MORPHOLOGICAL CHANGES AND ANTIHYPERGlyEMIC EFFECT OF M. CHAMPACA LEAVES EXTRACT ON BETA-CELL IN ALLOXAN INDUCED DIABETIC RATS

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Abstract
Rapidly increasing diabetes mellitus is becoming a serious threat to mankind health in all parts of the world. The control of treatment depends upon the availability medicines without any side effects. Traditional anti-diabetic plants, which can counter the high cost and poor availability of the current medicines. So an attempt was made to study the beneficial effects of M. champaca leaves extracts on pancreas morphology of diabetic rat model. M. champaca leaf extracts (Ethanolic, Chloroform and Petroleum Ether) were administered at dose levels of 200 mg/kg body weight orally. A positive control and normal group received distilled water orally. Blood samples were collected from retro-orbital plexus of each rat and analyzed by GOD-POD test. After 21 days treatment among different extracts, the maximum reduction of serum glucose level showed significantly (p<0.001) treated with ethanolic extract 200mg/kg b.w. compared to diabetic control group. The serum lipids level was reversed towards normal treated with all extracts when compared to diabetic control group. Histological study of the pancreas also assesses the islets of diabetic control group were damaged, shrunken while islets of herbal extracts group reverse the effect of alloxan induction which clearly showed the regeneration of beta cells and their size. Ethanolic extract at dose of 200mg/kg b.w. brings about significant beneficial effects in various physiological/histological parameters altered diabetic manifestations.

Keywords: Alloxan; Diabetes mellitus; Histopathology; M. champaca; Pancreas

Introduction
Diabetes mellitus is a complex and a multifarious group of disorders that disturbs the metabolism of carbohydrates, fats and proteins. It results from shortage or lack of insulin secretion or reduced sensitivity of the tissue insulin (1). As the numbers of people with diabetes multiply worldwide, the disease takes an ever increasing proportion of national and international health care budgets. It is projected to become one of the world main disabling and killers within the next 25 years (2). Unfortunately, after the introduction of bi-guanides and sulfonylureas about 50 years back no major success has been obtained in this direction of finding a proper drug for diabetes. These drugs have side effects and thus searching for a new class of compounds is essential to overcome these problems (3) but management of diabetes without any side effects is still a challenge to the medical community. So prior to discovery of insulin and other hypoglycemic synthetic drugs, herbal medicine has been long used for the treatment of diabetic patients and they are currently accepted as an alternative therapy for diabetic treatment and control (4). Traditional antidiabetic plants might provide new oral hypoglycemic compounds, which can counter the high cost and poor availability of the current medicines present day drugs for many rural population in developing countries. India is well known for its herbal wealth. Medicinal plants like Trigonella foenum graecum, Allium sativum, Gymnema slyvestre and Syzigium cumini have been studied (5) for treatment of diabetes mellitus. However, detailed studies on the efficacy, mechanism of action and safety of plant extracts are needed.

Michelia champaca (Magnoliaceae) having common name: Golden or yellow champaca, Champa, champaka (6). The tree is native from Asia-Tropical (Bangladesh, Arunachal pradesh, Assam, Bihar) and Indo China (Myanmar, Thialand, Vietnam, Sumatra, Malaysia) (7). Traditionally, it is being used in fever, colic, leprosy, post partum protection (8) and in eye disorders (9). It has been reported to possess antipyretic, anti-inflammatory (10) insecticidal (11), antimicrobial (8) and leishmanicidal activities (12). Juice of the leaves of Michelia champaca is given with honey in cases of colic. The flower oil is useful in cephalalgia, opthalmia and gout. The flowers and fruits are considered stimulant, antispasmodic, tonic, stomachic, bitter and cool remedies and are used in dyspepsia, nausea and fever. The bark is used as a stimulant, expectorant, astringent and febrifugal properties (13). The flowers mixed with sesamum oil forms an external application in vertigo and also

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applied to foetid discharges from the nostrils. They are useful as a diuretic in renal diseases and in gonorrhoea. The dried root and roots bark, mixed with curdled milk, is useful as an application to abscesses, clearing away or maturing the inflammation. In the form of an infusion it is valuable emmenagogue. It is also considered purgative. The flowers and fruits in combination with other drugs are recommended as an anti-dote to snake and scorpion venoms. (14). The methanol extracts of leaves, seeds, stem and root barks, stem and root heart-woods of *Michelia champaca* and the obtained fractions (petrol, dichloromethane, ethyl acetate and butanol) exhibited a broad spectrum of antibacterial activity. Fractionation drastically enhanced the level of activity particularly in all fractions of the stem bark and dichloromethane fraction of the root bark. Some fractions of the leaves stem and root bark demonstrated antifungal activity against some of the tested moulds. Liriodenine was the active constituent of the root bark, with a broader and, in some cases, better level of activity as compared to the standard (8).

On the basis of these facts, our present investigation was undertaken to study the histomorphological changes and anti-diabetic effect of *Michelia champaca* leaves extracts on the pancreas in alloxan induced diabetic model.

**Material and methods**

**Experimental animals**

Sprague Dawley rats of either sex, weighing around 150-250gm were purchased from Local Market. Male animals were housed separately in groups 6 per cage (Polycarbonate cage size: 29x22x14 cm) under laboratory conditions with alternating light and dark cycle of 12 h each. The animals had free access to food and water. The animals were acclimatized for at least five days before behavioural experiments which were carried out between 09:00 to 17:00 h. The experimental protocol (MMCP/IEC/10/02) was approved by Institutional Animals Ethics Committee (IAEC) and animals care was taken as per guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India.

**Drugs and chemicals**

All the solvents and chemicals used in this study were of pharmaceutical grade. Diagnostic kits were purchased from Ecoline, Merck Ltd., (Mumbai, India), Glbenclamide HCL was gifted by USV Ltd. Baddi, Himachal Pradesh., India. Alloxan monohydrate was purchased from Sigma Aldrich.

**Collection of plant materials**

Leaves of *M. champaca* as shown in Fig 1 were collected in the month of November 2009 from Botanical garden, Punjab University, Chandigarh, India. The specimen plant (NISCAIR/RHMD/consult/-2009-10/1337/139) was identified with the help of literature and authenticated by Dr. H. B. Singh, Scientist F & Head, Raw Materials Herbarium and Museum, N.I.S.C.A.I.R, New Delhi, India. The fresh plant material were cleaned with distilled water to dry at 35º-40ºC for 10 days and, pulverized in electric grinder and the powder was passed through sieve No.60 and used for further extraction.

![Fig. 1: Plant of Michelia Champaca](image)

**Preparation of extracts**

Dried and powdered plant material (1000 g) was successively Soxhlet extracted with petroleum ether (60-80º), chloroform, ethanol for 72 h each according to their polarity. The obtained extracts were evaporated in vacuum to give residues and their percentage yields were determined (15).

**Induction of diabetes**

Preparation of Normal Saline Solution: To make Normal Saline Solution (0.9%), weigh the 0.9 g of NaCl on physical balance and dissolved in sufficient distilled water to make volume up to 100 ml.

Preparation of Alloxan Monohydrate Solution: Weigh 2g of alloxan monohydrate was dissolved in 100 ml of 0.9% sodium chloride to obtain 2% alloxan monohydrate solution.

Preparation of 0.2% CMC: Weigh 200mg CMC were dissolved in 100ml of Normal Saline solution and heat at 50ºC with continuously shaking with stirrer.

**Experimental design**

The animals were divided into six groups of each containing six.

- **Group 1**: Normal operated + treated with Saline
- **Group 2**: Diabetic induced (Positive control)
- **Group 3**: Diabetic induced + treated with Glbenclamide (5mg / kg, b.w/day/oral)
- **Group 4**: Diabetic induced + treated with Pet. Ether extract of *M.champaca* 200 mg / kg, b.w/day/oral.
Group 5: Diabetic induced + treated with Chloroform extract of *M. champaca* 200 mg / kg, b.w/day/oral.

Group 6: Diabetic induced + treated with Ethanolic extract of *M. champaca* 200 mg / kg, b.w/day/oral.

Group 1 was treated as Normal rats and group-2 to group-6 were made diabetic by single intraperitoneal injection of alloxan monohydrate 120mg/kg. Following injection, animals were carefully observed for the first 24 hrs for any evidence of allergic reaction, behavioral changes and convulsions. No untoward reaction was observed in any animal. Blood was collected. The diabetic state was confirmed after 3 days of alloxan administration and rats with serum blood glucose levels of >140mg/dl were included in the study. Blood samples were drawn from retroorbital plexus at weekly interval till the end of study. Fasting blood glucose levels, HDL, LDL, Triglycerides and serum insulin levels were measured before & at the 7th, 14th & 21st day.

**Histopathology of pancreas**

The whole pancreas from each animal was removed after sacrificing the animal and washed on ice cold saline immediately. A portion of pancreatic tissue was fixed in 10% neutral formalin fixative solution for histological studies. After fixation tissues were embedded in paraffin, solid sections were cut at 5μm and the sections were stained with haematoxylin and eosin (16).

**Statistically analysis**

The data were expressed as mean ± SEM. The statistical significance between means was analyzed using one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test. A p<0.005 was considered as statistically significant.

**Results**

Administration of alloxan (120 mg/kg, i.p.) led to elevation of fasting blood glucose levels, which was maintained over a period of 3 weeks and daily treatment of various extract of *Michelia champaca* led to a dose-dependent fall in blood sugar levels. The plant extract were fed with diabetes induced rats. The serum glucose level of all the extracts of *M. champaca* treated groups were significantly (P<0.001) reduced when compared to the diabetic control group but ethanolic extract having the highest depletion power from 219 ± 1.44 to 151.71±1.93 at 21st day (Table 1).

Table 2 showed the data for serum Cholesterol, LDL, HDL and Triglycerides respectively. The serum cholesterol level of chloroform extract treated group (188.11 ± 29.4 mg/dl) and ethanolic extract treated group (169.25 ± 29.4mg/dl) showed statistically highly significant (P<0.001) compared to diabetic control group at 21st day similar results in case of Triglycerides at 21st day, the ethanolic extract demonstrated the better result and found significant (P< 0.001) compared to diabetic control group but in Pet. Ether treated group, it was found non-significant in serum cholesterol level and triglyceride levels. In LDL parameter, only Ethanolic extract treated group showed statistically significant (P< 0.001) compare to diabetic control group rather than other extracts which were non significant. In case of HDL level all the extracts showed non signficant reduction of HDL levels at 21st day.

<table>
<thead>
<tr>
<th>Group (n=6)</th>
<th>Serum Glucose level(mg/dl)</th>
<th>0 day</th>
<th>7th day</th>
<th>14th day</th>
<th>21st day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>89.37±11.19</td>
<td>80.74±14.71</td>
<td>85.17±21.43(***</td>
<td>84.21±10.46(***</td>
<td></td>
</tr>
<tr>
<td>Diabetic control</td>
<td>248.12±28.04</td>
<td>263.31±9.32(***</td>
<td>258.17±18.71(***</td>
<td>268.45±6.45(***</td>
<td></td>
</tr>
<tr>
<td>Glibenclamide (10mg/kg)</td>
<td>223.10±2.80</td>
<td>162.18±51.09(***</td>
<td>133.21±23.74(***</td>
<td>98.45±21.30(***</td>
<td></td>
</tr>
<tr>
<td>Pet. Ether (200mg/kg)</td>
<td>279.16±23.17</td>
<td>251.21±42.18(***</td>
<td>218.01±19.03(***</td>
<td>164.93±1.03(***</td>
<td></td>
</tr>
<tr>
<td>Chloroform (200mg/kg)</td>
<td>253.13±2.91</td>
<td>231.17±31.91(***</td>
<td>203.12±17.44(***</td>
<td>173.41±15.32(***</td>
<td></td>
</tr>
<tr>
<td>Ethanol (200mg/kg)</td>
<td>219±1.44</td>
<td>202.10±1.32(***</td>
<td>184.93±6.19(***</td>
<td>151.71±1.93(***</td>
<td></td>
</tr>
</tbody>
</table>

Values are Mean ± SEM
Data was analyzed by one-way ANOVA followed by Tukey’s t test
Statistically significant value:
* P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, ns- non significant
Table 2: Effect of extract of Michelia champaca on Serum Lipid Profile in diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum Lipid Profile (mg/dl)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum Cholesterol</td>
<td>Triglycerides</td>
<td>LDL</td>
<td>HDL</td>
</tr>
<tr>
<td>Normal</td>
<td>153±9.4***</td>
<td>82.91±2.1***</td>
<td>90.17±3.9***</td>
<td>39.02±17.3***</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>274±21.9</td>
<td>193.15±10.4</td>
<td>203.34±51.2</td>
<td>31.02±12.09</td>
</tr>
<tr>
<td>Gibenclamide (10mg/kg)</td>
<td>44.23±33.4***</td>
<td>99.82±11.3***</td>
<td>82.19±21.6***</td>
<td>48.99±10.14***</td>
</tr>
<tr>
<td>Pet. Ether (200mg/kg)</td>
<td>259.12±18.1***</td>
<td>180.55±1.2***</td>
<td>193.14±5.6***</td>
<td>34.86±3.04***</td>
</tr>
<tr>
<td>Chloroform (200mg/kg)</td>
<td>188.11±29.4***</td>
<td>141.06±44.2**</td>
<td>167.54±7.91***</td>
<td>42.13±19.1***</td>
</tr>
<tr>
<td>Ethanol (200mg/kg)</td>
<td>169.25±22.9***</td>
<td>123.77±52.9**</td>
<td>139.11±14.7***</td>
<td>52.23±1.03***</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM
Data was analyzed by one-way ANOVA followed by Tukey’s t test
Statistically significant value:

In histopathology of pancreas

The islets of langerhans of normal group (Fig. 2a) were unevenly scattered in the pancreatic tissue and they were often quite abundantly distributed and were of varying sizes in the same lobule of pancreas. The acinar cells which stained strongly were arranged in lobules with prominent nuclei. The islets cells were seen embedded within the acinar cells and surrounded by fine capsule. Pancreatic islets of diabetic control group 2 rats revealed significant architectural the acinar cells around the islets through seem to be in normal proportion does not look classical. (Fig. 2b) shows the islets were damaged, shrunken in size and infiltration of lymphocytes was observed. The islets cells of gibenclamide treated group (Fig. 2c) seen to be normal in position but few in numbers comparable to normal group. The size of cell is to be back in normal position after 21 days treatment of Gibenclamide. The islet cells were compactly arranged, with negligible intercellular space. In pet ether treated Group (Fig. 2d), the islets cells were seen in few numbers. The size of the cell is shrunken with architectural disarray and hydrolyses compared to diabetic control group. The islets cells (Fig. 2e) of chloroform extract treated group were shown to be in normal position restoration of beta cell size is to be back again after 21 days treatment compared to pet ether group. The islets of ethanolic extract treated group (Fig. 2f) were present with a large proportion of islets cells, though with a smaller volume compared to normal group. Ethanolic extract showed better restoration of beta cell in comparison of Pet ether and chloroform extract of Michelia Champaca.
Fig. 2 (c) Group C (Glibenclamide treated Group) Received 10mg/kg/day, p.o

Fig. 2(d) Group D (Pet ether treated group) Received 200mg/kg/day, p.o

Fig. 2(e) Group E (Chloroform treated Group) Received 200mg/kg/day, p.o

Fig. 2(f) Group F (Ethanol treated group) Received 200mg/kg/day, p.o

Discussion

The researchers conducted over last several decades has shown plant and plant based therapies have a potential to control and treat diabetes (17-19) and its complications (20). Only 15% of Pharmaceutical drugs are consumed in developing countries and relatively more affluent people take more a large proportion of herbal drugs because, they are safe, more dependable and do permanent cure of their diseases. They are better than Allopathic drugs, which have a lot of adverse side effects (21). For testing antidiabetic potential of plants, alloxan and STZ induced hyperglycemia in rats is considered to be a good preliminary screening model (18) and is widely used. Alloxan is well known for its selective pancreatic islet cell toxicity and has been extensively used induced diabetes mellitus in animals.

The unique capacity of alloxan to selectively destroy the pancreatic beta cells was first described by Dunn et al. Alloxan induces “chemical diabetes” in a wide variety of animal species by damaging the insulin secreting pancreatic β-cell, resulting in a decrease in endogenous insulin release (22). The majority of islet cells is formed by B cells which are responsible for producing insulin. Depletion of B cells will therefore result in insulin deficiency which will lead to a disorder in carbohydrate, protein and fat metabolism with resultant hyperglycaemia. In this study alloxan which selectively destroy B cells of the islets was used to induce type 1 diabetes (23). The plant Michelia champaca (Sampige), in this study is widely used in both Ayurveda and Homeopathic medicine. Michelia champaca (Magnoliaceae) is commonly used by many traditional healers in most of the herbal preparations for diabetes and kidney diseases.

Therefore the present study was to demonstrate the efficacy of Michelia champaca in the reduction of blood glucose concentration as well as to determine the recovery of beta cells of pancreas. This work evaluated biochemical parameters such as serum triglycerides, cholesterol, LDL, HDL in experimental diabetes caused by alloxan in rats. Our study indicated that Michelia champaca extracts have good anti-diabetic activity. Among different extracts, ethanolic extract was found to be declined very fast (P<0.001) on 7th, 14th and 21st day which showed statistically highly significant. Three weeks of daily treatment of various extract of Michelia champaca led to a dose-dependent fall in blood sugar levels.

They can also improve the conditions of Diabetes Mellitus as indicated by parameters like triglycerides, Serum LDL and HDL. The ethanolic extract and chloroform extract showed some improvement in serum triglycerides levels on 21st day when compare to diabetic control group which were found to be statistically significant (p<0.001). The damage of pancreas in alloxan-treated diabetic control rats Fig 2b
and regeneration of β-cells by glibenclamide Fig. 2c was observed. The comparable regeneration was also shown by Chloroform and Ethanolic extracts of M. champaca Fig. 2e & 2f resp. The possible mechanism by which M. champaca brings about its hypoglycaemic action may be due to flavonoids present in this plant which stimulates the receptor on the cytoplasm side of the membrane, a protein phosphokinase of the tyrosine-specific type. It phosphorylates itself with the aid of ATP, undergoes a conformational change, and activates via a G-proteins, which liberates several second messenger further activates protein P-kinases which open a Ca2+ influx gives insulin like effect (24). Another possible mechanism may be due to alkaloids causes inhibition of mitochondrial function that increases the AMP/ATP ratio, which could explain the activation pathway in the treatment of diabetes (25). The third most important probable mechanism of action may be by potentiating the insulin effects of plasma by increasing either the pancreatic secretion of insulin from the existing beta cells or by its release from the bound form. Though the exact mechanism is unknown but we are assuming that various active constituents of this plant help to improve in treatment of diabetes.

Conclusion

In Alloxan induce diabetic model, the treatment of M.Champaca extracts showed a significant decrease in the serum glucose level, which could be due to improvement in insulin secretion by recovery of pancreatic β-cell. Phenolic compounds, flavonoids have been found to be beneficial in controlling diabetes and many other diseases as evident from earlier studies. It is concluded from the data that M. champaca possess significant antidiabetic activity and it may prove to be effective for the treatment of Type 1 Diabetes.

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References


