1. Introduction

Natural products play a key role in the discovery and development of new drugs. About 25% of prescribed medicines are plant derivatives and about 80% of the world’s population relies on herbal medicines as a folk medicine and alternative remedies to cure different ailments and diseases (Batugal, 2004). The medicinal value of plants lies in plant borne chemical substances that produce a definitive physiological action on the human body (Edeoga et al., 2005). Vigorous research is now being focused on identifying various herbs possessing hypolipidemic, antiplatelet, antitumor or immune stimulating properties, that may prove helpful as adjuncts in reducing the risk of cardiovascular disease and cancer (Craig and Winston, 1999). Plants possessing therapeutic potential belong to nearly 30,000 species belonging to 5,000 genera (Akerele, 1998).

Diabetes mellitus is one of the five leading causes of death (Kumar et al., 2006) and its prevalence has increased exponentially. In fact, nearly 150 million people worldwide are diabetic, with that number expected to increase to 355 million by 2030 (Wild et al., 2004). Most importantly, the management of diabetes without any side effects is still a challenge (Kameswararao et al., 2003).

The ethnobotanical information reports that around 800 plant species may possess anti-diabetic potential. A

Abstract

Diabetes is a serious medical problem and affects millions of people worldwide. In Indian folk medicine, many plant species are suggested to be anti-diabetic but lack concrete scientific proof. The present study investigated the antidiabetic potential of one plant species namely Woodfordia fruticosa (Linn). Crude extract was administered acutely (3 g/kg b.w) and chronically (142 mg/kg b.w/day for 21 days) to alloxan monohydrate-induced diabetic rats. Controls were treated with DMSO and glibenclamide. Glucose levels were estimated at 0, 1, 3 and 5 hrs (for acute) and at day 1, 7, 14 and 21 (for chronic). At end of treatments rats were sacrificed. Serum samples were evaluated for creatinine, urea, liver enzymes, ALT, AST, ALP, cholesterol and triglycerides. Histology of pancreas was done to assess histomorphology. Results showed that Woodfordia fruticosa extract caused a greater reduction ($P < 0.001$) in plasma glucose concentration in both acute and chronic treatments. ALT, AST and ALP and serum urea were significantly reduced in the extract treated diabetic rats ($P < 0.001$). Cellular deformities in diabetic rat pancreas demonstrated restoration of pancreatic architecture. The present study demonstrates that Woodfordia fruticosa extract contains glucose lowering activity and can prove useful in diabetic and diabetes related pathologies.

Keywords: Antidiabetic agents; Diabetes mellitus; Diabetic nephropathy; Folk medicine; Plant extracts; Woodfordia sp.
wide array of plant-derived active compounds representing alkaloids, glycosides, polysaccharides, peptidoglycans, hypoglycans, guanidine, steroids, carbohydrates, glycopeptides, terpenoids, amino acids and inorganic ions have been demonstrated to possess anti-diabetic activity (Ajit kar et al., 1999; Jafri et al., 2000). Even the discovery of widely used hypoglycemic drug, metformin came from the traditional approach of using Galega officinalis. However, many of the plant species have proved not to be very effective in lowering glucose levels in severe diabetes (Davis and Granner, 1996).

Of several plant species Woodfordia fruticosa (Linn.) is also believed to have anti-diabetic potential. Its chemical constituents are flavonoids and tannins (Leaves, twigs and immature fruits). Its bark is being used in Garhawal (India) for treatment of menorrhagia. The flowers are being used in the preparation of Ayurvedic fermented drugs called 'Aristhas' and 'Asavas', and very popular in the Indian subcontinent and other South Asian countries. A popular crude drug (called 'Sidowaya' or 'Sidawayah') of Indonesia and Malaysia chiefly contains dried flowers of Woodfordia fruticosa. It has been used as an astringent to treat dysentery and sprue, and also for the treatment of bowel complaint, rheumatism, dysuria, hematuria and infertility in many South East Asian countries (Das et al., 2007).

Like other countries, the use of herbal medicine has increased enormously in Pakistan over the last few decades. Although hypoglycemic studies on Woodfordia have been done but relatively few studies encompass full physiological effect. The current study investigated acute and chronic exposure of crude extracts of Woodfordia fruticosa in experimentally induced diabetes on a more scientific and biochemical basis with emphasis on hepatic and renal function.

2. Methodology

The present study was conducted at the Department of Animal Sciences, Faculty of Biological Sciences, Quaid-i-Azam University Islamabad and the study was approved by the local ethics committee of the Department of Animal Sciences.

Animals and maintenance

Healthy adult male Sprague-Dawley rats (n=25, average body wt = 255±30 g) were purchased from the National Institute of Health, Islamabad. Five animals were housed in steel cages under standard laboratory conditions.

Plant collection and extract preparation

Leaves of a plant species Woodfordia fruticosa locally named as 'Dhawi' were collected from Margalla hills National Park and methanolic extracts were prepared through standard procedures.

Induction of Diabetes

Diabetes was induced to adult rats by a single intraperitoneal injection (i.p.) of alloxan monohydrate (150 mg/kg b.w) (Sigma Aldrich, USA) (Aruna et al., 1999).

Experimental design and dosage

Animals were divided into five groups (n=5). The experiments were carried out in acute as well as chronic doses. For acute experiments, rats were administered through gavage 3 g/kg b.w Woodfordia fruticosa extract (dissolved in dimethyl sulphoxide, DMSO). In chronic experiments, animals were administered crude plant extract through gavage at the rate of 142 mg/kg b.w per day for continuous 21 days (total dose=3 g/kg). Positive control group of rats were treated with an antidiabetic drug glibenclamide given at the rate of 10 mg/kg b.w/day for 21 days. Vehicle control group received oral doses of 70 µl DMSO day⁻¹ for 21 days.

In the acute experiments, for blood glucose measurement, blood samples were collected from the tail vein at 0, 1, 3, and 5 hrs post gavage of the plant extract. Blood glucose was measured using Dextrostix with glucometer (Accu-Chek Active. Roche, China). In the chronic experiments, treatment of diabetic rats with the plant extract was begun 48 hrs after alloxan injection. Blood glucose was measured at weekly intervals till end of the study (3 weeks). The animal weight and fasting glucose levels were estimated on day 1, 7, 14 and 21.

Blood and tissue collection

At end of experiments, rats were starved for 12 hrs, reweighed, and anesthetized with sodium pentabarbital 60 mg/kg b.w. Sera were prepared and stored -20°C until assayed. Lipid profile viz., serum total cholesterol, HDL, LDL, triglycerides, liver enzymes, serum urea and creatinine were estimated through commercial kits.
(Medizintechnik GmbH Austria; Globe Diagnostic, Italy and AMP Diagnostics, Austria) on a UV visible light spectrophotometer (Agilent 8453). Tissue samples of liver and pancreas were processed for histology.

**Statistical Analysis**

The results were analyzed and compared by one way ANOVA followed by post-hoc Tukey’s adjustment using the Statistical Package for Social Sciences (SPSS, version 16, Inc, Chicago, Illinois, USA). P < 0.05 was considered to be statistically significant difference.

3. Results

**Effect on body weight**

Throughout the experiments, body weight did not show any significant change in any of the experimental groups, treatment or otherwise.

**Plasma glucose levels**

**Effect of acute dose of Woodfordia fruticosa crude extract**

Compared to control animals, plasma glucose concentration that increased significantly after alloxan treatment showed significant fall at 1 hr and a highly significant decline at 3 hr and 5 hr (P < 0.001) post treatment with the **Woodfordia** extract. Glibenclamide treatment also led to a significant decrease in glucose levels at 3 hrs (P < 0.01) and 5 hr (P < 0.001) post treatment respectively (**Figure 1**).

**Effect of chronic dose of Woodfordia fruticosa crude extract**

A significant decrease was observed on the 7th day in glucose levels in diabetic rats (alloxan pretreated) treated with the extract (P < 0.001) followed by a further significant decline on the 14th day (P < 0.05), and a highly significant reduction on the 21st day (P < 0.001) post treatment with the extract as compared to day 1 (**Figure 2**).

Rats treated with glibenclamide also showed a highly significant decrease in blood glucose levels on the 7th, 14th and 21st day (P < 0.001) post treatment. Glucose levels remained significantly higher throughout the experiment in non-treated diabetic control group (**Figure 2**).

**Liver enzymes**

Application of **Woodfordia** plant extract to diabetic rats led to a significant decrease in serum ALT, AST and ALP (P < 0.001) as compared to negative control (only alloxan treated) who showed significantly concentrations. This significant reduction in glucose level was equivalent that occurred on application of glibenclamide (**Table 1**).
Renal function

A slightly significant reduction occurred in serum urea levels of diabetic rats treated with the *Woodfordia* extract (P < 0.05) but no change was noticeable in creatinine levels (*Table 1*).

Lipid profile

**Cholesterol and triglycerides**

Levels of serum cholesterol and triglycerides remained unaltered in the extract treated, and glibenclamide treated groups when compared with control rats (*Table 1*).

Histology

As compared to vehicle control rats (*Figure 3A*) the pancreas of diabetic rats showed gross deformities that included cellular lesions and reduced cellular dimensions. There occurred loss of connective tissue and a mild hyperplasia was noticeable. The cells were hyperchromatic and unevenly distributed. Cellular congestion was also observed in the histological sections of diabetic rat groups. There was mild infiltration of inflammatory cells (*Figure 3B*).

A restoration of normal cellular population size was noticeable in the pancreatic sections obtained from *Woodfordia* extract treated groups. The cellular congestion was reduced and no inflammatory cells infiltration was detected in this group of rats (*Figure 3C*). The findings were comparable with those of normal control group. Pancreas of glibenclamide treated rats had similar findings to extract treated group showing improvement of pancreatic cells (*Figure 3D*).

**Figure 3.** Photomicrographs of vehicle control (A), alloxan treated diabetic (B), *Woodfordia* extract treated (C) and glibenclamide treated (D) rats. A: Normal acini and normal cellular population in the islets of langerhans in pancreas of vehicle control rats. B: Alloxan treated diabetic rats showed extensive damage to the islets of langerhans and reduced dimensions of islets. C: Extract treated pancreas showed restoration of normal cellular population size of islets. D: Glibenclamide treated group showed restoration of normal cellular population size of islets. (Magnification 200x).
4. Discussion

The results of the present study on diabetic rats following acute and chronic treatments with *Woodfordia fruticosa* (Linn.) demonstrated significant reduction in plasma glucose, serum urea and liver enzymes, while pancreas showed restoration of normal cellular structure of islets. In both acute and chronic treatments, normoglycemia upon extract treatment indicated that this plant species possess definitive antihyperglycemic activity.

Different mechanisms of action of plant extracts to reduce blood glucose levels are already known. Some plants exhibit properties similar to the well-known sulfonylurea drugs like glibenclamide (Davis and Granner, 1996), while others do not affect blood glucose in normal state and act instead much like biguanides such as metformin which is a known antihyperglycemic compound (De Fronzo and Goodman, 1995; Stumvoll et al., 1995).

We presently suggest that *Woodfordia fruticosa* acted in a metformin-like mechanism as it did not decrease plasma glucose levels in normoglycemic rats.

Diabetic nephropathy is a microvascular complication of diabetes. A key morphological change associated with sustained hyperglycemia is the accumulation of glycogen granules in distal tubules, which leads to renal hypertrophy (Kang *et al.*., 2005). Presently, significant elevations of serum creatinine and urea levels were indicative of impaired renal function. A significant reduction in serum urea was noticeable in the extract treated rats, indicating this to be due to reduced hyperglycemia and a possible inhibition of glyconeogenesis.

### TABLE 1. Biochemical parameters in normal, diabetic and diabetic Woodfordia fruticosa extract treated rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal ctrl</th>
<th>Veh Ctrl</th>
<th>Db Ctrl</th>
<th>aDb ExTr</th>
<th>Db GlTr</th>
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<tbody>
<tr>
<td>ALT</td>
<td>38.69***</td>
<td>73.0 ± 12.03</td>
<td>45.20 ± 4.70</td>
<td>273.2 ± 24.44***</td>
<td>130.2 ± 12.05</td>
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<tr>
<td>AST</td>
<td>126.2 ± 8.85</td>
<td>109.06 ± 6.90</td>
<td>429.6 ± 22.49***</td>
<td>119.6 ± 10.1</td>
<td>84.4 ± 4.56</td>
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<tr>
<td>ALP</td>
<td>116.8 ± 6.71</td>
<td>141 ± 7.72</td>
<td>371.4 ± 20.7***</td>
<td>156.2 ± 14.32</td>
<td>64.4 ± 7.75</td>
</tr>
<tr>
<td>Urea</td>
<td>36.0 ± 2.68</td>
<td>38.2 ± 4.07</td>
<td>54.6 ± 5.35</td>
<td>34.2 ± 3.83*</td>
<td>43.28 ± 4.28</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.56 ± 0.02</td>
<td>0.5 ± 0.03</td>
<td>0.54 ± 0.02</td>
<td>0.52 ± 0.02</td>
<td>0.66 ± 0.02</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>117.0 ± 6.70</td>
<td>117.0 ± 5.63</td>
<td>105.4 ± 3.44</td>
<td>133.4 ± 6.70</td>
<td>111.0 ± 4.78</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>89.6 ± 3.30</td>
<td>94.73 ± 2.0</td>
<td>100.8 ± 5.42</td>
<td>95.0 ± 3.11</td>
<td>93.5 ± 2.15</td>
</tr>
</tbody>
</table>

Normal ctrl: Normal Control (non treated); Veh ctrl: Vehicle Control (DMSO treated); Db ctrl: Diabetic Control (Alloxan Treated); aDbExTr: Diabetic Extract Treated (aloxan pretreated); DbGlTr: Diabetic Glibenclamide Treated (aloxan pretreated)

Significant at *P < 0.05; ***P < 0.0001
As already known (Edem, 2009), presently diabetic rats showed prominent pathology of pancreas including hyperplasia, congestion of parenchymal cells and mild inflammation. Upon treatment with the extract of *Woodfordia*, rats showed reversal of abovementioned pathological changes equivalent to glibenclamide treatment indicating the efficacy of the extract as a hypoglycemic agent.

Our present findings indicate that the glibenclamide and the extract of *Woodfordia fruticosa* possibly use similar mechanism to induce hypoglycemic changes. This might be through potentiation of pancreatic secretion of insulin from the intact β-cells of islets. The mechanism may also be coupled with extra-pancreatic mechanisms like decreased glycogenolysis and enhanced glycogenesis by the liver and enhanced transport of blood glucose to the peripheral tissues.

**Conclusions and Recommendations**

It is concluded that *Woodfordia fruticosa* extract may possess potent antihyperglycemic activity and can prove useful to protect from hepatic and liver damage in diabetes. The present study provides supportive evidence in favor of the use of *Woodfordia fruticosa* leaves in traditional medicine to treat diabetes. Long-term studies on a more biochemical and molecular level are definitely required to understand the mechanism of action and to isolate the active ingredient to finally design an antidiabetic drug.

**References**


