HYPOGLYCEMIC ACTIVITY OF MEMECYLON UMBELLATUM LEAVES METHANOLIC EXTRACT

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ABSTRACT

Objective: To evaluate the hypoglycemic potential of methanolic extract of Memecylon umbellatum leaves. Methods: The methanolic extract was prepared by successive Soxhlet extraction with solvents of varying polarity. The diabetes was chemically induced in Swiss albino rats by a single dose of alloxan (120mg/kg, i.p.). After the development of diabetes, methanolic extract of the leaves was administered (i.p.) at two different concentrations (75 and 150mg/kg) and analyzed the change in sugar level. Acute toxicity studies were performed by checking the altered levels of urea, creatinine, serum glutamate oxaloacetate transaminase (SGOT), and serum glutamate pyruvate transaminase (SGPT). Results: The administration of methanolic extract of the plant resulted in significant reduction of blood glucose level (p<0.001) in diabetic rats. The acute toxicity studies revealed no side effects on the liver and kidney as evident by the level of urea and creatinine. The extract exhibited significant reduction in urea and creatinine level (p<0.001) as well as SGPT and SGOT values (p<0.01) as compared to diabetic control. The chronic studies confirmed a gain in body weight of the extract administered rats as compared to diabetic controls (p<0.01). Conclusion: Our studies indicate that M. umbellatum, an endangered medicinal plant from the Western Ghats of Karnataka, possesses a very good hypoglycemic activity. Isolation, purification and identification of the active ingredient present in the methanolic extract should reveal the usefulness of this molecule for further detailed studies and commercial exploitation.

Key words: Memecylon umbellatum, antidiabetic activity, alloxan, medicinal plant.
1. INTRODUCTION

The traditional medicine system across the world is gaining increased attention and has provided novel active molecules for the effective treatment of various diseases. In India, ancestors have effectively used various medicinal plants for the treatment of several diseases and the traditional healers in rural areas are still using many of these plants even today, although there is a lack of proper documentation. The traditional system of medicine such as Siddha, Ayurveda and Unani are based on the use of plants and its derivatives for improvement of health \[1,2,3\]. Among these, *Memecylon umbellatum*, a small shrub of melastomataceae family is a plant from Western Ghats in the state of Karnataka, which holds a very good medicinal value. This plant is being used for the treatment of fever and other illnesses. Although it has shown to be very effective as an anti-diabetic, no systemic and scientific evaluation has been carried out so far.

Diabetes mellitus (DM) is a chronic disease responsible for the death of more than two million people worldwide annually. DM is characterized by a reduced level of insulin or inefficiency of insulin resulting in deviation of normal carbohydrate, protein and fat metabolism thereby affecting nerve cells and other organs of the body. DM is also responsible for the increased risk of cardiovascular diseases. In Asia, it is estimated that the rate of diabetes may increase about two to three folds over the next 25 years \[4\]. Diabetes is becoming an epidemic in India and leading to largest number of diabetic patients across the world giving the crown as “diabetic capital of world” \[5,6\]. The WHO estimates that the number of diabetic cases is going to be more than 300 million by 2025\[7\]. Although insulin and other hypoglycemic drugs are available in the market, these drugs show significant side effects or prolonged use of these drugs reduces the efficiency \[8\]. Recently, the tendency to use traditional medicine is on the rise as these lack side effects compared to synthetic drugs. Various medicinal plants have been used in the traditional system to treat DM and more than 800 plants with hypoglycemic activity have been identified which lacks systemic evaluation and scientific validation \[2\]. The hypoglycemic activity of these plants has been attributed to the flavonoids, alkaloids, triterpenes and glycans content \[9\]. The hypoglycemic effect of many of plants such as *Aporosalindleyana* Baill (Euphorbiaceae), *Myrtus communis* L. (Myrtaceae), *Trigonella foenumgraecum* L. (Leguminosae), *Cuecuma longa* L. (Zingiberaceae) has been confirmed and further studies have been undertaken for determining the mechanism of action \[2,3,1\]. In this study, we prepared the methanolic extract of *M. umbellatum* leaves to assess its anti-diabetic activity.
2. MATERIALS AND METHODS

2.1 Preparation of Plant Extracts
The plant was collected from HulikalGhat near Hosanagara, a part of Western Ghats in the state of Karnataka, India. The plant was authenticated and voucher specimen is kept in the department of botany, Kuvempu University, India. The plant leaves were collected and washed with distilled water followed by 30% alcohol. The leaves were shade dried and grinded well to make it as a fine powder. This powdered material was used to prepare the extract in solvents of varying polarity.

A known amount of finely grinded powder was refluxed in Soxhlet’s apparatus for 48 h successively with petroleum ether (bp 40-60° C), chloroform and methanol (Merck, AR grade). The extracts were concentrated under reduced pressure, stored at 4° C until further use. Based on the preliminary results, only methanolic extract was further used for determining its hypoglycemic effect.

2.2 Determination of LD$_{50}$ Value
Swiss albino wistar rats of either sex were used to test the hypoglycemic activity of extract in vivo. Rats were obtained from Bangalore veterinary college, Bangalore, and housed in temperature controlled room (25±1°C), provided with 12 h light/dark cycle. The rats were fed with standard diet and had continuous access to water ad libitum unless it is restricted by protocol or assay.

LD$_{50}$ was determined according to method as described earlier$^{[10]}$. In the first phase, rats were randomly grouped into three groups of three animals each and were administered with different doses of extract intraperitonially (ip). The first group was administered with 1000mg/kg, second with 100mg/kg and third group with 10mg/kg body weight in one ml using Tween 20 as the vehicle. Change in the behavior, any signs of toxic symptoms and mortality was observed for the next 24 h. In the second phase four rats were divided into four groups of one rat each which received aipdosage of 500, 1500, 2000, 2500mg/kg, respectively. LD$_{50}$ value was calculated as geometric mean of highest non-lethal dose and lowest lethal dose.

2.3 Induction of Diabetes Mellitus
Diabetes was induced to rats by administering single dose of (150mg/kg, ip) alloxan monohydrate (prepared fresh in PBS)$^{[11,12]}$. Standard pellet diet and water were provided after
the administration. The diabetic condition was maintained for 21 days as described above. A drop of blood from prick at the tail vein was dropped on to glucometer (Ascensia Entrust, Bayers health care) to check the level of glucose. Rats showing glucose level >250mg/dl were considered for testing hypoglycemic activity.

The diabetes induced rats were grouped randomly into four groups of six each. They were provided with standard pellet and water supplied at *ad libitum*. The first group serves as healthy control; group II serves as diabetic control, group III and IV received methanolic extract (75mg/kg and 150mg/kg body weight, ip) respectively. The amount of glucose in the blood was recorded prior to administration of extract and was repeated at intervals of 4h for the next 24h after the administration of extract.

2.4 Estimation of Urea, Creatinine, SGOT and SGPT

After measuring the anti-diabetic activity of the Extract in the rats as described above, the animals were further administered with respective dosage of extract for the next seven days while maintaining the diabetic condition. On eighth day, the blood was collected by cardiac puncture under mild ether anesthesia. The blood was further processed to estimate the level of urea, creatinine, SGOT and SGPT to check for the proper functioning of kidney and liver. The estimations were performed using commercial kit from Lab care Diagnostics (India) Pvt. Ltd.

2.5 Determination of body weight

Two groups (n=6) of diabetic rats were administered daily with the extract (75 and 150mg/kg) for 21 days and change in the body weight of the animals was recorded on the 1,7,14 and 21st day and compared with the diabetic control.

2.6 Statistical analysis

All the values were expressed as mean ± standard deviation, and analyzed for variance using SPSS package (version 20).

3. RESULTS AND DISCUSSION

3.1. Hypoglycemic activity of *M.umbellatum* methanolic extract

The earlier study on the phytochemical analysis of different extracts of *M. umbellatum* has revealed the presence of various classes of the compounds such as terpenoids, flavonoids, tannins, phenols and glycosides in the methanolic extract which exhibited good antibacterial
and antioxidant activity\textsuperscript{[13]}. In addition, terpenes are widely implicated to exhibit different pharmacological activities and hence in the present study, the methanolic extract was subjected to antidiabetic activity in the rat model \textsuperscript{[3,7]}. The determination of LD\textsubscript{50} values indicate that no toxicity was recorded up to 2000mg/kg concentration of the extract and the rats were healthy and no mortality and change in behavior was observed. The LD\textsubscript{50} was found to be 2236 mg/kg body weight and the extracts were administered at concentrations less than one-tenth of LD\textsubscript{50} (75 and 150mg/kg) for all the experiments in this study.

Alloxan is a β-cytotoxin which induces chemical diabetes by destroying the insulin producing cells of the pancreas. It also causes time and concentration dependent degenerative lesions on pancreatic β-cells \textsuperscript{[8]}. After administration of alloxan, 80% of rats developed hyperglycemic condition having a glucose level of more than 250mg/dl. Development of hyperglycemic condition was associated with loss in weight, increased urinary output and sluggish activity. These diabetes induced rat model was used for testing the anti-diabetic activity of \textit{M. umbellatum} leaves methanolic extract.

Hypoglycemic activity is exhibited by a molecule either by mimicking activity of insulin or increasing the release of insulin from pancreas. In the present study, the methanolic extract of leaves exhibited good hypoglycemic activity in a dose dependent manner (both at 75mg/kg and 150mg/kg) which lasted for 24h. Both the concentrations of extract significantly (p<0.001) reduced the blood glucose level (Table 1). This anti-diabetic activity can be attributed to its high flavonoid and terpenoid content. In another study, after administration of single oral dose of \textit{M. umbellatum} leaves alcoholic extract, it has been shown that the level of serum glucose significantly lowered \textsuperscript{[14]}. This implies that the extract possesses an active hypoglycemic molecule which mimics the action of insulin or increases the sensitivity of the receptors towards glucose molecules.

3.2. Effect of \textit{M. umbellatum} methanolic extract on the functioning of kidney and liver

It is important to study the effect of \textit{M. umbellatum} methanolic extract on the functioning of kidney and liver. The level of urea and creatinine in the serum signifies the proper functioning of both kidney and liver as both are the end products of metabolism which has to be excreted through urine. Damage to kidney and liver significantly alters the level of urea and creatinine. The results obtained in this study clearly indicate that there is no damage to both these organs as observed by the values which remained almost same between the healthy control and experimental animals (Fig 1).
Table 1. Effect of *M. umbellatum* methanol extract on blood glucose level

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 hr</th>
<th>4 hr</th>
<th>8 hr</th>
<th>12 hr</th>
<th>16 hr</th>
<th>20 hr</th>
<th>24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>121±13.18</td>
<td>125.83±6.96</td>
<td>127.83±8.90</td>
<td>126.83±5.77</td>
<td>133±8.55</td>
<td>126.5±5.95</td>
<td>130.33±12.04</td>
</tr>
<tr>
<td>Db control</td>
<td>352.83±9.82</td>
<td>353.83±12.14</td>
<td>352.5±13.08</td>
<td>350±14.58</td>
<td>357.17±15.21</td>
<td>361±13.41</td>
<td>359.67±13.06</td>
</tr>
<tr>
<td>Extract (75mg/kg)</td>
<td>367.5±9.48</td>
<td>127±8.96 *</td>
<td>109.67±8.43 *</td>
<td>125.67±4.17 *</td>
<td>134.33±5.78 *</td>
<td>166.67±8.86 *</td>
<td>191.67±9.25 *</td>
</tr>
<tr>
<td>Extract (150mg/kg)</td>
<td>365.5±9.66</td>
<td>128±11.41 *b</td>
<td>102±8.55 *b</td>
<td>113.33±11.5 *b</td>
<td>131.17±16.79 *b</td>
<td>156.5±6.53 *b</td>
<td>195.67±8.36 *b</td>
</tr>
</tbody>
</table>

The values represented as mean ± SD, (n=6). The comparison of statistical significance is as follows: a, Groups 2 and 3; b, Groups 2 and 4. The values of **p<0.001 was considered as highly significant.
The blood from these animals was further analyzed for the level of enzymes, SGOT and SGPT. Significant alterations in the level of these enzymes also serve as marker for the liver damage. The level of these enzymes indicated a significant difference (p<0.01) between diabetic induced rats and healthy control. On the contrary, no significant difference was observed in the animals administered with the plant extract at two different concentrations.

3.3. Effect of *M. umbellatum* methanolic extract on the body weight of the animals

We have also determined the effect of plant extract on the body weight of the experimental animals. These were compared with the diabetic control and the values indicated a significant increase (p<0.01 and p<0.001) in the body weight of the animals as measured on 14th and 21st day, respectively. On the other hand, as expected, there was no significant difference between these values when compared between the rats administered with the extracts and healthy control (Table 2).

**Table 2. Effect of *M. umbellatum* methanolic extract on the body weight of experimental rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>1st Day</th>
<th>7th Day</th>
<th>14th Day</th>
<th>21st Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control/</td>
<td>255.67±8.43</td>
<td>259±7.89</td>
<td>265.83±3.57</td>
<td>271.5±7.86</td>
</tr>
<tr>
<td>2. Diabetic Control</td>
<td>251.83±2.56</td>
<td>248±2.60</td>
<td>240.83±5.41</td>
<td>236.5±5.68</td>
</tr>
<tr>
<td>3. Extract (75mg/kg)</td>
<td>255±5.69</td>
<td>260.83±5.53a</td>
<td>267.33±6.40a*</td>
<td>272.33±6.97a**</td>
</tr>
<tr>
<td>4. Extract (150mg/kg)</td>
<td>253.83±10.22</td>
<td>260.17±6.91b</td>
<td>263.17±11.62b*</td>
<td>271.83±7.47b**</td>
</tr>
</tbody>
</table>

The values represented as mean ± SD, (n=6). The comparison of statistical significance is as follows: a, Groups 2 and 3; b, Groups 2 and 4. The values of **p<0.001, highly significant; *p<0.01, significant; and ns, not significant
4. CONCLUSIONS
The need for a novel molecule for the treatment of diabetes is increasing, especially from the natural source which is expected to be free of side effects. In the present study, the results indicate that *M.umbellatum* methanolic extract possess highly promising hypoglycemic activity even at low concentrations (75 and 150 mg/kg), which lasted for 24h. The results of our study indicate that the ip administration of the extract is highly effective as compared to oral administration which showed the anti-diabetic activity only at higher concentrations 14. In literature, the extracts from several medicinal plants have been reported to possess anti-diabetic activity; some of these are comparable with that of *M.umbellatum* methanolic extract used in this study 15-18. Although the mechanism of action is unknown, the extract might possess molecules which may mimic the activity of insulin. The phytochemical analysis revealed different classes of compounds in the methanolic extract which include glycosides, terpenoids, flavonoids etc., 13. Based on the literature, the hypoglycemic activity exhibited by the extract may be attributed to its terpenoid content 7,8. The chronic studies conducted here confirmed the gain in body weight, which may be due to the antagonizing effect of the extract towards chemically induced diabetes. Further, *M.umbellatum* extract was found to have no side effect as evident from the level of SGOT and SGPT in serum. All these data indicate that the methanolic extract of the plant is rich in a novel molecule possessing hypoglycemic activity. However, further detailed and systematic studies are needed to validate the current findings.

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5. REFERENCES


