Anti-diabetic activity of rhizome extracts of *Imperata cylindrical* against alloxan-induced Diabetes Mellitus in rats

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**ABSTRACT**

The anti-diabetic effect of ethanolic and aqueous extracts of *Imperata cylindrical* rhizomes was investigated in alloxan-induced diabetes in rats. Diabetes was induced by a single 150 mg/kg intraperitoneal dose of alloxan. Rats were divided into five groups with six rats in each group i.e. the normal control group, diabetic control group, standard group (glibenclamide, 10mg/kg, p.o.), Test-I group (200 mg/kg ethanolic extract) and Test-II group (200 mg/kg aqueous extract). The above concerned groups were inoculated on 21st day. On the last day of the experiment, fasted rats were killed by cervical dislocation. The body weight was measured at the initial day and final day. The blood samples were collected for estimation of glucose. The loss of body weight in control group, but recovery was observed in drug treated group. The serum glucose level was significant increased in diabetic rats. However, significant improvement was observed in treated group. The biochemical parameters such as HDL and proteins level were decreased in the control group but maintained in drug treated group. LDL, cholesterol, triglyceride creatinine and urea were significant increase in control group however, reduced level in drug treated group. The present study concluded that ethanolic and aqueous extracts of *I. cylindrical* rhizome showed an appreciable effect in reducing the hyperglycemia and the complications associated with diabetes. However, aqueous extract is found more significant in decreasing blood glucose level in comparison to the ethanolic extract. The study results justify the traditional use of the plant as anti-diabetic.

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**INTRODUCTION**

Diabetes Mellitus (DM) is a group of metabolic disorders of altered carbohydrate metabolism specified by increased blood glucose level. It usually results from either inadequate secretion of insulin or an inadequate response of cells to insulin (Olokoba *et al.*, 2012). Insulin is produced by the endocrine part of pancreas inside the body (Maritim *et al.*, 2003). Diabetes is a disease affecting the majority of population globally from various walks of their life. In India, it is a major health problem with especially concern to urban areas. According to the International diabetes society, DM is classified into three categories i.e. Type-I, Type-II and Type-III. Type-I is insulin dependent DM and is caused due to immunological destruction of β-cells resulting in the reduction of hormone insulin. This type of diabetes is idiopathic without any specified etiology and is mostly inherited. Type II is non-insulin dependent DM...
and is due to the defect in insulin secretion or due to insulin resistance (Kaushik, 2017). The insulin resistance refers to the reduction in the sensitivity to insulin (Gonzalez et al., 2009). Type III is Gestational DM and is the form of glucose intolerance with onset or first recognition of pregnancy (Banoo et al., 2015; Kohei, 2010). The chronic hyperglycemia in diabetes may result in long term harm, dysfunction and organs failure mainly kidneys, eyes, heart, nerves and blood vessels. According to the American Diabetes Association, progressive disorder raises tissue and vascular damage leading to some severe impediments like neuropathy, nephropathy, retinopathy and cardiovascular complications etc (Banoo et al., 2015; Kohei, 2010; Grundy et al., 1999). The various therapy are effective in the treatment of DM, however a huge burden of diabetic mellitus in society is increasing day by day. There are various conventional therapies available for the treatment of diabetes however there is no complete cure as well as have serious side effects. There is not a single drug that cures DM completely at present day. Now a day, the researchers have a thrust area to develop the anti-diabetic drugs which cure DM with less side effect profile.

Herbal formulations are preferred now a day due to their lesser side effects and low cost than synthetic ones available in the market. There are a lot of herbal remedies recommended for diabetes and its complications (Modak et al., 2007). One of the etiological factors responsible for DM development and its complications are the damage of β-cells of Islets of Langerhans induced by free radicals and hence anti-diabetic compound with additional antioxidant property can be a better approach. The Imperata cylindrical, known as cogon grass, rhizome has been selected for the present study due to its traditional uses and reported phytochemicals. Traditionally, it is used as blood purifier, anthelmintic, sedative and diuretic; also in diabetes, wound healing, arthritis, inflammation, constipation and vaginismus. Especially, the roots of the plant are used to treat fever, cough, gonorrhea, asthma, cancer, dysuria, dropsy, jaundice, nephritis, diarrhoea, diabetes, gout, menorrhagia, burning sensation and anemia. The flowers and rhizomes are also used traditionally to treat blood in sputum, bleeding nose, lung and kidney diseases and UTIs (Modak et al., 2007; Simha et al., 2012). Imperata cylin-

drica contains various phytochemicals such as coumarins, triterpenoids, flavones, polyphenols, iron, potassium, calcium, cylindol, cylindrene, graminones, imperanene, phenylpropanoids, trimethoxyphenyl, propanetriol, coumaroylglycerol, coumarin-7-O-beta-D-glucopyranoside, vanillic acid, dihydroxybutyric acid, phenolic compound, salicin, Tristerpenes, arundoin, cylindrin, ferenol, simiarenol and glutinone (Simha et al., 2012; Keshava et al., 2016). The plant have already reported pharmacological activities like Inhibition of platelet aggregation, anti-pyretic, vasodilatation, hepatoprotective, antibacterial, immunomodulating, antihypertensive, antitumor, obesity, activity of regenerative capacity, antibacterial, antioxidant, homeostasis, anti-septic, neuroprotective and anti-anthelmintic activity (Keshava et al., 2016; Gowri et al., 2015; Lalthanpuii and Lalchhandama, 2018). The plant aqueous extract was found safe in dose range up to 1200 mg/kg body weight (Chunlaratthanaphorn et al., 2007). The present research work was focused to study the anti-diabetic activity of ethanolic and aqueous extracts of I. cylindrical rhizome in alloxan-induced diabetes model.

MATERIALS AND METHODS

Collection of plant material

The plant was procured in the month of April from Punjab region, Jalandhar, India which was authenticated by Department of Botany, National Institute of Pharmaceutical Education & Research, Mohali, India.

Extraction

The fresh rhizomes were cleaned before the drying process. Then the fresh rhizomes were divided into pieces and dried in shade at room temperature for two weeks. The dried rhizomes were powdered using the blender and stored in a closed container. The powdered material was packed into soxhlet apparatus for hot extraction. The successive extraction of packed material was done using petroleum ether and ethanol. To obtain the aqueous extract, the powdered material was extracted via maceration method using cold water. The extracts were concentrated in vacuum and dried under reduced pressure.

Phytochemical Investigation

The phytochemical screening of the rhizomes of Imperata cylindrical was carried out to identify various secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids, steroids, saponins, phenols, glycosides and carbohydrates according to standard phytochemical methods (Harborne, 1998; Lalthanpuii et al., 2018).

Animals

Adult healthy Wistar rats (250-300 g) of either sex were procured from Panacea Biotech Ltd, Lajru,
India. The rats were housed in polypropylene cage. After random allocation into the different groups and before the start of study, the rats were accommodated under the standard laboratory conditions of temperature, relative humidity and with 12 hours of dark and 12 hours of light cycle for a period of one week. The animals were fed with standard pellet diet and water ad libitum. The animals expressed as fasting were underprivileged of food and water for 16 hours.

**Acute toxicity studies**

The acute toxicity plant extracts was performed as per OECD Guideline 425. As per the guidelines, 2000 mg/kg dose of extract was given orally to rats and observed for any sign and symptom of toxicity for 24 hours with particular emphasis during the first four hours and daily after that daily for two weeks.

**Experimental Design**

All the animals were randomly allocated into five groups, each having six animals. The animals in different groups were scheduled following treatment:

- **Group I:** Normal control group (saline, 2 ml/kg, p.o.)
- **Group II:** Diabetic control group (Alloxan, 150 mg/kg, i.p.)
- **Group III:** Standard treatment (Alloxan, 150 mg/kg, i.p. + Standard drug, Glibenclamide 10 mg/kg, p.o.)
- **Group IV:** Test-I treatment group (Alloxan, 150 mg/kg, i.p. + *I. cylindrical* ethanolic extract, 200 mg/kg, p.o.)
- **Group V:** Test-II treatment group (Alloxan, 150 mg/kg, i.p. + *I. cylindrical* aqueous extract, 200 mg/kg, p.o.)

The saline, standard drug and plant extracts were given orally with the help of cannula. Group I represents normal control and given saline for 21 days. Group II considered as diabetic control and Group III to Group V were diabetic control animals which previously treated with alloxan and after that, given a fix dose of standard drug glibenclamide (10 mg/kg, p.o.), plant ethanolic extract (200 mg/kg, p.o.) and aqueous extract (200 mg/kg, p.o.) respectively for 21 days.

**Assessment of extracts on alloxan-induced diabetes**

The diabetes was produced in the experimental animals by giving a single intraperitoneal dose of alloxan, 150 mg/kg. Weighed quantity of alloxan for each animal as per their weight was dissolved in 0.2 ml saline (154 mM NaCl) just before the injection. After second day of alloxan treatment, the rats having blood glucose level of >140 mg/dl were selected for present study. The plant extracts treatment was initiated after 48 hour of alloxan injection. The blood samples were taken every week till the completion of the study i.e. 3 weeks (Latha and Pari, 2004).

**Collection of blood samples and determination of blood glucose level**

The blood samples were taken from the tip of the tail of the rats every week till the completion of the study. The fasting blood glucose level was determined on 1st, 7th, 14th and 21st day of the study. The blood glucose level was estimated through one touch electronic glucometer using glucose test strips. A body weight measurement of animals was done on the initial and final day of the study.

The rats were fasted overnight before the last day of study and on the 21st day, the blood sample was collected from retro-orbital plexus under mild anesthesia and glucose level was estimated (Giordano et al., 1989). Serum was segregated and examined for serum LDL (Friedewald et al., 1972), serum HDL (Allain et al., 1974), serum cholesterol (Roschau et al., 1974), serum triglycerides (TG) by enzymatic DHBS colorimetric method (Liebich et al., 1977), serum creatinine (Bowers and Wong, 1980), serum urea (Wilson, 1966) and total serum proteins.

**Statistical Analysis**

All the results of body weight, blood glucose levels and biochemical parameters were expressed as Mean ± standard error of mean (SEM). The statistical analysis was done by using one-way ANOVA followed by Tukey’s Multiple Comparison test. The level of significance were at \( P<0.05 \), \( **P<0.01 \) and \( ***P<0.001 \) in comparison to the control.

**RESULTS AND DISCUSSION**

The ethanolic and aqueous extracts exhibited the presence of carbohydrates, glycosides, triterpenoids, alkaloids, tannins, flavanoid glycosides and proteins in detectable amount which may be responsible for anti-diabetic activity. Acute oral toxicity study exhibited no mortality at the dose of 2000 mg/kg. Thus, \( 1/10^{th} \) dose of 2000 mg/kg i.e. 200 mg/kg dose of the extracts was chosen for the study.

The fasting blood glucose level in normal rats was measured 96 to 97 mg/dl on 1 day to 21 day. The administration of alloxan (150 mg/kg, i.p.) leads to increase in the blood glucose levels and was sustained over a period of 21 days. Daily treatment with each extracts of *Imperata cylindrical* rhizomes for 3 week result in fall in
Table 1: Effect of *Imperata cylindrical* Linn rhizomes extracts on body weight, blood glucose level in alloxan-induced diabetic rats.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Body weight</th>
<th>Blood Glucose level mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial day</td>
<td>Final day</td>
</tr>
<tr>
<td>Group I (Normal Control, Saline 2 ml/kg p.o.)</td>
<td>266.0 ± 5.85</td>
<td>267.0 ± 6.21</td>
</tr>
<tr>
<td>Group II (Diabetic Control)</td>
<td>264.7 ± 4.96</td>
<td>189.7 ± 5.66</td>
</tr>
<tr>
<td>Group III (Standard, Glibenclamide 10 mg/kg, p.o.)</td>
<td>263.2 ± 5.08</td>
<td>253.5 ± 7.11</td>
</tr>
<tr>
<td>Group IV (Test-I, Ethanolic extract 200 mg/kg, p.o.)</td>
<td>259.5 ± 5.82</td>
<td>256.8 ± 6.28</td>
</tr>
<tr>
<td>Group V (Test-II, Aqueous extract 200 mg/kg, p.o.)</td>
<td>264.2 ± 6.05</td>
<td>253.3 ± 6.59</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M (n=6). P values were determined using One-way ANOVA followed by Tukey’s Multiple Comparison test. The diabetic control group compared with that of normal group as well as to that of drug treated groups. P-value less than P* ≤ 0.05 P** ≤ 0.01 P*** ≤ 0.001 consider as significance levels.

Table 2: Effect of *Imperata cylindrica* Linn rhizomes extracts on biochemical parameters on last day

<table>
<thead>
<tr>
<th>Treatment groups and Dose</th>
<th>Serum LDL</th>
<th>Serum HDL</th>
<th>Serum TG</th>
<th>Serum Cholesterol</th>
<th>Serum Urea</th>
<th>Serum Protein</th>
<th>Serum Creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Normal Control, Saline 2 ml/kg p.o.)</td>
<td>56.50 ± 3.67</td>
<td>55.00 ± 2.47</td>
<td>82.17 ± 3.87</td>
<td>77.83 ± 3.01</td>
<td>30.83 ± 4.08</td>
<td>6.17 ± 0.48</td>
<td>0.60 ± 0.07</td>
</tr>
<tr>
<td>Group II (Diabetic Control)</td>
<td>94.00 ± 3.86</td>
<td>42.67 ± 2.96</td>
<td>121.8 ± 4.28</td>
<td>130.8 ± 4.02</td>
<td>60.67 ± 2.43</td>
<td>3.50 ± 0.34</td>
<td>2.58 ± 0.15</td>
</tr>
<tr>
<td>Group III (Standard Glibenclamide 10 mg/kg, p.o.)</td>
<td>58.67 ± 6.86***</td>
<td>56.33 ± 1.99***</td>
<td>83.50 ± 3.39***</td>
<td>90.83 ± 2.98***</td>
<td>32.33 ± 3.77***</td>
<td>6.00 ± 0.45**</td>
<td>0.64 ± 0.11***</td>
</tr>
<tr>
<td>Group IV (Test-I, Ethanolic extract 200 mg/kg, p.o.)</td>
<td>74.17 ± 4.58***</td>
<td>52.83 ± 2.18***</td>
<td>99.83 ± 6.11**</td>
<td>118.2 ± 3.36*</td>
<td>45.83 ± 2.68*</td>
<td>5.83 ± 0.40**</td>
<td>0.65 ± 0.09***</td>
</tr>
<tr>
<td>Group V (Test-II, Aqueous extract 200 mg/kg, p.o.)</td>
<td>61.0 ± 4.5***</td>
<td>51.00 ± 2.47***</td>
<td>77.67 ± 4.45***</td>
<td>89.33 ± 4.26***</td>
<td>33.17 ± 4.22***</td>
<td>5.33 ± 0.48*</td>
<td>0.76 ± 0.06***</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M (n=6). P values were determined using One-way ANOVA followed by Tukey’s Multiple Comparison test. The diabetic control group compared with that of normal group as well as to that of drug treated groups. P-value less than P* ≤ 0.05 P** ≤ 0.01 P*** ≤ 0.001 consider as significance levels.
blood glucose levels. However, plant aqueous extract at dose 200 mg/kg showed more significant anti-hyperglycaemic effects as compared to that of ethanolic extract at the same dose. Maximum anti-hyperglycaemic effect was seen after 3rd week of treatment. The highly anti-hyperglycaemic effect was observed after 3 week of treatment. The data corresponding to anti-diabetic activity of ethanolic and aqueous extracts of Imperata cylindrical rhizomes has been portrayed in Table 1. Alloxan is one of the chemical used to induce DM other than the streptozotocin. It has a distinctive effect on the β-cells of islets of Langerhans of the pancreas. It causes an enormous decrease in the release of insulin as a result of distraction of β-cells of the pancreas which resulted in the hyperglycemia. The alloxan treatment results in the elevation of serum LDL, triglycerides (TG), cholesterol, urea and creatinine but decreases the HDL and serum protein levels due to insulin deficiency as a result of β-cell destruction in control group. The standard treatment with glibenclamide (10 mg/kg, p.o.) and both extracts of Imperata cylindrical rhizomes revoked the above changes induced by alloxan treatment (Table 2).

The aqueous extract was found more significant in reversing the alloxan induced changes as compared to ethanolic extract. The level of serum HDL and protein are usually found elevated in DM and such an increased level represent the risk for coronary heart diseases. The abnormal high level of the serum HDL & proteins are mainly because of the abandoned action of lipolytics hormones on the fat stores especially due to the insulin action. In present study, the results showed significant decrease in the level of HDL and proteins in all treatment groups in comparison to the control group.

Pancreas is the main organ which senses the organism’s dietary and energetic condition through the blood glucose concentration. When there is an increase in blood glucose level, the pancreas secretes insulin as a result. Thus, it helps in regulating body weight. The vehicle control (Group I) rats were found to be steady in their body weight while diabetic rats (Group II) exhibited significant decrease in their body weight in comparison to the initial day. The alloxan induced body weight reduction was recovered by both extracts however the aqueous extract showed more significant effects as compared to ethanolic extract (Table 1).

The numbers of functional β-cells of islets of Langerhans of pancrease are of conclusive value in the development path and consequences of DM. The total numbers of β-cell mass represents the equilibrium between the renewal and damage of the cells. The renewal of β-cells in DM has already been studied in different animal models. It is also proposed that the regeneration of β-cells of the islets from the effects of the drug may be the prime reason for the improvement of alloxan-injected guinea pigs (Gorlay et al., 1986).

On the behalf of the results, the study indicated that both plant extracts exhibited significant anti-diabetic activities in alloxan-induced hyperglycaemic rats without remarkable changes in their body weight. The extracts of I. cylindrical treatment showed the improvement in the condition of DM as recommended by the parameters like body weight and lipid profile along with serum creatinin, serum urea, serum LDL, serum HDL, serum triglycerides and serum proteins. There are number of active constituents present in ethanolic & aqueous extracts of I. cylindrical rhizomes such as glycosides, carbohydrates, steroids, flavonoids, steroids and alkaloids which may be responsible for the activity.

CONCLUSIONS

The present study concluded that ethanol & aqueous extracts of Imperata cylindrical rhizomes have significant anti-diabetic activity via decreasing the blood glucose level and recovery was observed in the biochemical parameters also. Both the extracts showed potential anti-diabetic activity at dose 200 mg/kg however aqueous extract have more significant activity in alloxan-induced diabetic rats. This investigation supports the insertion of this plant in traditional anti-diabetic medicines. The mechanism of action and potential compound responsible for the activity are for future aspects.

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Conflict of interest

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