Antidiabetic activity of *Hibiscus syriacus* on alloxan induced diabetic rats

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**INTRODUCTION**

Diabetes mellitus is a group of metabolic diseases characterized by elevated blood glucose levels. It is a result of defects in insulin secretion, insulin action or both. Non-Insulin Dependent Diabetes Mellitus (NIDDM) is a multifactorial disease characterised by hyperglycemia and lipoprotein abnormalities. These traits are hypothesized to damage cell membranes, which results in excessive generation of reactive oxygen species. NIDDM has also been associated with an increased risk for developing premature atherosclerosis due to an increase in Triglyceride (TG) and Low Density Lipoproteins (LDL) and decrease in High Density Lipoprotein (HDL) levels [1]. Chronic hyperglycemia is associated with microvascular and macrovascular complications that can lead to visual impairment, blindness, kidney disease, nerve damage, amputations, heart disease, and stroke. In 1997 an estimated 4.5% of the US population had diabetes. Direct and indirect health care expenses were estimated at $98 billion [2].

**Epidemiology**

It is estimated that 366 million people had DM in 2011; by 2030 this would have risen to 552 million [3] the number of people with type 2 DM is increasing in every country with 80% of people with DM living in low- and middle-income countries. DM caused 4.6 million deaths in 2011. It is estimated that 439 million people would have type 2 DM by the year 2030 [4]. The incidence of type 2 DM varies substantially from one geographical region to the other as a result of environmental and lifestyle risk factors [5].

**MATERIALS AND METHOD**

**Plant material**

The whole plant of *Hibiscus syriacus* was collected in kanipakam, chittoor district. The plant material was authentified by Dr.K.Madhava chetty, Assistant professor, department of botany, S.V. University, Tirupathi. The plaat material was dried in shade at room temperature. Then dried material was powdered with the help of mechanical grinder and subjected to seiving for equal particles of coarse powder with the help of seiver.

**Preparation of extract**

The powdered plant material was packed in to Soxhlet apparatus and defatted with petroleum ether (60 - 80°C). Marc left was further extracted...
with ethanol. Extract was air dried until it comes to semi-solid to solid mass.

**Phytochemical screening**

The extract obtained was subjected to qualitative tests for identification of different constituents like tannins, alkaloids, saponins, glycosides, terpenes, phenolics, flavonoids, carbohydrates, proteins and steroids, by using simple and standard qualitative methods described by Trease and Evans [6].

**PHARMACOLOGICAL STUDY**

**Animals**

Healthy albino rats of either sex weighing about 120-150 g were used during the study. The animals were procured from Sree Vidyakethan College of Pharmacy, Tirupati, India. Before initiation of experiment, the rats were acclimatized for a period of 7 days. Standard environmental conditions such as temperature ranging from 18 to 32°C, relative humidity (70%) and 12 hrs dark/light cycle were maintained in the quarantine. All the animals were fed with rodent pellet diet and water under strict hygienic conditions. All procedures were performed in accordance to CPCSEA guidelines after approval from Sree Vidyakethan College of Pharmacy, Tirupati, India [Approval no. SVCP/IAEC/I-010/2013-2014, dated on 14 march 2014.].

**Acute oral toxicity study**

The acute toxicity of ethanolic extract of *Hibiscus syriacus* was carried out as per OECD-423 guidelines for safe dose administration to animals. The animals were kept fasting for overnight providing only water, after which the extracts were administered orally at the dose level 2000mg/kg body weight and observed for 14 days. The results showed no sign of toxicity till 14 days at a starting dose of 2000 mg/kg body weight. Hence, 1/10th were considered as test dose for the study.

**Induction of diabetes**

Alloxan monohydrate was obtained from BROS scientifics, tirupathi. To induce diabetes, alloxan 120 mg/kg, intraperitoneal (i.p.) Single dose will be administered intraperitoneal to groups II, III,IV&V. The fasting blood glucose was determined after 72 hours Rats showing a blood glucose level of >200 mg/dl were taken for the study.

**Experimental design**

Rats having blood glucose level more than 200mg/dl taken for the study. Then rats are divided into 5 groups, each group having 6 animals. Group I: received normal saline (10ml/kg/day/p.o) Group II: received alloxan (120mg/kg/i.p), Group III: received standard drug Metformin (150mg/kg/p.o) + alloxan (120mg/kg/i.p), Group IV: received ethanolic extract of *Hibiscus syriacus* (200mg/kg/p.o) + alloxan (120mg/kg/i.p.), Group V: received ethanolic extract of *Hibiscus syriacus* (400mg/kg/p.o) + alloxan (120mg/kg/i.p.). The study performed for 28 days.

**Sample collection**

28th day animals were sacrificed, pancreas was isolated and it were fixed in 10% neutral formalin solution. Fine sections were mounted on glass slides and stained with hematoxylin Eosin for light microscopic analysis.

**Blood glucose estimation**

Determination of blood glucose Fasting blood glucose was estimated by commercially available glucose strips (Accu- Chek) using One Touch Glucometer (Johnson – Johnson, India). Estimation of blood glucose level on 0th day, 7th day, 14th and 28th day.

**RESULTS**

Phytochemical screening: phytochemical screening of ethanolic extract was showed the presence of flavonoids, tannins, saponins, glycosides and phenols. Carbohydrates, proteins, aminoacids and alkaloids are absent.

**Table 1. Estimation of blood glucose level(mg/dl)**

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>0TH DAY</th>
<th>7TH DAY</th>
<th>14TH DAY</th>
<th>28TH DAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>99.17±0.87</td>
<td>100±0.91</td>
<td>99.8±0.87</td>
<td>99.33±0.76</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>230±2.9*a</td>
<td>230.5±2.91*a</td>
<td>235±1.68*a</td>
<td>239.7±0.98*a</td>
</tr>
<tr>
<td>Standard</td>
<td>230±3.1**b</td>
<td>157.7±1.9**b</td>
<td>116±1.40**b</td>
<td>113.2±0.90**b</td>
</tr>
<tr>
<td>EEHS(200mg/kg)</td>
<td>232±3.1**b</td>
<td>176±1.24**b</td>
<td>158±1.16**b</td>
<td>147.5±0.76**b</td>
</tr>
<tr>
<td>EEHS(400mg/kg)</td>
<td>232±3.1**b</td>
<td>162±1.72**b</td>
<td>129±2.62**b</td>
<td>128.2±0.70**b</td>
</tr>
</tbody>
</table>
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Values are expressed in ±SEM values (n=6). One-way ANOVA followed by Dunnet’s multiple comparison tests. Alloxan (120mg/kg.) was injected to except control, all other drug groups; a alloxan induced diabetic group vs normal group, *p* <0.001; b extract treated group vs alloxan induced diabetic group, ’*p* <0.01; ’’*p*<0.001.

**Fig 1. Estimation of blood glucose level**

![Comparative blood glucose level](image)

**Fig 2. Histopathology of pancreas**

![Histopathology of pancreas](image)
DISCUSSION
Pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose, insulin is secreted. Alloxan is one of the usual substances used for the induction of diabetes mellitus apart from streptozotocin by induce free radical production and cause tissue injury. It has a destructive effect of the beta cells of the pancreas [8]. A massive reduction in insulin release by the destruction of beta-cells of the islets of langerhans thereby inducing hyperglycemia [9]. Alloxan has been shown to Ethanolic Extract in the dose of 400 mg/kg reduced the reduction in the blood glucose levels compared to Extract in the dose of 200 mg/kg. The literature reports reveal that flavonoids and tannins present in the plant extract known to possess antihyperglycemic activity. Antihyperglycemic potential of test extract was observed may be due to presence of similar phytoconstituents which was evident by preliminary phytochemical screening. In the present investigation demonstrated ethanolic extract of Hibiscus syriacus has the significant anti-diabetic Activity.

CONCLUSION
Present study was concluded that the ethanolic extract of Hibiscus syriacus at the dose of 400 mg/kg body weight produced significant antidiabetic activity in alloxan-induced NIDDM in albino rats.

REFERENCES