FBG level within normal range.

Effect on liver glycogen content—The liver glycogen content was found to be significantly lowered (by 53.9%) in alloxan treated diabetic animals (Fig. 3) as compared to normal ones. However, glibenclamide, BE, C4 and C6 treatment markedly enhanced the hepatic glycogen content by 82.1, 85.2, 59.6 and 73.1%, respectively as compared to diabetic control group.

Effect on oxidative metabolism—Activity of various antioxidant enzymes such as SOD, CAT, GSH-Px and concentration of GSH was monitored to be markedly reduced in the liver, pancreas and kidney of diabetic control group (Table 1). Amongst various treatments only BE, C4, C5 and C6 exerted a protective effect on significantly decreased activity of these antioxidant enzymes in all the three tissues. However, all the herbal treatments, i.e. crude extract and 6 isolated compounds produced a pronounced escalation in the GSH content. Contrarily the elevated level of hepatic, pancreatic and renal TBARS of diabetic animals was significantly diminished in all the treatment provided groups. Furthermore, effect of various herbal treatments was better than the standard drug, glibenclamide.

Table 1— Effect of crude extract and isolated compounds on hepatic, pancreatic and renal antioxidant parameters of alloxan-induced diabetic mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TBARS</th>
<th>GSH</th>
<th>SOD</th>
<th>CAT</th>
<th>GSH-Px</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC Liver</td>
<td>43.9 ± 6.3</td>
<td>6.1 ± 0.6</td>
<td>308.5 ± 9.2</td>
<td>233.0 ± 12.7</td>
<td>689.5 ± 26.9</td>
</tr>
<tr>
<td>Pancreas</td>
<td>9.6 ± 1.3</td>
<td>5.6 ± 0.7</td>
<td>321.7 ± 20.4</td>
<td>266.4 ± 18.4</td>
<td>721.0 ± 7.7</td>
</tr>
<tr>
<td>Kidney</td>
<td>110.1 ± 4.9</td>
<td>5.9 ± 0.7</td>
<td>333.1 ± 29.2</td>
<td>272.1 ± 21.5</td>
<td>723.2 ± 57.2</td>
</tr>
<tr>
<td>DC Liver</td>
<td>1425 ± 122</td>
<td>3.9 ± 0.7</td>
<td>238.1 ± 22.3</td>
<td>176.7 ± 19.9</td>
<td>453.1 ± 20.7</td>
</tr>
<tr>
<td>Pancreas</td>
<td>419.6 ± 25.7</td>
<td>3.9 ± 1.0</td>
<td>317.5 ± 19.6</td>
<td>167.5 ± 6.7</td>
<td>481.7 ± 20.9</td>
</tr>
<tr>
<td>Kidney</td>
<td>964 ± 119.7</td>
<td>4.1 ± 0.7</td>
<td>299.6 ± 28.8</td>
<td>161.9 ± 10.6</td>
<td>377.7 ± 42.9</td>
</tr>
<tr>
<td>GT Liver</td>
<td>1001 ± 85.6</td>
<td>12.3 ± 1.0</td>
<td>192.2 ± 6.2</td>
<td>197.6 ± 12.0</td>
<td>287.5 ± 27.9</td>
</tr>
<tr>
<td>Pancreas</td>
<td>345.6 ± 30.5</td>
<td>12.2 ± 0.9</td>
<td>358.0 ± 46.8</td>
<td>277.1 ± 15.6</td>
<td>534.1 ± 19.1</td>
</tr>
<tr>
<td>Kidney</td>
<td>638.8 ± 67.7</td>
<td>12.3 ± 0.9</td>
<td>294.6 ± 25.2</td>
<td>245.8 ± 16.4</td>
<td>420.2 ± 16.6</td>
</tr>
<tr>
<td>BE Liver</td>
<td>323.2 ± 18.7</td>
<td>10.3 ± 0.3</td>
<td>269.3 ± 14.3</td>
<td>272.5 ± 15.8</td>
<td>580.1 ± 22.7</td>
</tr>
<tr>
<td>Pancreas</td>
<td>280.4 ± 19.4</td>
<td>10.2 ± 0.3</td>
<td>390.7 ± 23.8</td>
<td>415.9 ± 37.3</td>
<td>934.8 ± 104.7</td>
</tr>
<tr>
<td>Kidney</td>
<td>560.7 ± 21.9</td>
<td>10.6 ± 0.5</td>
<td>274.2 ± 16.5</td>
<td>267.7 ± 24.5</td>
<td>576.9 ± 22.3</td>
</tr>
<tr>
<td>C1 Liver</td>
<td>232.8 ± 24.1</td>
<td>9.7 ± 0.2</td>
<td>175.6 ± 8.9</td>
<td>203.3 ± 25.9</td>
<td>360.5 ± 34.0</td>
</tr>
<tr>
<td>Pancreas</td>
<td>292.6 ± 24.6</td>
<td>9.3 ± 0.2</td>
<td>370.9 ± 19.6</td>
<td>382.2 ± 33.7</td>
<td>664.5 ± 24.4</td>
</tr>
<tr>
<td>Kidney</td>
<td>441.9 ± 24.9</td>
<td>9.3 ± 0.5</td>
<td>276.8 ± 20.3</td>
<td>319.5 ± 22.7</td>
<td>491.7 ± 40.9</td>
</tr>
<tr>
<td>C2 Liver</td>
<td>416.7 ± 23.9</td>
<td>9.7 ± 0.2</td>
<td>66.6 ± 8.6</td>
<td>217.1 ± 14.8</td>
<td>313.1 ± 15.8</td>
</tr>
<tr>
<td>Pancreas</td>
<td>362.9 ± 21.1</td>
<td>9.2 ± 0.6</td>
<td>302.7 ± 34.7</td>
<td>282.8 ± 27.6</td>
<td>594.3 ± 42.1</td>
</tr>
<tr>
<td>Kidney</td>
<td>451.6 ± 23.6</td>
<td>9.6 ± 0.6</td>
<td>285.8 ± 23.8</td>
<td>328.1 ± 16.8</td>
<td>512.2 ± 38.5</td>
</tr>
<tr>
<td>C3 Liver</td>
<td>348.9 ± 31.9</td>
<td>6.1 ± 0.7</td>
<td>189.1 ± 16.8</td>
<td>194.1 ± 9.1</td>
<td>395.6 ± 15.7</td>
</tr>
<tr>
<td>Pancreas</td>
<td>345.9 ± 28.9</td>
<td>6.2 ± 0.4</td>
<td>319.6 ± 13.9</td>
<td>287.2 ± 23.1</td>
<td>544.4 ± 60.8</td>
</tr>
<tr>
<td>Kidney</td>
<td>386.7 ± 20.6</td>
<td>6.2 ± 0.6</td>
<td>295.7 ± 12.7</td>
<td>310.1 ± 29.6</td>
<td>645.6 ± 53.5</td>
</tr>
<tr>
<td>C4 Liver</td>
<td>518.5 ± 37.3</td>
<td>13.3 ± 0.4</td>
<td>343.4 ± 40.3</td>
<td>375.8 ± 38.6</td>
<td>729.6 ± 52.7</td>
</tr>
<tr>
<td>Pancreas</td>
<td>287.1 ± 29.6</td>
<td>11.7 ± 0.6</td>
<td>448.6 ± 54.9</td>
<td>454.3 ± 42.8</td>
<td>880.7 ± 60.2</td>
</tr>
<tr>
<td>Kidney</td>
<td>495.3 ± 32.2</td>
<td>12.1 ± 0.3</td>
<td>472.4 ± 46.4</td>
<td>492.7 ± 14.9</td>
<td>827.3 ± 51.6</td>
</tr>
<tr>
<td>C5 Liver</td>
<td>337.5 ± 35.7</td>
<td>7.9 ± 0.2</td>
<td>213.5 ± 17.9</td>
<td>247.7 ± 6.1</td>
<td>550.6 ± 49.6</td>
</tr>
<tr>
<td>Pancreas</td>
<td>256.9 ± 24.3</td>
<td>7.9 ± 0.2</td>
<td>407.4 ± 38.9</td>
<td>363.5 ± 15.3</td>
<td>954.3 ± 41.1</td>
</tr>
<tr>
<td>Kidney</td>
<td>432.7 ± 8.6</td>
<td>7.7 ± 0.3</td>
<td>393.3 ± 32.4</td>
<td>385.0 ± 36.6</td>
<td>903.9 ± 93.5</td>
</tr>
<tr>
<td>C6 Liver</td>
<td>638.0 ± 47.6</td>
<td>8.1 ± 0.4</td>
<td>292.9 ± 29.5</td>
<td>488.1 ± 34.8</td>
<td>879.4 ± 62.4</td>
</tr>
<tr>
<td>Pancreas</td>
<td>318.1 ± 20.5</td>
<td>8.0 ± 0.5</td>
<td>411.3 ± 22.3</td>
<td>498.6 ± 42.3</td>
<td>1084.8 ± 84.4</td>
</tr>
<tr>
<td>Kidney</td>
<td>893.1 ± 34.9</td>
<td>8.2 ± 0.7</td>
<td>355.1 ± 26.2</td>
<td>443.1 ± 26.6</td>
<td>804.1 ± 65.6</td>
</tr>
</tbody>
</table>

Values are significant at $P<0.05$ compared to NC; $^*$ compared to DC; $^\dagger$ compared to GT.
Values are represented as GSH = (mg g$^{-1}$ tissue); TBARS = (nmol TBARS mg$^{-1}$ protein); SOD = Units min$^{-1}$mg$^{-1}$ protein; CAT = $\mu$ mole H$_2$O$_2$ decomposed min$^{-1}$mg$^{-1}$ protein; GSH-Px = $\mu$g GSH consumed min$^{-1}$mg$^{-1}$ protein
NC-normal control; DC-diabetic control; GT-(alloxan + glibenclamide); BE-crude extract; and C1-C6-isolated compounds treated
From the present results it was deduced that amongst the six isolated compounds, C6 was most potent in reducing the elevated level of FBG in diabetic animals. Moreover, it was also capable in combating the oxidative damage. So along with normal control, diabetic control, glibenclamide and BE treated groups, C6 treated group was also selected for histopathological study of liver and pancreas. Further, C6 was also chosen for identification and further characterization via HPLC and various spectroscopic methods.

Histopathological study—Liver of normal animals (group I) displayed normal histological details (Fig. 4a); contrarily in the liver of diabetic mice (group II), dilation in the sinusoids, atrophic hepatocytes, necrosis, degeneration and congestion was observed.

Fig. 4—(a)-Normal liver; (b)-Diabetic liver (showing degenerated structure); (c, d, e)-Glibenclamide, BE and C6 treated liver (showing normal structure); (f)-Normal pancreas (showing normal islet); (g)-Diabetic pancreas (showing degenerated islet); (h)-Glibenclamide and (i) BE treated pancreas (showing normal structure); and (j)-C6 treated pancreas (showing hyperplasia).
(Fig. 4b). However, the above deformities were not witnessed in glibenclamide treated (group III, Fig. 4c), BE treated (group IV, Fig. 4d), and C6 treated (group X, Fig. 4e) animals. This regenerative response may be due to beneficial and protective effect of the above three treatments.

Normal histological details were also monitored in the pancreas of group I (Fig. 4f). However, the pancreas of alloxan-administered diabetic mice of group II displayed damaged islets. Mild to severe atrophy of the islets of Langerhans was found to be the most striking feature in these animals (Fig. 4g). No discernible islets were found in severely and persistently involved diabetic animals. In some of the sections, the dimensions of the islet was considerably reduced and shrunken. However, in glibenclamide (Fig. 4h), BE (Fig. 4i) and C6 (Fig. 4j) treated mice, the islets were comparable to normal ones, nevertheless, traces of degenerative changes were still visible in some of the sections. In these three respective treatment provided groups, rare evidence of hyperplasia marked by increase in the cellular components was also witnessed. Thus, from the histological examination of pancreas it can be culminated that all the above three treatments restored the activity of islets of Langerhans as compared to diabetic control group II.

From the histopathological study of liver and pancreas, it can be outlined that alloxan administration severely deteriorated the histology of these tissues in group II. But glibenclamide, BE and C6 treatment to a certain extent restored the detected deformities, It can be concluded that further extension of these treatments for a prolonged period of time may prove fruitful in healing the damages completely.

**HPLC profile of crude extract, terpenoid standard (α-amyrin) and C6—**HPLC chromatogram of the crude extract shows 4 prominent peaks. One of the most prominent peaks was observed at the retention time 3.592 (Rt min, Fig. 5), which was similar to that observed in case of C6 and the standard. It might be terpenoid present in the extract. Other prominent peaks were recorded with the retention time 3.267, 3.417 and 4.442 (Rt min), respectively.

However, in HPLC chromatogram of C6, only 1 prominent peak was visible, at Rt (min) 3.675 (Fig. 6). In the chromatogram of C6 apart from this...
prominent peak, few inconspicuous peaks were also detected, which might be attributed to the presence of certain impurities in small concentration along with the isolated compound.

In the HPLC chromatogram of the triterpene standard (α-amyrin), 1 prominent peak was witnessed at the retention time 3.658 (Rt min, Fig. 7), which to an extent corresponded with that of C6.

Thus, the present results of HPLC confirmed triterpene nature of the isolated compound (C6).

**Characterization of compound 6 by spectral studies**

**IR spectrum**—IR spectrum of C6 on KBr pellet exhibited peaks at 3447.4 cm\(^{-1}\) for hydroxyl (-OH) group with H-bonded primary alcohol. The IR spectrum also exhibited a broad peak in the range of 3000 cm\(^{-1}\) to 2500 cm\(^{-1}\) for acidic group as well as a strong peak at 2924 cm\(^{-1}\) which clearly verified the presence of –CH\(_2\) group. The carboxylic group can also be verified by C-O peak at 1241 cm\(^{-1}\). Peak at 2853 cm\(^{-1}\) indicated the presence of –CHO group, whereas >C=O peak at 1735 cm\(^{-1}\) instead at 1745 cm\(^{-1}\) indicated the ring involvement or aromatic structure of the compound. Further, a peak at 1166 cm\(^{-1}\) was a clear evidence for the presence of ester group (νC-O) in the extracted compound. Moreover, two strong absorption peaks at \(\approx 920\) cm\(^{-1}\) and \(734\) cm\(^{-1}\) showed the presence of trans and cis olifinic bond, respectively in the present extracted compound (Fig. 8).

**\(^1\)NMR spectrum**—\(^1\)NMR spectrum of the extracted compound revealed a strong peak at 1.18 ppm which corresponds to chain of methylene proton. Further, the spectrum also confirmed the presence of olifinic functional group whose peak corresponded to value data 5.20 ppm and ring protons at 7.0 to 7.64 ppm. However, a shoulder of peaks at 4.0 to 4.5 ppm gave

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Fig. 7—HPLC chromatogram of triterpene standard, α-amyrin [showing 1 prominent peak at 3.658 min which corresponds to the only prominent peak of C6 (3.675 min) and third peak of crude extract (3.592)].

Fig. 8—IR spectrum (showing the presence of hydroxyl, acidic, carboxylic, aldehyde and ester groups).
an indication of the acidic proton present in the extracted compound (Fig. 9).

From the above spectral data it could be concluded that there might be an aldehydic group present at the saturated ring of the studied compound.

**Mass spectrum**—The highest molecular ion peak in the mass spectral data was recorded at ~ 469 m/z (Fig. 10) which corresponded to the molecular weight of the extracted compound.

**Discussion**

In the present study, a marked hike in the level of fasting blood glucose was witnessed in groups II to X subsequent to alloxan administration. The observed hyperglycemia may be due to glycogenolysis or gluconeogenesis\(^\text{17}\). However, 45 days treatment of diabetic animals with crude extract and compounds C1, C4 and C6 caused a significant reduction in FBG level. This antihyperglycemic action may be due to insulin potentiating effect via stimulation of the
undamaged or residual pancreatic islets to release insulin. Other possible mechanisms that can be attributed for the antihyperglycemic potential of the isolated compounds may be an inhibiting effect on aldose reductase, the first enzyme in Polyol pathway (as noticed in triterpenes mangiferin, tingenine B etc. isolated from the stems of Salacia chinensis) or insulin sensitizing potential (as witnessed for the triterpene dehydrotrametenolic acid isolated from dried sclerotia of Poria cocos). Further, level of glycogen, the primary intracellular storable form of glucose in various tissues is a direct reflection of insulin activity as insulin promotes intracellular glycogen deposition by stimulating glycogen synthetase and inhibiting glycogen phosphorylase. The observed depletion of liver glycogen stores in diabetic mice is consistent with an earlier study, indicating that it could be due to the loss of glycogen synthetase activating system and/or increased activity of glycogen phosphorylase which, in turn is due to marked decrease in insulin levels. Administration of crude extract and compounds C4 and C6 for 45 days showed a trend towards significant increase in glycogen content when compared to diabetic mice, thus confirming its insulin potentiating effect to a certain extent, which is comparable to that of glibenclamide. Whereas severity of diabetes mellitus entails an increasing resistance to the movement of glucose into the cells of extra hepatic tissues, crude extract and its active constituents seem to restore this process to the extent that are proportional to their antihyperglycemic efficacies. The antihyperglycemic efficacy of this herbal drug and the isolated compounds can be correlated with the findings of Chen et al. and Eddouks et al.

High reactivity of ROS in many diseases including diabetes may trigger disorders in the body resulting in tissue damage and necrosis in many instances. In the present study, necrosis was visible in the histological sections of liver and pancreas. Excessive oxidative stress and depleted antioxidant status also interfere with critical cellular activities and result in lipid peroxidation (LPO) that has shown to be increased in both insulin dependent diabetes (IDDM) and non-insulin dependent diabetes (NIDDM), even in patients without complications. Lipid peroxidation products are regarded as one of the major sources for endogenous genotoxic compounds. Oxidative stress during diabetes was evaluated in the present study by analyzing pro-oxidants and anti-oxidants (both enzymatic and non-enzymatic).

In this study, a considerable fall in the level of hepatic, pancreatic and renal GSH of diabetic mice was witnessed, which was consistent with an earlier report. Decrease in tissue GSH content could be the result of decreased synthesis or increased degradation of GSH by oxidative stress that prevails during diabetes. Furthermore, a marked increase in the concentration of LPO products (TBARS) was also monitored in the tissues of diabetic mice; however, treatment of these animals with crude extract as well as the isolated compounds decreased the elevated level of TBARS. Simultaneously, reduced GSH content was also increased significantly which indicates that these herbal treatments could either increase the biosynthesis of GSH and/or reduce the oxidative stress that ultimately reduced the degradation of GSH. Similar results have also been witnessed in an earlier study.

Oxidative stress results in the loss of key antioxidant enzymes. Data of the present study also revealed a declined content of antioxidant marker enzymes such as CAT, GSH-Px and SOD in hepatic, pancreatic and renal tissues of diabetic animals. Glucose that forms Schiff’s base with proteins has been reported to have high affinity for proteins specially those containing transition metal ions, thus reducing their activities during glycation-induced oxidative stress. Since, glycation mediated reaction of glucose with proteins, including antioxidant enzymes is inevitable under diabetic conditions; therefore, formation of ROS is also unavoidable. However, crude extract of B. monosperma seeds increased the activity of CAT and GSH-Px in the tissues of treated diabetic animals. These findings can be complemented with the study of Gokce & Haznedaroglu. The antioxidant activity of the test drug might be due to inhibition of glycation of antioxidant enzymes. Increased activity of CAT and GSH-Px suggested a compensatory response to oxidative stress as it reduced the endogenous H2O2 produced, thus diminishing the toxic effects due to this radical or other free radicals derived from secondary reactions. However, elevation monitored in the activity of SOD, in any of the tissues of crude extract treated mice was statistically insignificant. This perhaps is due to different metabolic actions of the tissues and their different responses to oxidative stress. Furthermore, amongst the six isolated compounds, C4, C5 and C6 were more influential than rest of the three (C1, C2 and C3), regarding their
antioxidant potential. When the anti-oxidative aptitude of crude extract was contrasted with the individual isolated compound, the superior consequence of the latter was explored out. The greater antioxidative potential of the individual isolated compound compared to crude extract can be attributed to the antagonistic effect of C1, C2 and C3 over C4, C5 and C6.

Histopathology of pancreas from crude extract, C6 and glibenclamide treated groups was performed to detect any protective or harmful effect of these treatments under diabetic conditions. In these treatments provided groups, there were more islets (in comparison to diabetic control group II) and they were comparable to the islets of normal control group I. Somewhat similar observations have been also reported in earlier studies. Apart from pancreatic β-cells, alloxan has also been reported to attack the cells of liver and kidney. In the liver of alloxan-diabetic mice, dilation in the sinusoids and inflammation was noticed. The histological findings were in agreement with the degenerative structural changes reported in the liver tissues as a result of insulin depletion in diabetic animals. However, the degenerative changes witnessed were restored in glibenclamide, crude extract, C4 and C6 treated groups. This might be due to the beneficial and protective effect of the undertaken treatments on the liver and pancreas of diabetic mice. These observations were also consistent with an earlier report.

The functional groups detected in the isolated compound C6 were in accordance with the study of Sharma et al. who have also reviewed the presence of similar functional groups in the Laccijalaric ester-I, a triterpene present in soft resin of B. monosperma seeds. Further, Rf of C6 and Laccijalaric ester-I was almost similar (0.90) in the solvent system benzene-acetic acid (for C6) and benzene-ethyl acetate-acetic acid (for Laccijalaric ester-I), thus indicating somewhat similar nature of the two compounds.

It can be concluded that crude extract of B. monosperma seeds and some of its active constituents possess significant antihyperglycemic and antioxidative properties. However, further studies are needed to investigate and elucidate the possible mechanism of action of the active ingredients, establish complete safety profiles and evaluate the potential value of crude extract and its active constituents for the management of diabetes and associated complications in the clinic.

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