Antidiabetic Antioxidant and Hematological Effect of Ethanolic Extract of Muntingia Calabura on Streptozoticin Induced Diabetic Rats

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ABSTRACT

Diabetes has emerged as major problem International Diabetes Federation (IDF), there have been an estimated 40 million persons with diabetes in India in 2007 and this number is predicted to rise to almost 70 million people by 2025. Fresh stem bark of muntingia calabura was collected in Tadepalligudem in the plant was identified and authenticated by Dr. D.V. Swamy Professor in Horticulture Dr. YSR Horticulture university. The bark of muntingia calabura was peeled out from stem and was washed cleanly. The bark muntingia calabura was air dried to a constant weight in room. 750 grams of bark was collected. Thirty male wistar 200-250 gms rats were randomized in to five groups consisting six animals in each group. Streptozotocin is used as an agent to induce diabetes mellitus by selective cytotoxicity effect on pancreatic beta cells. Thus it affects endogenous insulin release and as a result increases blood glucose level. Streptozotocin was confirmed in this study in relation to the normal control rats. Long term reduction of this parameter may result in internal and external haemorrhage and finally leads to death. However, after plant extract administration, the level of platelet was improved markedly especially at the dose of 100 mg/kg while that of 200 mg/kg did not have strong effect as compared with diabetic untreated rats. This effect indicated the ability of the plant extract to stimulate the biosynthesis of clotting factors due to the presence of active muntingia calabura.

INTRODUCTION

Diabetes has emerged as major problem International Diabetes Federation (IDF), there have been an estimated 40 million persons with diabetes in India in 2007 and this number is predicted to rise to almost 70 million people by 2025 [1]. The countries with the most important number of diabetic people are going to be India, China and USA by 2030. It is estimated that each fifth person with diabetes are going to be an Indian. Due to these sheer numbers, the economic burden thanks to diabetes in India is amongst the very best within the world [2,3]. The real burden of the disease is however thanks to its associated complications which cause increased morbidity and mortality. WHO estimates that mortality from diabetes, heart condition and stroke costs about $210 billion in India within the year 2005 Much of the heart disease and stroke in these estimates was linked The world of diabetes among adults aged 20-79years will be 6.4% affecting 285 million adults, in 2010, and will increase to 7.7%, and 439 million adults by 2030 [4,5]. Between 2010 and 2030, there’ll be a 69% increase numbers of adults with diabetes in developing countries and a 20% increase in developed countries [6].

Diabetes mellitus is an endocrine, metabolic disorders caused by relative or an absolute lack of insulin. According to International Diabetes Federation (IDF), worldwide 382 million people were affected by diabetes in 2013 and it’s expected to boost to 592 million by 2035 [7]. IDF estimates 65 million diabetic patients in India in 2013 and it is expected to cross 109 million by 2030. 20% diabetes patients are increasing day by day may be because of the change in food pattern, i.e. fast food diet intake and change in lifestyle [8]. Management of diabetes is a tough task. The medicines utilized in diabetic treatment are either too costlier or have adverse effects like hypo glycemic coma, insulin resistance, hypersensitivity and metallic taste etc. Hence, within the recent years, herbal compounds are gaining popularity in both developed and developing countries because of their natural origin, low adverse effects. Ethnobotanical information indicates that around
800 medicinal plants having hypo glycemic or anti diabetic potential [9]. Herbal plants are abundant in India. Hence the search for safer and effective anti diabetic agents has become the current research area. It’s a well-established fact that diabetes is a risk factor for cardiovascular disease. While micro vascular complications of diabetes include nephropathy and retinopathy, macro vascular complications resulting in atherosclerotic cardiovascular disease such as coronary artery disease, cerebrovascular disease and peripheral vascular disease are the cause of death in the diabetic population. The Diabetes Control and Complications trial (DCCT) demonstrated that tight control of blood sugar is effective in reducing clinical complications significantly, but even optimal control of blood sugar could not prevent complications suggesting that alternative treatment strategies are needed [10]. Since numerous studies demonstrated that oxidative stress, mediated mainly by hyperglycemia-induced generation of free radicals, contributes to the development and progression of diabetes and related contributions, it became clear that ameliorating oxidative stress through treatment with antioxidants could be an efficient strategy for reducing diabetic complications [10].

An ethno botanical study was carried on the medicinal plants often used for the management of diabetes in Andhra Pradesh by traditional healer’s. muntingia calabura the one of the used by the traditional healers for diabetes. Even though medicinal plants are widely used, the effective treatment of the disease has not been verified with scientific standards. Only a couple of plants used for diabetes in traditional medicine are scientifically audited in vivo.

MATERIALS AND METHODS:

Plant Material:
Fresh stem bark of muntingia calabura was collected in tadepalligudem in the plant was identified and authenticated by Dr D.v swamy professor in horticulture Dr YSR Horticulture university.

Preparation of Plant Extract:
The bark of muntingia calabura was peeled out from stem and was washed cleanly. The bark muntingia calabura was air dried to 10 days in horticulture Dr YSR Horticulture university. The bark muntingia calabura was peeled out from stem and washed and then peeled. The bark of muntingia calabura was air dried to 10 days in the plant was identified and authenticated by Dr D.v swamy professor in horticulture Dr YSR Horticulture university.

Preparation of Serum:
Blood sample collection method:
For haematological analysis, small quantity of potassium oxalate and sodium flouride as anticoagulant. The tip of the capillary was then slightly withdrawn, so that the blood flows into the vessels are ruptured, blood wells up in the peri-orbital space. The tip of the capillary was inserted at the medical canthus into the retro-orbital plexus. Capillary tube: 1ml (bore size).

Preparation of Serum:
Blood was collected through blood sample was collected in EDTA sample bottles. For haematological analysis Results of blood glucose levels in diabetic rats:
Effect of muntingia calabura bark extract on blood glucose levels in diabetes induced rats was given in Table 1. Effect of ethanolic extract of muntingia calabura comparing with control in blood glucose levels was given Table 2. Evaluation of anti-oxidant activity (In vitro): in muntingia calabura was calculated by T-paired test

RESULTS

Experimental design:
Thirty male wistar 200-250 gms rats were randomized in to five groups consisting six animals in each group.

<table>
<thead>
<tr>
<th>Days</th>
<th>Normal</th>
<th>Control</th>
<th>Standard 100mg</th>
<th>Standard 200mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>86.661±0.687</td>
<td>225.761±7.97</td>
<td>225.761±7.97</td>
<td>219.500±7.97</td>
</tr>
<tr>
<td>7</td>
<td>86.733±1.715</td>
<td>206.33±5.220</td>
<td>182.167±1.75</td>
<td>148.317±5.94</td>
</tr>
<tr>
<td>14</td>
<td>85.467±0.648</td>
<td>176.272±4.56</td>
<td>145.67±2.616</td>
<td>106.00±1.03</td>
</tr>
</tbody>
</table>

Table 1: Effect of muntingia calabura bark extract on blood glucose levels in diabetes induced rats.

<table>
<thead>
<tr>
<th>Days</th>
<th>Diabetic control 100mg</th>
<th>Diabetic control 200mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>225.761±5.797</td>
<td>225.761±5.797</td>
</tr>
<tr>
<td>7</td>
<td>206.33±5.220</td>
<td>182.167±1.75</td>
</tr>
<tr>
<td>14</td>
<td>176.272±4.56</td>
<td>145.67±2.616</td>
</tr>
</tbody>
</table>

Table 2: Effect of ethanolic extract of muntingia calabura comparing with control in blood glucose levels.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50(µg/ml)</th>
<th>Standard</th>
<th>IC50(µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLJ</td>
<td>45.17</td>
<td>Gallic acid</td>
<td>45.17</td>
</tr>
<tr>
<td></td>
<td>45.17</td>
<td>Vitamin C</td>
<td>1.722</td>
</tr>
</tbody>
</table>

Table 3: Evaluated parameters of GLJ with Standard Gallic acid and Vitamin C.
such as enzymes, metabolic products, haematology, normal including plant extracts on the blood constituents of animals. They are could be used to reveal the deleterious effect of foreign compounds peroxides that lead to haemolysis of RBC [16]. In this study, the oxidation of these proteins and hyperglycaemia due to the increased non-enzymatic glycosylation of RBC membrane proteins [14,15]. Oxidation of these proteins and hyperglycaemia in diabetes mellitus causes an increase in the production of lipid peroxides that lead to haemolysis of RBC [16]. In this study, the determination of hematological parameters was calculated using standard solutions.

**Table 4: Determination of Hematological Parameters.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal</th>
<th>Diabetes</th>
<th>100mg muntingia calabura</th>
<th>200mg muntingia calabura</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>14.5±0.00</td>
<td>12.667±0.00</td>
<td>13.23±0.43</td>
<td>13.483±0.683</td>
<td>13.483±0.683</td>
</tr>
<tr>
<td>RBC</td>
<td>4.500±0.00</td>
<td>3.90±0.00</td>
<td>3.98±0.893</td>
<td>4.067±0.267</td>
<td>4.067±0.267</td>
</tr>
<tr>
<td>WBC</td>
<td>8225.00±275.0</td>
<td>6450±0.00</td>
<td>7158.33±641.667</td>
<td>740.33±1091</td>
<td>740.33±1091</td>
</tr>
<tr>
<td>Platelet</td>
<td>3.90±0.00</td>
<td>2.3±0.00</td>
<td>4.308±1.808</td>
<td>4.475±1.642</td>
<td>4.475±1.642</td>
</tr>
<tr>
<td>PCV</td>
<td>42.00±0.739</td>
<td>39.0±0.00</td>
<td>39.58±0.917</td>
<td>39.58±0.917</td>
<td>39.58±0.917</td>
</tr>
<tr>
<td>MCV</td>
<td>95.1±0.00</td>
<td>93.767±0.00</td>
<td>94.60±0.167</td>
<td>39.58±0.917</td>
<td>39.58±0.917</td>
</tr>
<tr>
<td>MCH</td>
<td>87.767±0.00</td>
<td>29.250±0.00</td>
<td>31.80±2.533</td>
<td>31.95±2.533</td>
<td>31.95±2.533</td>
</tr>
<tr>
<td>MCHC</td>
<td>34.13±0.00</td>
<td>30.5±0.00</td>
<td>31.58±2.917</td>
<td>31.58±2.717</td>
<td>31.58±2.717</td>
</tr>
</tbody>
</table>

4. Mean and SEM VALUES are calculated

**Discussion:**

Streptozotocin is used as an agent to induce diabetes mellitus by selective cytotoxicity effect on pancreatic beta cells. Thus it affects endogenous insulin release and as a result increases blood glucose level [12]. The continuous administration of ethanolic extract of muntingia calabura bark at 200 mg per kg or glibenclamide for 14 days significantly reduced the blood glucose concentration in STZ induced diabetic rats. The plant extract (200 mg/kg) showed a comparable activity with the glibenclamide treated groups.

Glibenclamide is standard antidiabetic drug that stimulates insulin secretion from beta cells of islets of Langerhans. The probable mechanisms of action of the plant extract at higher dose could be linked to potentiation of insulin from beta cells or by increasing peripheral glucose uptake [13] the assessment of haematological parameters could be used to reveal the deleterious effect of foreign compounds including plant extracts on the blood constituents of animals. They are also used to determine possible alterations in the levels of biomolecules 222such as enzymes, metabolic products, haematology, normal functioning and histo morphology of the organs.

The occurrence of anaemia in diabetes mellitus has been reported due to the increased non-enzymatic glycosylation of RBC membrane proteins [14,15]. Oxidation of these proteins and hyperglycaemia in diabetes mellitus causes an increase in the production of lipid peroxides that lead to haemolysis of RBC [16]. In this study, the RBC membrane lipid peroxide levels in diabetic rats were not measured. However, there d blood cells parameters such as Hb, MCHC, MCH, PCV,MCV and were studied to investigate the beneficial effect of muntingia calabura extract on the anaemic status of the Oyedemi S.O et al./Asian Pacific Journal of Tropical Biomedicine (2011)353-358357 diabetic rats. The levels of RBC, Hb, haematocrit, LUC andMCHC in the diabetic animals were drastically reduced which may be attributed to the infections on the normal body systems. This observation agrees with report of Baskaret all [17] who reported anti hyperglycemic activity of ethanolic bark extract of Rubia cordifolia in streptozotocin-induced diabetic rats.

The alterations of these parameters are well known to cause anaemic condition in man [18] Following plant extract administration, the level of RBC and its related indices were appreciably improved especially at200 mg/kg. This gives an indication that the plant extract may contain some phytochemicals that can stimulate the formation or secretion of erythropoietin in the stem cells of the animals. Erythropoietin is a glycoprotein hormone which stimulates stem cells in the bone marrow to produce red blood cells [19] The stimulation of this hormone enhances rapid synthesis of RBC which is supported by the improved level of MCH and MCHC [20].

These parameters are used mathematically to define the concentration of haemoglobin and to suggest the restoration of oxygen carrying capacity of the blood. Though, the action mechanism of this plant is not investigated in this study. However, it may be attributed to the plant extract to lower lipid peroxidation level that causes haemolysis of erythrocytes [21] Previous study on this plant revealed the presence of flavonoids proanthocyanidins, tannins, phenols and flavonoids in this plant. These compounds have been reported to possess strong antioxidant capacity therefore, could inhibit per oxidation of polyunsaturated fatty acids in the cell membrane and haemolysis of red blood cells in the diabetic animals reported by Torell and Faure et all [22,23] Streptozotocin is a well known chemical that suppresses the immune system by damaging WBC and certain organs in the body [24]. The intraperitoneal injection of streptozotocin into rats significantly...
reduced the WBC count and its differentials such as basophils, monocytes, eosinophils, lymphocytes and neutrophils. The reduction of these parameters could be linked to suppression of leucocytosis from the bone marrow which may account for poor defensive mechanisms against infection [25]. Consequently, they might have effects on the immune system and phagocytic activity of the animals [22]. The white blood counts and its related indices were significantly restored to near normal after plant extract administration at both doses. The presence of some phytochemicals with ability to stimulate the production of white blood cell count in the extract could be responsible for the observed result in the treated rats. The extract at both dosages significantly improved the levels of WBC, monocytes, lymphocytes, eosinophils and neutrophils as compared with glibenclamide treated group. However, the extract did not have any significant effect on basophils in this study. Platelet aggregation ability has been shown in diabetic patient with long term poor glycaemic control due to lack or deficiency of insulin Platelets known as thrombocytes help to mediate blood clotting, which is a meshwork of fibrin fibres. The fibres adhere to any vascular opening and thus prevent further blood clot. It plays a crucial role in reducing blood loss and repairing of vascular injury [26] the reduction of platelets levels in diabetic rats induced with muntingia calabura.

CONCLUSION

Streptozotocin was confirmed in this study in relation to the normal control rats. Long term reduction of this parameter may result in internal and external haemorrhage and finally leads to death. However, after plant extract administration, the level of platelet was improved markedly especially at the dose of 100 mg/kg while that of 200 mg/kg did not have strong effect as compared with diabetic untreated rats. This effect indicated the ability of the plant extract to stimulate the biosynthesis of clotting factors due to the presence of active muntingia calabura.

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Conflict of interest

No

Funding

Self

REFERENCES


