Hypoglycemic activity of ethanolic leaf extract of *Phyllanthus amarus* in alloxan induced diabetic mice

A. Shetti and B. B. Kaliwal*

Department of Biotechnology and Microbiology, Karnatak University, Dharwad, India

ABSTRACT

*Phyllanthus amarus* is an indigenous medicinal plant, with a folk reputation as hypoglycemic agent in Central and Southern India. In the present investigation hypoglycemic activity of ethanolic leaf extract of *Phyllanthus amarus* was evaluated using alloxan induced hyperglycemic mice. Blood glucose levels of all the mice of each group were tested before the treatments on fasting mice and then tested after 1h, 2h and 4h of administration of a single dose of the extract (400 mg/kg body weight) or standard antidiabetic drug glibenclamide (600 µg/kg body weight). The blood glucose level was measured using glucometer. The diabetic mice treated with extract showed a significant (P ≤ 0.05) decrease in blood glucose level after the intervals of 1h, 2h and 4h. The hypoglycemic activity was comparable to that of the reference drug glibenclamide. The present investigation revealed that the ethanolic leaf extract of *Phyllanthus amarus* possesses a potent hypoglycemic activity. The possible mechanism may be the stimulation of β cells and subsequent release of insulin and activation of the insulin receptors. The plants hypoglycemic action may be by potentiating of pancreatic secretion of insulin.

Keywords: Diabetes mellitus, *Phyllanthus amarus*, hypoglycemic, glibenclamide

INTRODUCTION

Diabetes mellitus is one of the common metabolic disorders with micro-and macro vascular complications that results in significant morbidity and mortality. It is considered as one of the five leading causes of death in the world [1], [2]. India has largest number of diabetic patients in world and is termed as diabetic capital of the world. According to International Diabetes Federation currently around 40.9 millions of peoples are diabetic and number is expected to rise to 69.9 million by 2025 unless urgent preventive steps are taken. Different types of oral hypoglycemic agents such as biguanides and sulphonylurea are available along with insulin for the treatment of diabetes mellitus, but have side effects associated with their uses [3]. There is a growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experience and relatively low costs. Herbal drugs or their extracts are prescribed widely, even when their biological active compounds are unknown. Even the World Health Organization (WHO) approves the use of plant drugs for different diseases, including diabetes mellitus. Traditional plant medicines are used throughout the world for treatment of diabetes mellitus.

*Phyllanthus amarus* is a small herb common to Central and Southern India. All parts of the plant are employed therapeutically. It is reported to contain lighans, alkalids, flavonoids, galloutnoids and glycosides [4]. *Phyllanthus amarus* possesses antiviral, antiparasitic, antimalarial, antimicrobialal, and anticacelus properties. *Phyllanthus* species show antidiabetic properties, *phyllantus emblica* has proven antidiabetic effect [5]. There for the present investigation was undertaken to determine the hypoglycemic activity of ethanolic leaf extract of *Phyllanthus amarus* in alloxan induced diabetic mice.
MATERIALS AND METHODS

Plant Material and preparation of extract
The leaves of *Phyllanthus amarus* (Euphorbiaceae) were collected in Feb 2009 from Botanical garden of Karnataka University, Dharwad Karnataka state, India and authenticated at department of studies in Botany Karnataka University Dharwad of Karnataka state, India. The fresh leaves of the plant were shade dried on a laboratory table for 6 days and reduced to powder. This powder was packed into soxhlet apparatus and extracted using absolute ethanol. The extraction was carried out for 38 hrs till the total extraction was obtained. The extract obtained was dried at 45°C in hot air oven till semisolid mass was obtained [6]. The yield was 4.5%. The extract was stored in a refrigerator at 4°C until used.

Animals
Laboratory bred 3-4 months old adult virgin male Swiss albino mice weighing 25-30 g maintained on standard animal pellets and water ad libitum in conventional animal house located at department of studies in Zoology of Karnataka University, Dharwad, Karnataka, India. Animals described as fasting were deprived of food with free access to water for 16 h.

Induction of experimental diabetes
Diabetes was induced by intraperitoneal administration of alloxan monohydrate (150 mg/kg body weight) dissolved in normal saline to the male Swiss albino mice, after an overnight fast (access to only water) of 12 hours to make them more susceptible to developing diabetes [7]. After 72 h mice with diabetes mellitus indicated by glycosuria (indicated by Benedict’s test) and hyperglycemia with blood glucose range of 200 to 350 mg/dl were used for present study [8]. The mice were weighed, and randomly divided into four groups (Group I-IV) of 10 mice each. Group I consisted of Normal untreated mice given only vehicle (1% percent gum acacia), Group II served as Diabetic control, in Group III Diabetic mice were given single dose ethanolic extract of *Phyllanthus amarus* leaves (400mg/kg body weight) with vehicle by oral administration and finally in Group IV Diabetic mice were given single dose of glibenclamide (600mg/kg) with vehicle by oral administration.

Evaluation of hypoglycemic activity
The blood glucose level (BGL) was monitored after alloxanisation in blood samples collected by amputation of the tail tip under mild an aesthesia [9]. A drop blood was placed on a blood glucose test strip (Glucocard tm 01 sensor) and was inserted into a glucometer ARKRAY Glucard 01-mini Blood glucose testing system. After 72 h Swiss albino mice having BGL beyond 200 mg/dl were selected for the study.

The 400 mg/kg of body weight dosage of *Phyllanthus amarus* ethanolic extract and 600 mg/kg of body weight of Glibenclamide was given to mice of respective groups. Blood glucose levels were tested before the treatments on fasting mice and after 1h, 2h, and 4h of administration of a single dose of the extract or Glibenclamide.

Statistical analysis
All the values of tasting blood glucose were expressed as mean ± standard error of mean (S.E.M) and analyzed for ANOVA and post hoc dumet’s t-test. Differences between groups were considered significant at P≤ 0.05 levels.

RESULTS AND DISCUSSION

Diabetes mellitus is a collection of disorder which results from either lack of insulin or factors, which interfere with the action of this hormone. In animals, it can be induced by partial pancreatectomy or by the administration of diabetogenic drugs such as alloxan, streptozotocin, dituzonx and anti-insulin serum [10]. Alloxan causes massive distraction of the β cells of islets of Langerhans, not only destroys pancreatic β cells but also damages the kidney [11]. The disease is progressive and is associated with high risk of atherosclerosis, kidney and nerve damage as well as blindness. Abnormalities in the regulation of peroxide and transition metal metabolism are postulated to result in the development of the disease as well as its long term complication [12]. The mechanism of alloxan has been fully described much earlier [13].

Although *P. amarus* has been advocated as a traditional plant treatment in folkloric medicine in southern India, detailed scientific studies to evaluate its efficiency and mechanism of action are lacking. The result of the present study on blood glucose level revealed that administration of ethanolic leaf extract of *P. amarus* to alloxan induced diabetic control mice produced a significant reduction in blood glucose level compared to diabetic control group. The hypoglycemic activity of the extract was sustained throughout the monetarily period almost comparable to that of the reference drug glibenclamide. The present study indicates the scientific basis for its use. As we know Diabetes mellitus is a metabolic disorder characterized by insufficient insulin secretion and/or insensitive target tissues to
metabolic action of insulin though, insulin is presently one of the most important therapeutic agent known to medicine, efforts have continued to seek for insulin substitutes from synthetic or plant sources for treatment of diabetes and reported to have hypoglycemic effect.

The *Phyllanthus amarus* ethanolic leaf extract of extract is also known for its liver protective action [14]. Liver is an insulin dependent tissue, which plays a vital role in glucose and lipid homeostasis and is severely affected during diabetes. Decreased glycolysis impeded glycogenesis and increased gluconeogenesis are some of the changes of glucose metabolism in the diabetic liver [14], [15]. The results of the present study is also supported by the earlier reports on hypoglycemic activity of *zygophyllum gaulcetium* extract [16], *Artemisia herba alba* [17], against alloxan induced Diabetes mellitus. Similar results have been reported on the hypoglycemic activity of plant extract [6], [7], [18].

The hypoglycemic effect of *Phyllanthus amarus* extract could be linked to more than one mechanism. The possible mechanism includes the stimulation of β cells and subsequent release of insulin and activation of the insulin receptors. The plants anti- hyperglycemic action may be by potentiating of pancreatic secretion of insulin. In this context a number of other plants have also been reported to have anti-hyperglycemic and insulin release stimulatory effect [19], [20]. Hence, in the present study the *Phyllanthus amarus* extract also may act as a hepatoprotective agent so this evidently, improves the function of liver and maintains glucose uptake, enhanced transport of blood glucose to peripheral tissue and utilization, which may be another mechanism of action [21].

Table 1. Effect of ethanolic leaf extract of *Phyllanthus amarus* on blood glucose level in alloxan induced diabetic mice after a single dose

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Blood glucose level mg/dl (mean ± S.E.M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>Initial: 105.20 ±3.92</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic control</td>
<td>310.20±8.24</td>
</tr>
<tr>
<td>III</td>
<td>Glibenclamide control (600 µg/kg/day)</td>
<td>310.20±7.88*</td>
</tr>
<tr>
<td>IV</td>
<td><em>P. amarus</em> extract (400 mg/kg/day)</td>
<td>312.60±7.67*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>1h</th>
<th>2h</th>
<th>4h</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>95.60±6.98</td>
<td>84.20±5.94</td>
<td>79.60±3.76</td>
</tr>
<tr>
<td>II</td>
<td>287.60±8.08</td>
<td>271.40±6.17</td>
<td>261±7.63</td>
</tr>
<tr>
<td>III</td>
<td>246.20±12.6*</td>
<td>178.±8.80*</td>
<td>149.20±3.72*</td>
</tr>
<tr>
<td>IV</td>
<td>251.20±2.15*</td>
<td>185.60±4.98*</td>
<td>142.80±3.26*</td>
</tr>
</tbody>
</table>

In the glucose loaded hyperglycemic model used in the present studies, the *P. amarus* ethanolic leaf extract exhibited significant (P<0.05) hypoglycemic activity at a dose of 400 mg/kg in 1, 2, and 4 h. It is well established that after glucose loading in glucose tolerance test, along with blood glucose, blood insulin level is also increases. Insulin brings down blood glucose in about 1 h to 4 h by its utilization through different mechanisms. The present results with extract and glibenclamide are in support of this. In the case of the aqueous extract and drug treated groups, the glucose level was not increased like the alloxan induced diabetic control group, giving an indication regarding the supportive action of the extracts, fraction and drug in the glucose utilization. Glibenclamide is reported to enhance the activity of β cells of the pancreas resulting in secretion of larger amount of insulin which in turn brings down glucose level [22]. However, insulin level was not estimated in the present study.

CONCLUSION

In the diabetic control group no significant change in blood glucose level was observed during monitoring period of 4 hours. The control group also did not show significant change in the blood glucose level during monitoring period of 4 hours. The diabetic mice treated with 400 mg/kg body weight of *Phyllanthus amarus* ethanolic leaf extract showed significant (P<0.05) decrease in the blood glucose level after 4 h. There was a study decrease in glucose level after 1h, 2h, and 4h of treatment. The decreased blood glucose levels were comparable to the values obtained with that of reference drug glibenclamide. The present investigation revealed that the ethanolic leaf extract of *Phyllanthus amarus* has hypoglycemic activity. Further, comprehensive chemical and pharmacological investigation should be carried out to isolate the active compound and appropriate elucidation of its mechanism of action.

REFERENCES

A. Shetti and B. B. Kaliwal
