ANTIOXIDANT AND HYPOGLYCEMIC ACTIVITY OF SOME INDIAN MEDICINAL PLANTS

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Summary

The present study was designed to evaluate comparative antioxidant and hypoglycemic activities of 10 herbal samples referred in Indian system of medicine by using alloxan induced diabetic albino rats. The 80% alcoholic extracts of *Casearia esculenta*, *Coccinia indica*, *Tragia involucrate*, *Moringa oleifera*, *Tinospora cordifolia*, *Ficus benghalensis*, *Murraya koenigii*, *Sesbania aegyptiaca*, *Mucuna prurita* and *Zingiber officinale* were separately suspended with 1% gum acacia and employed for assessing anti-diabetic activity at a dose of 200mg/kg for 21 days and glibenclamide tablet was used as a standard drug. DNA nicking assay was performed by using supercoiled pUC 18 DNA and analyzed on 1% agarose gel. *T. cordifolia* was found to be most potent and showed blood glucose lowering effect from 298 to 235 mg/dl, 186 mg/dl and 95 mg/dl after 1, 2 and 3 weeks of treatment respectively. The effect after 3 weeks in terms of hypoglycemic activity in increasing order was *M. prurita*, *S. aegyptiaca*, *M. koenigii*, *Z. officinale*, *F. benghalensis*, *C. esculenta*, *M. oleifera*, *T. involucrate*, *C. indica* and *T. cordifolia*. The total phenolic contents showed variation from 1.07 (Zingiber officinale) to 45.6 mg GAE/g extract (Murraya koenigii) and antioxidant activity from 28.9 (Coccinia indica) to 75.6% (Moringa oleifera) in the extracts of different plants. In the protection of DNA damage experiment *Moringa oleifera* and *Tinospora cordifolia* showed significant reduction in the formation of nicked DNA and increased native DNA.

Keywords: Diabetes mellitus, Alloxan, Hypoglycaemic activity; Medicinal plants, Phenols, Antioxidant activity, Free radical scavenging activity

Introduction

Diabetes mellitus (DM) is a metabolic disorder associated with increased morbidity, mortality rate and can be defined as a group of metabolic diseases characterized by chronic hyperglycemia due to defect in insulin secretion, insulin action or both, resulting in impaired carbohydrate, lipid and protein metabolism. It is a major health problem worldwide; approximately 5% of the world’s population suffers from diabetes. Since DM is a multi-factorial disease, the treatment is aimed to not only controlling blood sugar level to normal limit, but also at correcting the associated metabolic defects. Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn leads to secondary complications affecting eyes, kidney, nerves and arteries.
Along with hyperglycemia and abnormalities in serum lipid, diabetes is associated with micro and macro vascular complications the major causes of morbidity and death due to diabetes[1-4]. Besides medicine, exercise and diet play key role in the management of diabetes mellitus. There is a great scope for exploiting the anti-diabetic potency of natural sources that appear to hold promise as potential anti-diabetic agents. More than 150 medicinal plants are mentioned in the Indian system of medicines including folk medicines for the management of diabetes, which are effective either singly or in combinations as compound formulation[5-8]. The presence of specific Phytochemicals, inorganic micro nutrients, vitamins and antioxidants play important role to control blood sugar level and associated disorders. The minerals are not hypoglycaemic in themselves but most of the essential trace mineral elements act primarily as catalysts or co-factors in enzyme systems[9-11].

The earlier reports by different investigators showed wide variations in their experimental results for the same plant(s). That might be due to inadequate experimental design, incomplete extraction procedure, and insensitive animal model for showing great variation and some time in some cases even negative results have been reported[12-21]. The present investigations were undertaken with the objective to take care against all such factors and 10 medicinal plants known for their hypoglycaemic activities were selected. Some of these plants had been investigated earlier and some others are less known for their hypoglycaemic activity[5, 12-21].

In the present study, comparative hypoglycaemic activities of 80% ethanol/water extracts of herbal samples referred in Indian system of medicine have been thoroughly evaluated by using alloxan induced diabetic albino rats.

Materials and Methods

The specific parts of the full grown matured plants (Identified by Dr S.K. Tewari, Scientist, National Botanical Research Institute, Lucknow) were collected, cleaned thoroughly, dried and powdered. The experimental plants, part used and their family are Casearia esculenta Wight and Arn. (stem, Flacourtiaceae), Coccinia indica Wight and Arn (matured unripe fruits, Cucurbitaceae), Tragia involucrate L. (whole plant, Euphorbiaceae), Moringa oleifera Lam. (aerial parts, Moringaceae), Tinospora cordifolia (Willd.) Miers (stem, Menispermaceae), Ficus benghalensis L. (stem bark, Moraceae), Murraya koenigii (L) Spreng (aerial parts, Rutaceae), Sesbania aegyptiaca Pers. (leaves, Papilionaceae), Mucuna prurita Hook. (seeds, Papilionaceae) and Zingiber officinale Rosc. (rhizomes, Zingiberaceae). The powdered (40 Mesh) plant samples (200 g) were extracted with 80% ethanol/water (V/V, 600 ml) in a soxhlet at controlled temperature. The collected plant extracts were separately concentrated under reduced pressure below 60°C by using a vacuum pump and rotary evaporator ensuring complete removal of the solvent. The concentrated and dried ethanolic extracts of the samples thus obtained were stored at 4°C until used.

Animal selection and induction of diabetes:

Wistar albino male rats weighing between 150-200 g, obtained from animal house of the college were used for the experiment. Throughout the study, animals were maintained under normal laboratory conditions and were given standard animal feed. Animal study protocol was approved by institutional animal ethical committee (IAEC). The animals were kept on fasting for 24 hrs and rendered diabetic by injecting a single dose of alloxan 150 mg/kg body weight (Loba, Mumbai) administered as a 5% w/v in distilled water.
It produced diabetes by selective necrosis of β-cells of islets of langerhans of pancreas. After one week, diabetes was confirmed by testing blood glucose by using o-toluidine method\(^{[20, 22, 23]}\). The animals with sugar level more than 200 mg/dl were considered as experimental diabetic. The 80% alcoholic extract of the plants were separately suspended with 1% gum acacia and employed for assessing anti-diabetic activity at a dose of 200mg/kg for 21 days and glibenclamide tablet (Aventis Pharmaceuticals, Mumbai) was used as a standard drug.

**Experimental design:**
A total 78 rats were divided into 13 groups each consisting of six rats. **Group-I:** received only the vehicle (1% gum acacia) served as normal control, **Group-II:** untreated diabetic animals served as a negative control, **Group-III:** diabetic animals treated with standard drug (glibenclamide, 10mg/kg body weight) served as positive control, **Group-IV to XIII:** diabetic animals treated with 200mg/kg of 80% ethanol/water (v/v) plant extracts of different plants once a day. After an overnight fasting, each group was treated for 21 days as mentioned above. The blood samples were collected from the tail vein puncture, for the measurement of blood glucose at 0, 7, 14 and 21 days by using o-toluidine method\(^{[22, 23]}\).

**Total phenolic contents (TPC) and antioxidant activity (AOA):**
The powdered plant material (1.0 g) was extracted with 50% MeOH: H\(_2\)O (2 X 20 ml), overnight at room temperature and solvent from combined extract was removed under reduced pressure. The total phenolic contents (TPC) in the extracts were measured by the method of Ragazzi and Veronese\(^{[24]}\) and were expressed as mg gallic acid equivalent (GAE)/g extract. The antioxidant activity (AOA) of extracts was performed by auto oxidation of β-carotene and linoleic acid coupled reaction according to Emmons and Peterson\(^{[25]}\) and was expressed as per cent inhibition relative to control. Free radical scavenging activity (FRSA) was measured by using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical according to Yen and Duh\(^{[26]}\) and the inhibitory concentration (IC\(_{50}\)), efficiency concentration (EC\(_{50}\)) and anti radical power (ARP) were estimated and calculated as described by Kroyer\(^{[27]}\).

Reducing capacity of extracts was determined (ASE/ml = absorbance of 1 mM ascorbic acid/ absorbance of 1 mg/ml sample) by ferric reducing - antioxidant power assay\(^{[28]}\) using quercetin as reference standard and expressed as ascorbic acid equivalent (1mM = 1 ASE). DNA nicking assay were performed using supercoiled pUC 18 DNA by the method of Lee et al.\(^{[29]}\) and analyzed on 1% agarose gel.

**Results and Discussion**

In Indian system of Ayurvedic medicine or indigenous folk medicines, the hypoglycemic plants have been mentioned to be used generally in their natural forms such as fresh juice, paste or dry powder. Dispensing natural plants in this form will retain both the inorganic and organic constituents of the concerned herbs. It is also important to mention here that the inorganic part of a medicinal plant containing mainly mineral elements, sometimes plays a contributory role in enhancing medicinal properties of that particular plant or their products\(^{[9-10]}\). There are a number of essential minerals (Ca, Zn, K, Mn and Cr) that are known to be associated with the mechanisms of insulin release and its activity or glucose tolerance factor in different laboratory animals and also in human beings\(^{[9-11]}\).
To study the comparative hypoglycemic effect on final blood glucose levels of each sample specific fixed doses (200mg/kg) of the concerned 80% ethanolic extracts of *C. esculenta*, *C. indica*, *T. involucrata*, *M. oleifera*, *T. cordifolia*, *F. benghalensis*, *M. koenigii*, *S. aegyptiaca*, *M. prurita* and *Z. officinale* were given to experimental animals. The blood glucose levels were measured in fasting animals at 0, 7, 14 and 21 days (Table 1). The present result indicated that most of the experimental samples showed specific blood glucose lowering effects within 2 and 3 weeks. The experimental sample of *T. cordifolia* was found to be most potent and showed blood glucose lowering effect from 298 to 235 mg/dl (21%), 186 mg/dl (38%) and 95 mg/dl (68.1%) after 1, 2 and 3 weeks of treatment respectively (Table 1).

The next higher hypoglycaemic activity, a reduction of 60.3% was observed on similar dose after 3 weeks by *Coccinia indica*. The standard drug glibenclamide used for comparison showed 63.5% decrease from initial level after 3 weeks of treatment. The marked effect of most of the samples was observed after 3 weeks and in terms of hypoglycemic activity in increasing order it was *M. prurita* (36.4), *S. aegyptiaca* (37.8), *M. koenigii* (41.7), *Z. officinale* (45.7), *F. benghalensis* (50.7), *C. esculenta* (53.9), *M. oleifera* (57.0), *T. involucrata* (58.8), *C. indica* (60.3) and *T. cordifolia* (68.1% decrease from initial level). Some of the medicinal plants are among the numerous plant adjuncts tried for the treatment of diabetes mellitus. There is scope for more extensive research in this area, especially to examine their long term beneficial effect to identify the active principle, and to understand the mechanism of action.

WHO has pointed out that prevention of diabetes and its complications is not only a major challenge for the future, but essential if health for all is to be an attainable target. The WHO study groups in its report had emphasized strongly the need of optimum, and rational uses of traditional and natural indigenous systems of medicines in health care of general public of any specific country [1, 2]. There is still insufficient evidence to draw definite conclusions about the efficacy of individual herbs and supplements for diabetes; however, they appear to be generally safe. The available data suggest that several supplements may warrant further study. The best evidences for efficacy from adequately designed randomized controlled trials (RCTs) are available for several plants of Indian system of natural medicines [12-21].

### Table 1: Effect of 80% ethanolic extracts of different plants on blood glucose level of diabetic albino rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Yield of Extract</th>
<th>0 Day</th>
<th>7 Days</th>
<th>14 Days</th>
<th>21 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Normal)</td>
<td>--</td>
<td>96±2.3</td>
<td>99±3.2</td>
<td>97±1.4</td>
<td>99±10.2</td>
</tr>
<tr>
<td>Control (diabetic)</td>
<td>--</td>
<td>300±11.1</td>
<td>305±6.0</td>
<td>295±8.0</td>
<td>282±10.4</td>
</tr>
<tr>
<td>Glibenclamide (Std)</td>
<td>--</td>
<td>294±11.4</td>
<td>169±23.1</td>
<td>138±28.1</td>
<td>101±5.2*</td>
</tr>
<tr>
<td><em>Casearia esculenta</em></td>
<td>6.7</td>
<td>308±5.0</td>
<td>190±11.0</td>
<td>141±13.7</td>
<td>106±5.7*</td>
</tr>
<tr>
<td><em>Coccinia indica</em></td>
<td>8.5</td>
<td>303±24.4</td>
<td>220±17.9</td>
<td>142±23.3</td>
<td>106±8.7*</td>
</tr>
<tr>
<td><em>Tragia involucrata</em></td>
<td>4.3</td>
<td>306±9.6</td>
<td>191±14.8</td>
<td>140±16.6</td>
<td>107±7.7*</td>
</tr>
<tr>
<td><em>Moringa oleifera</em></td>
<td>9.8</td>
<td>296±21.9</td>
<td>209±16.9</td>
<td>145±15.4</td>
<td>107±7.7*</td>
</tr>
<tr>
<td><em>Tinospora cordifolia</em></td>
<td>10.6</td>
<td>304±13.0</td>
<td>203±13.9</td>
<td>122±16.0</td>
<td>97±2.7*</td>
</tr>
<tr>
<td><em>Ficus benghalensis</em></td>
<td>12.3</td>
<td>303±4.7</td>
<td>222±18.8</td>
<td>149±12.9</td>
<td>100±3.3*</td>
</tr>
<tr>
<td><em>Muraya koenigii</em></td>
<td>7.9</td>
<td>289±13.0</td>
<td>204±3.7</td>
<td>141±7.6</td>
<td>96±4.3</td>
</tr>
<tr>
<td><em>Sesbania aegyptiaca</em></td>
<td>10.2</td>
<td>297±7.6</td>
<td>232±28.7</td>
<td>151±15.1</td>
<td>104±7.4</td>
</tr>
<tr>
<td><em>Mucuna prurita</em></td>
<td>5.3</td>
<td>294±6.0</td>
<td>237±28.2</td>
<td>190±21.3</td>
<td>130±3.3</td>
</tr>
<tr>
<td><em>Zingiber officinale</em></td>
<td>11.8</td>
<td>298±10.6</td>
<td>240±20.7</td>
<td>143±23.3</td>
<td>102±2.8</td>
</tr>
</tbody>
</table>
Blood glucose values (in mg/dl) are the mean ± S.D. of six rats; Std=Standard anti-diabetic drug; *Significant compared with diabetic untreated control group at P< 0.05.

To find antioxidant potential, all the ten plants were studied (Table 2) for their total phenolic contents (TPC) and antioxidant activity (AOA). TPC showed wide variation from 10.2 (Zingiber officinale) to 45.6 mg GAE/g extract (Muraya koenigii), and AOA measured by auto oxidation of β-carotene and linoleic acid coupled reaction from 28.9 (Coccinia indica) to 75.6% (Moringa oleifera) in the extracts of different plants. The amounts of TPC 23.5, 34.7, 34.8 GAE/g and AOA 41.9, 75.6 65.5% was respectively in Tragia involucrate, Moringa oleifera and Sesbania aegyptiaca. In case of Moringa oleifera the AOA was high in spite of low levels of TPC that might be due to presence of some other antioxidant phytochemicals. Phenols are known to be responsible for free radical scavenging activity (FRSA). The selected plants were further subjected to FRSA assayed by DPPH free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule and was expressed (Table 2) in terms of IC$_{50}$ (inhibitory concentration) that ranged from 0.11 to 0.73 mg/ml, EC$_{50}$ (efficiency concentration) from 5.23 to 31.4 mg/mg DPPH and ARP (antiradical power) from 3.12 to 19.87. Moringa oleifera showed the highest FRSA followed by Muraya koenigii as evident by their low IC$_{50}$, EC$_{50}$ and high ARP values, than rest of the plants. The reducing power expressed as ascorbic acid equivalent (ASE/ml) varied from 0.73 to 1.73 ASE/ml that indicates their potential as electron donor to scavenge free radicals.
Figure 1. Inhibitory effects of some plant extracts (20 µg/ml) with hypoglycemic activity on native pUC18 DNA, nicking caused by hydroxyl radicals. **Lane 1.** pUC18 DNA; **Lane 2.** DNA + Fenton; **Lane 3.** DNA + Fenton + SOD (2U); **Lane 4.** DNA + Fenton + *Casearia esculenta*; **Lane 5.** DNA + Fenton + *Moringa oleifera*; **Lane 6.** DNA + Fenton + *Tinospora cordifolia*; **Lane 7.** DNA + Fenton + *Ficus benghalensis*; **Lane 8.** DNA + Fenton + *Tragia involucrata*; **Lane 9.** DNA + Fenton + *Cocinia indica*

In the protection of DNA damage (Fig. 1) experiment *Moringa oleifera* (Lane 5) and *Tinospora cordifolia* (Lane 6) showed significant reduction in the formation of nicked DNA (form II, circular) and increased native (form I, supercoiled) DNA. Transition metal ions are known to catalyze the formation of free radicals and reduction in the formation of single-stranded nicked DNA (form II, circular), double-stranded nicked DNA (form III, linear) and increased form I (supercoiled) DNA. Antioxidant effect of *Opuntia ficus-indica* against oxidative DNA damage had shown similar results at the same concentration \[29\].
The most probable reason for their potential as free radical scavengers and protection of DNA damage might be related to polyphenol and other antioxidant phytochemicals contents as they have been reported to inhibit lipid peroxidation by scavenging reactive oxygen species, chemiluminescence reactions and tumorigenesis\(^{30, 31}\). They are also known as powerful protecting agents against the lethal effects of oxidative stress and offer protection of DNA by chelating redox-active transition metal ions. Present studies together with the previous works suggest the triple synergistic action of phenols in scavenging free radicals, repairing DNA and metal chelation\(^{29}\). The phenols have well documented free radical scavenging activities and metal ion chelating capacity. They might also be responsible for their efficient free radical scavenging activity. Reactive oxygen species can cause damage to cellular bio-molecules like DNA, RNA, enzymes, lipids and carbohydrates and consequently may adversely affect immune functions. Oxidation of bases in DNA, deoxyribose lesions and strand breaks may lead to mutagenic changes and a variety of diseases\(^{29, 32}\). Phenols due to their strong antioxidant and a range of biological properties are also known to diffuse the toxic free radicals\(^{33, 34}\).

From the above discussion it may be concluded that for a final co-ordinated result, the effects of both the organic and inorganic constituents of the concerned medicinal plant may be taken into consideration. Therefore, to get more potent and optimum hypoglycaemic herbs of Indian origin can be selected for their use in indigenous systems of medicine for the preparations in crude forms either singly or in combinations as compound formulations. Further besides controlling the blood sugar level the antioxidant and free radical scavenger activities may be of importance in for the protection against oxidative stress.

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**References**


