ANTIDIABETIC ACTIVITY STUDIES ON CASSIA FISTULA FRUITS

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ABSTRACT
Cassia fistula commonly known as Indian laburnum has found to have numerous medicinal properties for almost all the parts of the plants. In Ancient literatures, Cassia fistula fruits were documented to have anti diabetic activity. But, till now, no scientific studies have done on this part whereas the reports were on the antidiabetic activity of terpenoids present in the stem bark and hence, in the present study, petroleum ether extract of Cassia fistula fruit pulp was screened pharmacologically for its antidiabetic activity. Acute toxicity studies were performed at a dose of 2000mg/kg b.w and the extract was found to be safe without any signs of toxicity and mortality. In vivo antidiabetic study was conducted using streptozotocin antidiabetic model. In the study, bodyweight analysis, blood glucose level analysis and SGOT, SGPT, cholesterol, triglycerides levels were monitored and confirmed that plant has significant antidiabetic activity. Hence, the present study proved the importance of traditional knowledge; as the traditionally used antidiabetic plant selected for the study found to have significant activity. Therefore, it is justifiable to promote these plant extracts which are comparatively cheap, safe and reliable for the treatment of chronic disease, diabetes.

Keywords: diabetes, Cassia fistula, streptozotocin, traditional drug

INTRODUCTION
Cassia fistula commonly known as Indian laburnum has found to have numerous medicinal properties for almost all the parts of the plants. The root is prescribed as a tonic, astringent, febrifuge and strong purgative. Roots also used in chest pain, joint pain, migraine, blood dysentery and diabetes. The extract of root bark with alcohol can be used for backwart fever. The leaves are laxative and used externally as emollient, a poultice used for chilblains, in insect bites, swelling, rheumatism and facial paralysis. Leaves are used in ulcers, external skin eruptions and eczema. Fruits are used as cathartic, in snake bite and asthma. Flowers and pods are used as purgative, febrifugal, biliousness and astringent. Pulp is used as analgesic, antipyretic and applied in liver disorders, blood poisoning, anthrax, leprosy and diabetes. Barks possess tonic and antidysentric properties. It is also used for skin complaints, leprosy, jaundice, syphilis and heart diseases [1]. Hypoglycaemic and hypocholesterolemic

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effects of hexane extract of stem bark of Cassia fistula were studied and a dose of 0.45g/kg b.w. was found to have comparable results with glibenclamide [2]. Antioxidant activity of different parts of Cassia fistula plant was investigated and reported the antioxidant power in the decreasing order as stem bark, leaves, flowers and pulp [3] whereas in another investigation done by Amitabye et al, reported highest total phenolics, proanthocyanidins, flavonoids and antioxidant potentials for the pods when compared with other vegetative organs and reproductive parts of the plant [4]. Methanolic extract of buds of Cassia fistula was studied for its antipyretic activity on normal body temperature and yeast induced pyrexia in rats at doses of 200 and 400mg/kg b.w. Extract caused significant lowering of normal body temperature up to 3h and 6h after administration for 200 and 400mg/kg b.w. respectively. In the model of yeast provoked elevation of body temperature, extract showed dose dependent lowering of body temperature up to 4h at both dosage levels and the results were comparable to paracetamol, a standard antipyretic agent [5]. Methanolic extracts of fruits of Cassia fistula inhibited lipoxygenase catalysed formation of leukotriene B4 in bovine polymorphonuclear leukocytes and found to have an IC50 value of 38µg/ml. A linear correlation was obtained between the effects of the extracts suggesting a redox-based mechanism for the inhibition of 5-lipoxygenase enzyme [6]. In vitro effect of infusion of Cassia fistula pod was examined on isolated guinea pig ileum for its laxative property and found to be a safe laxative agent [7]. Hepatoprotective and antioxidant effect of Cassia fistula leaf extract on liver injury induced by diethyl nitrosamine was investigated and observed that the extract could protect the liver at a dose of 500 mg/kg b.w. [8]. Effects of methanolic extract of Cassia fistula seed on the growth of Ehrlich ascites carcinoma was studied on the life span of tumour bearing mice and concluded that the extract at a dose of 100mg/kg b.w. could decrease the tumour volume and viable tumour cell count with increasing the life span of mice than at a doses of 200 and 300mg/kg b.w. [9].

In another study, Cassia fistula bark extract was orally administered to 7,12 dimethyl benz(a)anthracene (DMBA) induced hamster buccal pouch carcinogenesis. Study was concluded reporting the ability of the extract to prevent the formation of oral squamous cell carcinoma on DMBA painted animals. Petroleum ether extract of seeds of Cassia fistula was screened for antifertility activity in proven fertile female albino rats at doses 100, 200 and 500 mg/kg b.w. on days of 1-5 of pregnancy. Results indicated decline in the fertility index, number of uterine implants and live foetuses in a dose dependent manner on 15th day of pregnancy [10]. Anti inflammatory activity studies of aqueous and methanolic extracts of Cassia fistula bark were performed in both acute and chronic models and both the extracts were found to possess significant anti inflammatory activity [11]. The methanolic extract of Cassia fistula leaves was examined for its wound healing property in the form of ointment in two types of wound
models in rats; excision wound model and incision wound model. Results obtained for 5 and 10%w/w ointment of leaves extract were comparable with that of the standard drug, nitrofurazone in terms of wound contraction ability, epithelisation period, tensile strength and regeneration of tissue at wound area [12]. Methanol extract of leaves of *Cassia fistula* was investigated for its effect on a cough model induced by sulphur dioxide gas in mice. Anti tussive activity of the extracts at 400 and 600mg/kg b.w. was comparable with that of codeine phosphate [13]. Aqueous and alcoholic extracts of stem bark of *Cassia fistula* were evaluated using disc diffusion method against S.aureus and alcoholic extracts showed better effect than the aqueous extract [14]. Jaffary et al investigated the effectiveness of *Cassia fistula* in the treatment of leishmaniasis and comparable results were obtained with intralosomal injection of glucantime. In his another study, efficacy of intralosomalmeglumine antimonite – *Cassia fistula* fruit gel combination for the treatment of cutaneous leishmaniasis. Combination therapy with gel increases the efficacy of intralosomalmeglumine antimonite and should be considered for the treatment of acute cutaneous leishmaniasis [15, 16].

In Ancient literatures, *Cassia fistula* fruits were documented to have anti diabetic activity. But, till now, no scientific studies have done on this part whereas the reports were on the antidiabetic activity of terpenoids present in the stem bark and hence, in the present study, petroleum ether extract of *Cassia fistula* fruit pulp was screened pharmacologically for its antidiabetic activity.

**METHOD**

**Extraction of Cassia fistula fruits**

Fruits were collected and the fruit pulp was taken out manually. Fruit pulp was shade dried and 1kg was extracted with petroleum ether by simple maceration technique. It was carried out in amber coloured bottle with intermittent shaking for 5 days. After 5 days, extract was decanted off and allowed to evaporate using a rotary evaporator [17]. The residue obtained (PC) was weighed and calculated the yield.

**Evaluation of Antidiabetic Activity Using In Vivo Model**

*Experimental induction of diabetes* [18,19]

Diabetes was induced by a single intraperitonial (ip) injection of freshly prepared Streptozotocin (60mg/kg b.w.) in normal saline to a group of overnight fasted rats. After 2 days of administration, the animals showing fasting blood glucose levels > 200mg/dl were selected for the study.

*Experimental design*

In the experiment, total 24 animals were used, in which 18 diabetic surviving rats and 6 normal rats. The 30 diabetic induced animals were then randomly divided in to five groups of six animals each and included in Group II, III and IV. Normal rats (6) were kept in Group I as negative control (treated with vehicle only).

Group I - Negative control
Group II - Diabetic control
Group III - Diabetic rats treated with 0.5mg/kg b.w. of glibenclamide
Group IV - Diabetic rats treated with 200mg/kg b.w. of PC

The standard drug (Glibenclamide) was given once a day orally for 14 days to Group III animals and the plant extract, PC, was given once a day orally for 14 days to Group IV.

**Body weight analysis**

Initial body weight of the diabetic animals and final weight after treatment were noted and compared.

**Sample collection & estimation** [20]

The blood samples were collected by retro-orbital plexus puncture method and blood glucose levels were estimated on 0th, 5th, 10th, and 15th day. Serum was analyzed for SGOT, SGPT, cholesterol and triglycerides levels on the 15th day with commercial kits.

**Data and statistical analysis**

Data obtained were expressed as mean ± standard error of mean. Statistical comparisons between all groups were performed by Anova followed by Dunnett’s test using Graph pad IV software.

**RESULTS**

The percentage yield of the plant extract was found to be 2.2% w/w.

**Acute toxicity studies**

Acute toxicity study on petroleum ether extract of *Cassia fistula* at 2000mg/kg b.w. in rats for a period of 14 days did not produce any mortality. There was no significant change in the body weight and food consumption of the animals of both the groups. The animals treated with plant extract did not show any signs of toxicity and no difference to that of normal animals. It indicates the safety of extracts of in the experimental species.

**In Vivo antidiabetic study**

*In vivo* antidiabetic activity of the plant extracts PC was evaluated using streptozotocin diabetic induced model. Various parameters; body weight, blood glucose levels, SGOT, SGPT, cholesterol and triglycerides were monitored. Data obtained were statistically evaluated and the level of significance was incorporated in the respective tables. The body weight of the animals was monitored on 0th and 15th day and data were expressed as mean ± Standard deviation in the table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean Body weight (g) 0th day</th>
<th>Mean Body weight (g) 15th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>183.04 ± 4.97</td>
<td>185.0 ± 5.36</td>
</tr>
<tr>
<td>Group II</td>
<td>195.16±2.32</td>
<td>146.66±3.56</td>
</tr>
<tr>
<td>Group III</td>
<td>188.83± 3.06*</td>
<td>167.5± 2.73*</td>
</tr>
<tr>
<td>Group IV</td>
<td>190.33± 7.85*</td>
<td>154.33± 9.45*</td>
</tr>
</tbody>
</table>

From the figure 5.9, body weight difference of the animals on 0th and 15th day can be easily noted. The blood glucose level of animals was monitored for each group and values obtained were tabulated in the table 2.
From the numerical data tabulated in the table, the capability of extracts in lowering the glucose level was understood. From the blood samples, serum was separated and subjected for estimation of liver enzymes; GOT and GPT. The SGOT, SGPT values obtained for each group were tabulated in the table 3.

**Table 3: Results of liver function test**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver function test</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0th day</td>
<td>5th day</td>
</tr>
<tr>
<td>Group I</td>
<td></td>
<td>24.95±1.71</td>
<td>27.1±1.97</td>
</tr>
<tr>
<td>Group II</td>
<td></td>
<td>42.86±2.21</td>
<td>46.83±2.82</td>
</tr>
<tr>
<td>Group III</td>
<td></td>
<td>23.21±2.19*</td>
<td>26.76±2.15*</td>
</tr>
<tr>
<td>Group IV</td>
<td></td>
<td>28.35±2.15*</td>
<td>29.31±2.03*</td>
</tr>
</tbody>
</table>

Lipid profile was also estimated in the blood serum samples. Cholesterol and triglyceride values obtained for each group were tabulated in the table 4.

**Table 4: Lipid profile**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lipid profile</th>
<th>Cholesterol (mg/dl)</th>
<th>TG (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td></td>
<td>88.64</td>
<td>92.65</td>
</tr>
<tr>
<td>Group II</td>
<td></td>
<td>158.40</td>
<td>172.97</td>
</tr>
<tr>
<td>Group III</td>
<td></td>
<td>94.66*</td>
<td>98.61*</td>
</tr>
<tr>
<td>Group IV</td>
<td></td>
<td>102.76*</td>
<td>106.05*</td>
</tr>
</tbody>
</table>

*P<0.05 when compared to control

**DISCUSSION**

Medicinal plants have been used as sources of medicine in virtually all cultures. Of late, the use of traditional medicines has increased globally and is gaining popularity. Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost [21]. Therefore, investigation on traditional medicinal plants offers exciting opportunities as it offers unlimited resources for the discovery of new drug leads which could be used for the benefit of mankind. In this context, *Cassia fistula*, was selected and analyzed in the present study.

Prior to antidiabetic studies using streptozotocin induced diabetic model, acute toxicity studies were performed at a dose of 2000mg/kg b.w and the extract was found to be safe without any signs of toxicity and mortality. Streptozotocin injection induces overproduction (excessive hepatic glucogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues which are the fundamental basis of hyperglycemia in diabetes mellitus. In the present study, apart from extract treated groups, normal group, positive control group and standard drug (Glibenclamide) treated groups were present for comparison. Induction of diabetes with streptozotocin is associated with characteristic loss of body weight due to increased muscle wasting and due to loss of tissue proteins and fats [22]. Extract treated group at a concentration of 200mg/kg b.w was found to have prevented the loss of body weight significantly (P<0.05). This may be due...
to its protective effect in controlling muscle wasting i.e. reversal of gluconeogenesis and may be also due to the improvement in insulin secretion and glycaemic control.

Significant hypoglycaemic effect ($P<0.05$) was observed in the extract treated group when compared to positive control and results were found to be comparable with the standard drug. Diabetic complications such as increased gluconeogenesis and ketogenesis may be due to the elevated transaminase activity. As a result, elevation of biomarker enzymes such as SGOT and SGPT will occur [22]. Therefore in the study, SGOT and SGPT levels of the different groups were monitored. A significant reduction ($P<0.05$) in the SGOT and SGPT levels were observed in the extract treated group when compared to positive control.

Diabetes is usually associated with hypercholesterolemia and hyper-triglyceridaemia. Insulin has an inhibitory action on HMG Co-A reductase, a key rate limiting enzyme responsible for the metabolism of cholesterol rich LDL particles. Also, hypertriglyceridaemia may be due to the number of metabolic abnormalities that occur sequentially thereafter [23]. Since, extract treated group was monitored for cholesterol and triglyceride levels. A significant reduction ($P<0.05$) in cholesterol and triglyceride levels was observed in the extract treated group when compared with positive control.

**CONCLUSION**

Diabetes mellitus is one of the major diseases currently affecting an estimated 143 million people worldwide and the number is growing rapidly. Oral hypoglycaemic agents available in market are often unable to restore a normal pattern of glucose homeostasis and also the usage of these drugs are restricted due to their pharmacokinetic properties, secondary failure rates and accompanying side effects. Therefore the World Health Organisation expert committee on diabetes has listed as one of its recommendations that traditional methods of treatment for diabetes should be further investigated. Hence, from the traditionally used antidiabetic plants, *Cassia fistula* was selected for the study. Acute toxicity confirmed the safety of drug extracts at a dose of 2000mg/kg b.w. In the *in vivo* antidiabetic study, bodyweight analysis, blood glucose level analysis and SGOT, SGPT, cholesterol, triglycerides levels were monitored and confirmed that plant has significant antidiabetic activity. Hence, the present study proved the importance of traditional knowledge; as the traditionally used antidiabetic plant selected for the study found to have significant activity. Therefore, it is justifiable to promote these plant extracts which are comparatively cheap, safe and reliable for the treatment of chronic disease, diabetes.

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