ANTIDIABETIC ACTIVITY OF ETHANOLIC EXTRACT OF HUGONIA MYSTAX LEAVES LINN IN STREPTOZOTOCIN-NICOTINAMIDE INDUCED DIABETIC RATS

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ABSTRACT
Objective: To evaluate the antidiabetic potential of ethanolic extract of Hugonia mystax leaves in Streptozotocin-nicotinamide induced diabetic rats. Methods: Group I = Control (Animal received Normal saline- 1ml/kg), Group II = Diabetic Control (Animal received Streptozotocin-nicotinamide 150mg/kg), Group III = Animal received ethanolic extract of Hugonia mystax leaves (200mg/kg), Group IV =Animal received ethanolic extract of Hugonia mystax leaves (400mg/kg) and Group V=Animal received Glibenclamide (5mg/kg) respectively. Results: The results of the study indicates that Hugonia mystax leaves extract significantly (P<0.01) reduced the blood sugar level. The leaf extract also significantly reduced the levels of serum cholesterol, triglycerides, and low density lipoprotein and increase HDL levels in diabetic rats. The leaf extract significantly (P<0.01) decrease in the elevated alkaline phosphatase, SGOT and SGPT in Streptozotocin-nicotinamide treated rats and the level significantly (P<0.01) restored to normal level after treatment. Conclusions: Ethanolic extract of Hugonia mystax leaves has protective effects on the protection of vital tissues (pancreas, kidney, liver, heart and spleen), thereby reducing the causation of diabetes in experimental animals.

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INTRODUCTION

The genus *Hugonia* L. of family Linaceae comprise about 40 species in the world; of which *Hugonia mystax* L. was reported from India\(^1\)\(^,\)\(^2\). This plant *Hugonia mystax* is locally known as Modirakanni. Ethnobotanically, the fruits are used by the tribals of Kalakad Mundanthurai for the treatment of Rheumatism\(^3\). Roots were used as anthelmintic, astringent and also used for dysentery, snake bite, fever, inflammation and rheumatism. Biological activities such as analgesic, anti-inflammatory and ulcerogenic were also reported\(^4\)\(^,\)\(^5\)\(^,\)\(^6\)\(^,\)\(^7\). Roots of *Hugonia mystax* were evaluated for preliminary phytochemical screening and antimicrobial activity. Preliminary phytochemical screening showed the presence of various classes of secondary metabolites such as flavonoids, phenols, saponins, steroids, tannins and terpenoids. Antimicrobial activity of petroleum ether, chloroform, ethanol and aqueous extracts of root extracts showed significant activity against various human pathogens\(^8\).

Taking into consideration of medicinal value and utility, the present study was planned to explore Antidiabetic potential of the medicinal plant named *H. mystax*.

MATERIALS AND METHODS

Collection, identification and preparation of plant materials

Fresh leaves of *H. mystax* were collected from velliangiri hills from Coimbatore DT. It was identified by a scientific officer, Dr. P.Samydurai Asst.Prof. Department of Botany konghu nadu arts and science college Coimbatore.

Preparation of Extracts

Leaves of *Hugonia mystax* was dried in shade for two weeks. Dried leaves were coarsely powdered, sieved (#40) and stored in an air tight container at room temperature. Dried powder was then extracted sequentially with petroleum ether, chloroform, and ethanol using soxhlation method. The extracts were concentrated to dryness using rotary evaporator. The yields of various extracts were found to be 4.5% w/w (petroleum ether), 4.7% w/w (chloroform) and 10.5% w/w (ethanol). All the extracts were preserved in a refrigerator at 4 °C. However, only ethanolic extract of the leaves was selected and evaluated for antidiabetic study.

Phytochemical Analysis

The phytochemical analysis of ethanolic extract of *Hugonia mystax* leaves showed the presence of carbohydrates, flavonoids, steroids, saponins, terpenoids and absence of alkaloids, proteins and amino acids.

Anti-Diabetic Activity

Selection of animals

Wistar Albino Rats (Male) weighing around 150-200 gm was selected for the experiment. The animals were checked for the free of any disease, only healthy rodent is accepted for the experiments. The male rats are preferred so that they occurs no interference in between the experiment because of the pregnancy. The rats are collected from the animal house of Nandha College of pharmacy, Erode 52.

Induction of experimental Diabetes

Hyperglycemia was induced by injecting single intraperitoneal injection of 60mg/kg of streptozotocin (STZ), freshly dissolved in cold citrate buffer, pH 4.5 after 15 min of i.p. injection of nicotinamide (110 mg/kg) prepared in normal saline. The animals were kept under observation. After 48 h, the animals were tested for glucosuria using Diastex strips. The blood glucose level was checked before and 72 h after STZ injection to confirm the development of diabetes. The diabetic animals were stabilized for seven days and the experiment was started on the next day (day 0). Only those animals which showed blood glucose levels >250 mg/dL were separated and used for the study.

Experimental design

All the animals were randomly divided into five groups with six animals each. The extract and standard group animals were treated once a day for 14 days as follows:

**Groupings of animals**

- **Group I** = Control (Normal saline- 1ml/kg orally)
- **Group II** = Diabetic Control (STZ 60mg/kg + Nicotinamide 110mg/kg, i.p)
- **Group III** = Diabetic Control + Animal received ethanolic extract of *Hugonia mystax* leaves (200mg/kg, orally)
- **Group IV** = Diabetic Control + Animal received ethanolic extract of *Hugonia mystax* leaves (400mg/kg orally)
- **Group V** = Diabetic Control + Standard (Glibenclamide 5mg/kg orally)

The drugs were dissolved in normal saline and it was administered orally via a standard orogastric cannula. Anti-hyperglycemic activity in diabetic rats was assessed by fall in fasting blood glucose level. Blood samples were collected from the tip of the tail on 0 Day, 3rd day, 7th day and 14th day by without sacrificing the animals, from the tail vein by snipping off the tip of the tail and blood glucose were checked by (An Accu-Chek system, Glucotrend. Made in Germany). After the completion of experiment, the blood were collected through the retro orbital puncture of eye of animals under mild ether anesthesia in Eppendorff’s tube (1ml).

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containing 50μl of anticoagulant (10% trisodium citrate) and the serum was separated by centrifuging at 3000rpm for 15min. The biochemical parameters blood glucose level, Lipid profile, SGPT, SGOT, ALP were determined by using the commercial kit available (Ecoline, Manufactured by Merck Specialties, Private limited, Ambernath).

### Statistical analysis

Statistical significance was determined by one way analysis of variance (ANOVA) followed by Dunnet’s test.

### RESULTS

Effect on blood glucose level of ethanolic extract of *Hugonia mystax* leaves in experimental rats in Table 1 showed the blood glucose level in different groups. The glucose level was significantly (P<0.01) high in STZ control rats compared with normal control. But the level of blood glucose was significantly (P<0.01) decrease in diabetic rats treated with extract on repeated administration of extract for 14 days. A significant decrease in the glucose level was observed in the diabetic rats as compare to normal control.

Effect on SGOT, SGPT and SALP of ethanolic extract of *Hugonia mystax* leaves in blood serum of experimental rats showed in Table 2. The SALP, SGOT, SGPT levels were found to be increased significantly (P<0.01) in STD treated diabetic rats compare to normal control. The extract significantly (P<0.01) decrease in the elevated alkaline phospatase, SGOT and SGPT in STD treated rats and the level significantly (P<0.01) restored to normal level after treatment.

The lowering the value of SGOT and SGPT from higher value after the treatment also indicated the revival of insulin secretion. SGOT and SGPT levels are indicators of liver function, hence the restoration of normal level indicate the normal function of liver.

Effect on lipid profile of ethanolic extract of *Hugonia mystax* leaves in blood serum of experimental rats showed Table 3. The level of lipid profiles in control, diabetic control and experimental rats was depicted. In STZ induced diabetic rats there was a significant (P<0.01) increase in cholesterol, triglycerides and LDL in serum compare to normal control. The plant extract used in the experimental study significantly (P<0.01) decrease the level of cholesterol, triglycerides and LDL. This indicated the leaf extract of *Hugonia mystax* had favorable effect on lipid metabolism of diabetic rats and HDL was significantly increased in diabetic rats.

### Table 1: Effect on blood glucose level of ethanolic extract of *Hugonia mystax* leaves in experimental rats.

<table>
<thead>
<tr>
<th>S. No</th>
<th>GROUPS</th>
<th>Blood glucose level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 day</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>116.32±5.16</td>
</tr>
<tr>
<td></td>
<td>(Normal saline 1ml/kg)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Diabetic Control</td>
<td>401.06±7.17</td>
</tr>
<tr>
<td></td>
<td>(STZ 60 mg/kg + Nicotinamide 110mg/kg)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EEHM 200 mg/kg</td>
<td>352.19±5.06</td>
</tr>
<tr>
<td>3</td>
<td>EEHM 400 mg/kg</td>
<td>348.12±6.37</td>
</tr>
<tr>
<td>4</td>
<td>Glibenclamide</td>
<td>340.74±5.42</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean± SEM; n=6.

*p<0.01 when compared to control

b*p<0.01 when compared to STZ control (one way ANOVA followed by Dunnett’s test).
Table 2: Effect on SGOT, SGPT and SALP of ethanolic extract of *Hugonia mystax* leaves in blood serum of experimental rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGOT (U/l)</th>
<th>SGPT (U/l)</th>
<th>SALP (U/I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Normal saline 1ml/kg)</td>
<td>17.49±0.94</td>
<td>24.16±0.91</td>
<td>116.32±1.16</td>
</tr>
<tr>
<td>Diabetic Control (STZ 60 mg/kg + Nicotinamide 110mg/kg)</td>
<td>29.32±1.02</td>
<td>38.47±0.89</td>
<td>239.74±1.30</td>
</tr>
<tr>
<td>EEHM 200 mg/Kg</td>
<td>23.07±1.17</td>
<td>26.05 ±0.87</td>
<td>149.12 ±1.12</td>
</tr>
<tr>
<td>EEHM 400 mg/Kg</td>
<td>19.84±0.98</td>
<td>23.18±0.79</td>
<td>120.40±1.08</td>
</tr>
<tr>
<td>Glibenclamide 5 mg/Kg</td>
<td>19.11±0.98</td>
<td>22.26±0.91</td>
<td>112.06±1.09</td>
</tr>
</tbody>
</table>

Values are mean± SEM; n=6.

*p<0.01 when compared to control

Table 3: Effect on lipid profile of ethanolic extract of *Hugonia mystax* leaves in blood serum of experimental rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>HighDensity Lipoprotein(HDL)</th>
<th>Low Density lipoprotein(LDL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control (Normal saline 1ml/kg)</td>
<td>68.15 ± 0.54</td>
<td>93.74 ± 1.17</td>
<td>32.16 ± 1.36</td>
<td>17.16 ± 0.81</td>
</tr>
<tr>
<td>Diabetic control (STZ 60 mg/kg + Nicotinamide 110mg/kg)</td>
<td>80.42 ± 1.42</td>
<td>96.32 ±1.32</td>
<td>24.62 ±1.86</td>
<td>22.32 ± 0.79</td>
</tr>
<tr>
<td>EEHM 200 mg/kg</td>
<td>71.46 ± 1.92 b</td>
<td>92.17 ± 1.06 b</td>
<td>31.02 ± 1.32 b</td>
<td>18.69 ± 0.62 b</td>
</tr>
<tr>
<td>EEHM 400 mg/kg</td>
<td>65.94 ± 1.09 b</td>
<td>86.02 ± 1.23 b</td>
<td>31.28 ± 1.16 b</td>
<td>18.06 ± 0.67 b</td>
</tr>
<tr>
<td>Glibenclamide 5 mg/kg</td>
<td>66.32 ± 1.46 b</td>
<td>88.36 ± 1.29 b</td>
<td>31.76 ± 1.32 b</td>
<td>17.59 ± 0.80 b</td>
</tr>
</tbody>
</table>

Values are mean± SEM; n=6.

*p<0.01 when compared to control

*p<0.01 when compared to STZ control (one way ANOVA followed by Dunnett’s test )
DISCUSSION

Management of diabetes with the agents with devoid of any side effects is still challenge to medical system. This led to an increase the demand for natural products with antihyperglycemic and fewer side effects.

Plants may act on regulate the blood glucose level through different mechanism. Some of them may have insulin like substances and some may inhibit insulinase activity. Stimulation of beta cells to produce more insulin and others may increase beta cells in the pancreas by activating regeneration of pancreatic cells.

The mechanism by which STZ brings about the diabetic state include the cytotoxic action of STZ is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration leading to a rapid destruction of beta cells which leads to poor glucose utilization by tissues. This suggest that extract may possess as insulin like effect on peripheral tissues by either promoting glucose uptake (or) metabolism, by inhibiting hepatic gluconeogenesis or absorption of glucose into the muscles and adipose tissues, by stimulation of regeneration process and revitalization of the remaining beta cells.

The levels of serum lipids are usually elevated in diabetes mellitus and such an elevation represents a risk factor for coronary heart disease. This abnormal high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots. Under normal circumstances insulin activates the enzyme lipoprotein lipase which hydrolyses the triglycerides. However in the diabetic state lipoprotein lipase is not activated due to insulin deficiency, resulting in hypertriglyceridemia. In addition treatment of animals with Hugonia mystax caused a decrease in cholesterol levels. It indicates that the extract of Hugonia mystax was more useful in the treatment of diabetes as it has hypolipidemic effect. Since the diabetes is always associated with hyperlipidemia. Moreover its hypolipidemic effect caused represents a protective mechanism against the development of atherosclerosis which is usually associated with diabetes (or) drug therapy seems to be associated with is decreased in the risk of vascular disease.

The animals treated with Streptozotocin-nicotinamide developed hepatic damage which was evident from the increase in the enzyme activities. Treatment with ethanolic extract of Hugonia mystax and Glibencamide resulted in a decrease of transaminase activities in Streptozotocin-nicotinamide treated animals. In this study, it was observed that the levels of ALP, SGPT and SGOT in Streptozotocin-nicotinamide induced diabetic rats were elevated. It may be due to leaking out of enzymes from the tissues and migrating in to the circulation by the adverse effect of Streptozotocin-nicotinamide. In present study, the ethanolic extract of Hugonia mystax regulated the activity of SGOT, SGPT and ALP in liver of animals intoxicated with alloxan. SGOT and SGPT levels are indicators of liver function hence restoration of normal levels indicate normal function liver. The results indicates that the ethanolic extract of Hugonia mystax can reduce the level liver marker enzymes and confirms the possibility that the major function of the extract are on the protection of liver tissues, there by improve the liver function.

However, the present study further supports to the using Hugonia mystax for routine treatment of diabetes mellitus. So the present investigation reveals that ethanolic extract of Hugonia mystax leaves has significant hypoglycemic action in Streptozotocin-nicotinamide induced diabetic rats.

REFERENCE