Antidiabetic effect of aqueous extract of butea monosperma (LAM) Taub bark

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ABSTRACT
Herbal preparation of Butea monosperma (Lam.) Taub. bark had been considered as effective, economical and safe ethnomedicine for various ailments in Indian traditional system of medicine. The present study was aimed to investigate scientifically the antidiabetic potential of B. monosperma bark. Both kinds diabetes; insulin dependent diabetes mellitus (IDDM) and non insulin dependent diabetes mellitus (NIDDM) were induced in the rats by treating with alloxan monohydrate (150 mg/kg b.wt; ip) and hydrocortisone (5mg/100 g b.wt; ip) respectively. Fasting plasma glucose (FPG) levels were measured at periodic intervals during the test period. The blood samples were collected with care sino-ocular puncture method and serum was isolated by centrifugation to analyze plasma glucose and serum lipid profile. The results of preliminary phytochemical analysis depicts that B monosperma bark has the presence of steroids and tannins and absence of terpenoids, glycosides, alkaloids and flavonoids. The moisture content and total ash values of bark was 3.0%and 9.7% respectively. The treatment with bark aqueous extract of B monosperma substantially declined the plasma glucose level in both IDDM and NIDDM animal subjects by 7.2% and 26.6% respectively. This treatment also appreciably (P= 0.05 and P=0.01) lowered the serum lipid profile. In conclusion, the aqueous extract of Butea monosperma reflected hypoglycemic and hypocholesterolemic potential through glucose and lipid profile lowering activity in experimental animals. It supported the folklore state of antidiabetic potential of the plant.

Introduction
Diabetes is an intricate and multifarious group of disorders characterized by hyperglycemia that has reach epidemic extent in the present century1. The prevalence of diabetes is rapidly rising all over the globe at an alarming rate2. Over the past 30 yrs, the status of diabetes has changed from being considered as a mild disorder of the elderly to one of the major causes of morbidity and mortality affecting the youth and middle aged people. It is important to note that the rise in prevalence is seen in all six inhabited continents of the globe3. Several drugs such as biguanides and sulfonylureas are presently accessible to reduce hyperglycaemia in diabetes mellitus. These drugs have side effects and thus searching for a new class of compounds is essential to overcome this problems4. Management of diabetes without any side effects is still a challenge to the medical community. Plant kingdom represents a rich house of organic compounds, many of which have been used for medicinal purposes and could serve as lead for the development of novel agents having good efficacy in various pathological disorders in the coming years5,6. Herbs have always been the principal form of medicine in India and presently they are becoming very popular throughout the world, as people strive to stay healthy in the face of chronic stress and pollution, and to treat illness with medicines that work in count with the body’s own defense. Given a reasonable probability that medicinal plants with a long history of human use will eventually yield novel drug prototypes, systematic and intensive search in plants for new drugs to treat Type 2 diabetes mellitus seem to be of great utility.

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matters and commonly used as tonic, astringent, aphrodisiac and diuretics\(^1\). Roots are useful in filariasis, night blindness, helminthiasis, piles, ulcer and tumours. It is reported to possess antifertility, aphrodisiac and analgesic activities\(^1\). Flowers are free radical scavenging, anti-diabetic, hepatoprotective and anti-diarrheal\(^2\). The stem bark is useful in indigenous medicine for the treatment of dyspepsia, diarrhoea, dysentery, ulcer, sore throat and snake bite. The shoots are clothed with gray or brown silky pubescence. The bark is fibrous and ash coloured and reported to possess astringent bitter, pungent, alliterative, silky pubescence. The bark is fibrous and ash coloured and the treatment of dyspepsia, diarrhoea, dysentery, ulcer, sore

**Experimental design**

Puncture method and serum was separated by centrifugation.

**Sample collection**

Rats were made IDDM diabetic by single intraperitoneal injection of alloxan monohydrate (Koch light laboratories ltd; 150mg/kg body weight) for 3 consecutive days\(^9\). Alloxan was first weighted individually for each animal according to weight (solubilized with 0.2ml saline) first prior to injection. Three days after alloxan injection, rats with plasma glucose levels of >200 mg/dl were included in the study. Like wise, rats were made NIDDM diabetic by administration of dissolved hydrocortisone sodium succinate (Glaxo Smith Kline, Pharmaceutical Ltd; 5 mg/100g b w, i.p. for eight consecutive days). NIDDM were confirmed in 48h after last cortisone dose administration. Only rats with glucose level >140mg/dl were used for the study.

**Induction of diabetes in rats**

Rats were made IDDM diabetic by single intraperitoneal injection of alloxan monohydrate (Koch light laboratories ltd; 150mg/kg body weight) for 3 consecutive days\(^9\). Alloxan was first weighted individually for each animal according to weight (solubilized with 0.2ml saline) first prior to injection. Three days after alloxan injection, rats with plasma glucose levels of >200 mg/dl were included in the study. Like wise, rats were made NIDDM diabetic by administration of dissolved hydrocortisone sodium succinate (Glaxo Smith Kline, Pharmaceutical Ltd; 5 mg/100g b w, i.p. for eight consecutive days). NIDDM were confirmed in 48h after last cortisone dose administration. Only rats with glucose level >140mg/dl were used for the study.

**Biochemical Analysis**

Biochemical parameters plasma glucose estimated by GOD-POD method\(^21\), Serum total cholesterol (S. TCh) estimated by CHOD/PAP method\(^22\), Serum Triglyceride (S. TG) carried out by enzymatic method\(^23\) and Serum high density lipoprotein – cholesterol (S. HDL-C)\(^24\) and Serum low density lipoprotein – cholesterol (S. LDL-C) and very low density lipoprotein-cholesterol (S VLDL-C)\(^25\) calculated as per equation:

\[
\text{VLDL-C} = \text{Serum TG/5} \\
\text{LDL-C} = \text{Serum T Ch-(Serum VLDL-C + Serum HDL-C)} \\
\text{Serum albumin was estimated by BCG method}\(^26\) and Serum urea was carried out by enzymatic method\(^27\).

**Statistical Analysis**

All the values of plasma glucose level and other biochemical estimations were expressed as mean±SD are analysed for student’s t test differences between the groups were considered significant at \(P \leq 0.05\) & \(P \leq 0.01\) levels.

**Abbreviations**

AllXX, Alloxan; DM, Diabetes Mellitus; Bmb, Butea monosperma Bark; FPG, Fasting plasma glucose, TCh, Total cholesterol; TG, Triglyceride, HDL-C, High density lipoprotein-Cholesterol; LDL-C, Low density lipoprotein-Cholesterol, IDDM, Insulin dependent diabetes mellitus; NIDDM, Non insulin dependent diabetes mellitus; GOD–POD, Glucose oxidase peroxidase ; CHOD/PAP, Cholesterol hydrolysis and oxidation.
Results
Preliminary phytochemical screening

The phytochemical parameters like foreign matter (2.0%), Extractive alcohol and water soluble values (15.5 and 16.0%), moisture content (3.0%), total ash (9.70%) and acid insoluble ash (1.3%) of bark was investigated. Prelude phytochemical screening of bark showed the existence of steroids, tannins and glycosides, terpenoids, alkaloids and flavonoids were lacking in bark of Butea monosperma as depicted in table1.

Effect of aqueous extract Bmb on normoglycemic rats

Table 2 reveals the effect of aqueous extract of Bmb on fasting plasma glucose of both alloxan & hydrocortisone induced diabetic rats. In test animals suffering from IDDM (alloxan; group D), the fasting plasma glucose level was 241.80±7.70mg/dl for group D, which substantially declined to 224.20±5.21 mg/dl after 28 days of intervention. Hence, 7.2% reduction was noticed due to treatment of aqueous extract of Bmb in a dose of 1.25 g kg⁻¹ b w.t. Similarly, NIDDM subjects (group F) were also significantly reduced as marked from144.6±18.90 to 106.01±22.01 mg/dl after 28 days of intervention i.e 26.6%, notably reduction was seen in comparison to group E (diabetic control C3 for NIDDM).

The data has been pinpointing the momentous effect of intervention at P<0.01 level when the values were compared to the onset values. In other evaluation at the end (28th day) of the study, the final values of both experimental groups (D&F) were compared with that of placebo groups (C&E) and again it went to point towards a significant difference at P<0.01.

Effect on fasting Plasma glucose (FPG) level on diabetic rats

In all groups, prior to alloxan and hydrocortisone administration, the basal blood glucose levels of rats were not significantly different. However after alloxan and hydrocortisone blood glucose level were significantly higher i.e. above >200 mg/dl for IDDM and >140 mg/dl for NIDDM group and these animals were selected for the study. The non diabetic control (group A) and treated (group B) with aqueous extract of Bmb remained constantly euglycemic through the course of the study.

Table 2 depicts the effect of aqueous extract of Butea monosperma bark on fasting plasma glucose level of both alloxan & hydrocortisone induced diabetic rats. In test animals suffering from IDDM (alloxan; group D), the fasting plasma glucose level was 241.80±7.70mg/dl for group D, which substantially declined to 224.20±5.21 mg/dl after 28 days of intervention. Hence, 7.2% reduction was noticed due to treatment of aqueous extract of Bmb in a dose of 1.25 g kg⁻¹ b wt. Similarly, NIDDM subjects (group F) were also significantly reduced as marked from144.6±18.90 to 106.01±22.01 mg/dl after 28 days of intervention i.e 26.6%, notably reduction was seen in comparison to group E (diabetic control C3 for NIDDM).

The results indicate that the extract of Butea monosperma bark decreases the serum glucose in normal rats as compared to the normal control C1 groups. The maximum hypoglycemic activity of extract was observed in NIDDM animal models that is 26% This is might be due to increased peripheral glucose utilization or potentiating of insulin effect. Stigmasterol, is sterol isolated from the bark of Butea monosperma (2.6 mg/kg/d for 20 days) was evaluated for thyroid hormone and glucose regulatory efficacy in mice. The result showed its thyroid inhibiting and hypoglycemic properties. Antioxidative potential due to decrease in the hepatic lipid peroxidation and an increase in the activities of catalase, superoxide dismutase and glutathione 33. Similar study indicated by34 that the single dose treatment of ethanolic extract of Butea monosperma flowers at the dose of 200mg/kg P.O. significantly improved glucose tolerance and cause reduction in blood glucose level in alloxan induced diabetic Rats. Oral administration of the ethanolic extract of the Butea monosperma seeds at the dose of 300mg/kg b.w., exhibited significant anti diabetic, hypolipidaemic and antiperoxidative effects35. A similar kind of study conducted in the bark of Ficus hispida has shown a significant blood glucose reducing effect in normal and diabetic rats36. The oral administration of bark extract at 1.25mg kg⁻¹ showed the significant decrease in the fasting glucose level at the end of study after 28 days. According to study conducted by37, treatment of diabetes mice with ethanolic extract of Butea monosperma (300mg/kg body wt) for 45 days caused significant reduction in fasting blood glucose level.

The elevated T-Ch, TG, LDL-C and VLDL-C decreased HDL-C level in both alloxan and hydrocortisone induced diabetic rats were in agreement with previous reports regarding alteration of these parameters under diabetics induced
hyperlipidemia might be due to excess mobilization of fat from the adipose tissue because of underutilization of glucose\textsuperscript{38}. The mean serum T-Ch of the experimental groups had been significantly reduced after administration of Bmb aqueous extract when compared with placebo groups. TG and VLDL-C which influence lipid deposition are clotting mechanism have been reduced significantly in hydrocortisone diabetic rats through the reduction was not sizable in alloxan diabetic rats. As the Bmb aqueous extract have been found to be positive modulator of lipid profile of diabetic subjects in being hypocholesterolemic with respect to T Ch,TG, LDL-C and VLDL-C level in serum, they also have static effect on HDL-C which can build synergy of their effects. HDL-C appears to remove cholesterol from the walls of arteries and returns it to the liver and reduces the risk of heart attack\textsuperscript{39}. Thus, they can improve the lipid profile along with serum urea and serum albumin. In study conducted by \textsuperscript{40}, has been observed that administration of Azadirachta indica seed kernel powder significantly decreased the concentration of serum lipids are blood glucose in alloxan diabetic rats. In another study concluded by\textsuperscript{41} that leaf extract of Aegle marmelos and aqueous extract of Terminalia arjuna were reported to act as hypocholesterolemic\textsuperscript{32}. The efficacy of the bark extract of the test plant in lessening diabetes mainly depends on the presence of certain active principles. However, few active principles of Butea monosperma are already known. From the study, we can conclude that aqueous extract of Butea monosperma bark has beneficial effect on blood glucose level. It has the credible to report therapeutic effect in diabetes. Further pharmacological and biochemical studies are required to elucidate the mechanism action of the extracts in details at molecular level and also need to investigate the antioxidant potential are free radicals.

References:

### Table 1: Preliminary Phytochemical Analysis of Butea monosperma (Lam.)Taub Bark

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Butea monosperma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foreign matter(%w/w)</td>
<td>2.0%</td>
</tr>
<tr>
<td>Extractive value(%w/v)</td>
<td></td>
</tr>
<tr>
<td>Alcohol soluble</td>
<td>15.5%</td>
</tr>
<tr>
<td>Water soluble</td>
<td>16.0%</td>
</tr>
<tr>
<td>Moisture content</td>
<td>3.0%</td>
</tr>
<tr>
<td>Ash values</td>
<td></td>
</tr>
<tr>
<td>Total Ash (%w/w)</td>
<td>9.7%</td>
</tr>
<tr>
<td>Ash insoluble ash (%w/w)</td>
<td>1.3%</td>
</tr>
<tr>
<td>Chemical Tests</td>
<td></td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-ve</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-ve</td>
</tr>
<tr>
<td>Steroids</td>
<td>+ve</td>
</tr>
<tr>
<td>Saponins</td>
<td>-ve</td>
</tr>
<tr>
<td>Tannins</td>
<td>+ve</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-ve</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-ve</td>
</tr>
</tbody>
</table>

### Table 2: Effect of daily oral dose of aqueous extract of Butea monosperma bark (Bmb) on serum glucose of alloxan & hydrocortisone rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatments</th>
<th>Mean Serum glucose level mg/dl</th>
<th>%Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Treatment</td>
<td>AT1</td>
<td>AT2</td>
</tr>
<tr>
<td>A</td>
<td>Normal</td>
<td>80.98±10.8</td>
<td>82.98±9.80</td>
</tr>
<tr>
<td>B</td>
<td>Normal+Aqueous Extract Bmb</td>
<td>85.35±6.24</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>Diabetic Control C2 (Alloxan)</td>
<td>66.91±7.5</td>
<td>237.12±10.1</td>
</tr>
<tr>
<td>D</td>
<td>Alloxan+Aqueous Extract Bmb</td>
<td>66.13±3.81</td>
<td>241.80±7.70</td>
</tr>
<tr>
<td>E</td>
<td>Diabetic Control C3 (HYD CORT)</td>
<td>71.50±4.30</td>
<td>138.70±17.21</td>
</tr>
<tr>
<td>F</td>
<td>HYD CORT+Aqueous Extract Bmb</td>
<td>75.37±10.9</td>
<td>144.61±18.9</td>
</tr>
</tbody>
</table>

Value: mean±SD (n=8)
ap ≤ 0.05, bpc ≥ 0.05 when AT values compared with AT4 values of respective groups .
dpc ≤ 0.05, cp ≤ 0.01, fp ≥ 0.05 when AT4 values of control (C2 & C3) compared with experimental groups (D & F)
Group A- Normal control (C1)
Group B- normal treated + Aqueous extract of Bmb.
Groups (C&E)-Diabetic control (C2& C3)
Groups (D&F) given test sample (Aqueous extract of Bmb)
BT- Before treatment, AT- After Alloxan/ Hydrocortisone
AT1, AT2, AT3, AT4 are alloxan/ Hydrocortisone treated after 7, 14, 21 and 2 days intervention.
Table 3. Effect of aqueous extract of Butea monosperma Bark (Bmb) on serum lipid profile (150mg/kg bw) and hydrocortisone (5 mg/100g bw) induced diabetic rats after 28 days of treatment.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatments</th>
<th>Serum TCh</th>
<th>Serum TG</th>
<th>Serum HDL-C</th>
<th>Serum LDL-C</th>
<th>Serum VLDL-C</th>
<th>Serum Albumin</th>
<th>Serum Urea</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Normal Control C1</td>
<td>98.6±4.80</td>
<td>106.31±10.70</td>
<td>34.6±4.20</td>
<td>44.90±5.4</td>
<td>23.27±2.01</td>
<td>2.86±0.24</td>
<td>34.45±2.25</td>
</tr>
<tr>
<td>B</td>
<td>Normal Control C1 + Aqueous Extract Bmb</td>
<td>86.78±8.0</td>
<td>107.45±8.9</td>
<td>35.8±5.2</td>
<td>42.9±4.8</td>
<td>21.72±0.92</td>
<td>3.7±1.48</td>
<td>35.82±3.20</td>
</tr>
<tr>
<td>C</td>
<td>Diabetic Control C2 (ALLXN)</td>
<td>225.80±14.0</td>
<td>107.10±7.8</td>
<td>39.60±3.43</td>
<td>164.78±9.8</td>
<td>21.42±1.61</td>
<td>2.21±0.34</td>
<td>37.81±2.80</td>
</tr>
<tr>
<td>D</td>
<td>ALLXN+ Aqueous Extract Bmb</td>
<td>186.70±7.20</td>
<td>106.80±8.21</td>
<td>36.30±1.71</td>
<td>129.04±6.2</td>
<td>21.36±1.91</td>
<td>2.31±0.24</td>
<td>36.28±2.81</td>
</tr>
<tr>
<td>E</td>
<td>Diabetic Control C3 (HYD CORT)</td>
<td>174.80±14.0</td>
<td>126.20±15.2</td>
<td>35.80±3.41</td>
<td>113.76±9.80</td>
<td>25.4±2.41</td>
<td>2.4±0.25</td>
<td>35.82±3.81</td>
</tr>
<tr>
<td>F</td>
<td>HYD CORT+ Aqueous Extract Bmb</td>
<td>117.0±15.8</td>
<td>106.20±15.21</td>
<td>32.83±2.82</td>
<td>59.96±10.8</td>
<td>21.21±2.82</td>
<td>5.88±0.26</td>
<td>34.92±2.62</td>
</tr>
</tbody>
</table>

Value: mean±SD (n=8)
ap≤0.05, b≤0.01, c≥0.05 when AT4 value of control groups A, C&E (normal alloxan and hydrocortisone induced diabetic rats) compared with experimental groups (B,D&F).

Group A- Normal control (C1)
Group B- Normal + Aqueous extract of Bmb.
Groups (C&E)- Diabetic control (C2&C3)
Groups (D&F) given test sample (Aqueous extract of Bmb)