PRELIMINARY STUDIES ON ALLOXAN INDUCED ANTI-DIABETIC ACTIVITY OF SOME SIDDHA MEDICINAL PLANTS IN MALE ALBINO RATS.

Elango V.* and Eazhisai Vallabi D.

Department of Siddha Medicine, Faculty of Sciences, Tamil University, Thanjavur-613010.

ABSTRACT

Siddha traditional system of medicine, a science that uses herbal medicines extensively, originated in India. The present studies focus the anti-diabetic activity in *Boerhavia diffusa*, *Momordica charantia* and *Carica papaya* leaves; have been reported to be beneficial for treating type 2 diabetes induced by alloxan. Diabetes mellitus is one of the common metabolic disorders acquiring around 2.8% of the world's population and is anticipated to cross 5.4% by the year 2025. Since long back herbal medicines have been the highly esteemed source of medicine, there were many plant and plants phyto-constituents were used for anti diabetic activity. In this preliminary experimental study blood glucose, glycogen, cholesterol and the antioxidant activity (LPO) were studied and statistical analyses were carried out.

KEYWORDS: Diabetes mellitus, glucose, alloxan, antioxidant, glycogen.

INTRODUCTION

Diabetes- mellitus encountered as a major public health produces different complications and also responsible for increased number of mortality and mortality. Diabetes mellitus is characterized by elevated plasma glucose concentrations resulting from insufficient insulin and insulin resistance, or both, leading to metabolic abnormalities in carbohydrates, lipids and proteins. If not cured or controlled it may even lead to acute or chronic complications causing ketoacidosis, micro-angiopathy and other related infections (Tsuneki *et al.*, 2007).

The signs and symptoms present as a result of *hyperglycaemia* (excessive sugar in the blood). There is an increase in urine output (polyuria) which results from the glycosuria (glucose in
urine) secondary to hyper-glycaemia. Patients experience increased thirst (polydipsia) which is secondary to osmotic diuresis and hyper-osmolality. Increased appetite (polyphagia) results because of cellular starvation, and decreased storage of calories. Weight loss in the presence of polyphagia is due to the ineffective metabolism of carbohydrate, protein and fat. Weakness and lethargy are experienced as a result of inadequate energy production. Fatigue is another symptom of diabetes mellitus. Vaginitis may be an early complaint in females. Dry mucous membranes, dry skin, tachycardia and nausea may also manifest if the patient is unable to take in enough fluids to replace the losses through osmotic diuresis (Phipps et al., 1993).

Oxidative stress is involved in the development and progression of diabetes-associated complications. In hyperglycemic condition continuous generation of reactive oxygen species (ROS) occurs and the evidence showed diabetes induced changes in the activities of antioxidant enzymes in various tissues. Antioxidants play an important role in scavenging the free radicals and protect the human body from oxidative stress (Eurich et al., 2007).

Several antioxidants of plant origin have been screened for their ability to scavenge free radicals and are useful as protective agents against oxidative stress (Sabu and Kuttan, 2000). Many Siddha traditional plant used to reduce the serum glucose level had significant antioxidant activity in vitro as it found to significantly reduce lipid per oxidation radicals. In this experimental investigation Boerhavia diffusa, Momordica charantia and Carica papaya leaves were used.

Numerous animal models have been developed for the past few decades for studying diabetes mellitus and testing anti-diabetic agents that include chemical, surgical and genetic manipulations. One of the most potent methods to induce experimental diabetes mellitus is chemical induction by Alloxan (Etuk, 2010).

Alloxan (2,4,5,6-tetraoxypyrimidine) is an oxygenated pyrimidine derivative which is present as alloxan hydrate in aqueous solution (Wohler and Liebig, 1838), this causes selective necrosis of the β- cells of pancreatic islets. In addition, it has been widely used to produce experimental diabetes in animals such as rabbits, rats, mice and dogs with different grades of disease severity by varying the dose of alloxan used (Iranloye et al., 2011). The drug has been noted to exert its diabetogenic action when administered parenterally, i.e., intravenously, intraperitoneally or subcutaneously. Furthermore, the dose of alloxan required for inducing diabetes depends on the animal species, route of administration and nutritional status.
Moreover, alloxan has been demonstrated to be non-toxic to the human beta-cells, even in very high doses, the reason of which may be attributed to the differing glucose uptake mechanisms in humans and rodents (Eizirik et al., 1994). The toxic action of alloxan on pancreatic beta cells involve oxidation of essential sulphhydryl (-SH groups), inhibition of glucokinase enzyme, generation of free radicals and disturbances in intracellular calcium homeostasis (Dhanesha et al., 2012). The underlying mechanism involves the selective uptake of the compound due to its structural similarity to glucose as well as highly efficient uptake mechanism of the pancreatic β-cells (Viswanathaswamy et al., 2011). The aim of the present experimental study is to explicate the mechanisms involved in alloxan induced induction of experimental diabetes mellitus.

Hence, drug with both antioxidant and antidiabetic property would be useful for the treatment of the diabetes mellitus. In recent times, many medicinal plants have been reported to cure diabetes worldwide and have been used widely as antidiabetic remedies (Maritim et al., 2003).

Many traditional medicines in use are derived from medicinal plants, minerals and organic matter. Diabetes and herbs have got a long relation from the past. Thus, plants are a potential source of anti-diabetic drugs which can be proved by the ethnobotanical information reports about 800 plants that may possess anti-diabetic potential (Rao et al., 2010).

The use of plants in religious rituals as well as for magic and medicinal purposes is well known and widespread. In the modern era also most of the people believe the plants and phyto constituents are better choice to treat diseases than the allopathic drugs, even most of the drugs used in primitive medicine were instigated from plants (Sen et al., 2009).

**Collection of the Plant Material**

Fresh plant sample of *Boerhavia diffusa*, *Momordica charantia* and *Carica papaya* leaves were collected from various parts of Thanjavur district. The leaves were washed, shade dried, powdered and the crude powder of these plant leaves (BMC) in water suspension was administered (P.O) to the experimental rats at the dose of 100mg/100g body weight.

**Animals and Treatment**

Male albino rats of 8 – 10 weeks of age weighing between 150 and 200g were used for this present anti diabetic study. The animals were purchased from Sri Venkateswara Enterprises
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(Ltd), Bangalore and housed in polypropylene cages. Animals were provided with normal rat’s feed and normal water *ad libitum*. Animals were divided into five groups of 4 animals. Group I: normal animals provided with usual rat feed and water. Group II: as control animals provided with rat feed and water along with 0.5ml/100g.b.wt alloxan in saline (0.9), the Group III as Drug treated animals provided with rat feed and water along with alloxan and crude powder of *Boerhavia diffusa*, Group IV as Drug treated animals provided with rat feed and water along with alloxan and crude powder of *Momordica charantia* and Group IV as Drug treated animals provided with rat feed and water along with alloxan and crude powder of *Carica papaya* in water suspension and the drug followed by it.

**Induction**

Alloxan induced acute diabetes mellitus was produced by the subcutaneous injection dose of 0.5ml/100g.body.wt to the groups of overnight fasted rats. Rats with above 150mg were selected.

**Collection of Sample**

At the end of treatment after 30th day, animals were fasted overnight, anaesthetized with ether and scarified by cervical decapitation. Blood and liver were collected immediately. Blood was collected in a dry test tube for serum separation. Blood collected using anti coagulant (heparin) was used for the estimation of glucose.

**Biochemical Estimation**

Glucose was estimated by GOD/POD method. Glucose is oxidized by glucose oxidase (GOD) to gluconate and hydrogen peroxide. In a subsequent per oxidase (POD) catalyzed reaction, 4- aminophenazone and phenol reacts with hydrogen peroxide to form red coloured quinoneamine compound 4- (p- benzoquinone –mono- imino) phenazone. The color so developed is measured at 510nm and is directly proportional to the glucose concentration. (Trinder, 1969).

Cholesterol estimation is based on the following enzyme catalyzed reactions. Cholesterol reacts with water in the presence of cholesterol esterase to give cholesterol and fatty acids. So formed cholesterol is oxidized by cholesterol oxidase to give cholestenone and hydrogen. Peroxide subsequent peroxide catalyzed reaction 4 – amino phenazone and phenol reacts with hydrogen peroxide to form lavender coloured quinoneamine compound 4 – phenazone. The colour developed is measured at 546nm against reagent blank (Siedel, 1983 and Kappermann,
Glycogen was estimated by Anthrone reagent method (Kimora, 1981). Lipid peroxide content in the serum was assayed by thiobarbituric acid procedure. The byproducts of lipid peroxidation (Aldehyde especially malonic dialdehyde) formed by degradation of hydroperoxide react with thiobarbituric acid forming pink coloured trimethine complex which was measured spectrophotometrically at 530nm (Desai et al., 1964).

**Statistical Analysis**
Mean values standard were calculated for all the values carried out. Based on this student’s test were analyzed and probability of error was assessed (Fisher, 1950).

**RESULTS AND DISCUSSION**
The blood glucose is a biomarker for diabetes. The criteria for the diagnosis of diabetes mellitus in clinical practice is fasting plasma glucose that is equal or greater than 126 mg/dL or two-hours post prandial plasma glucose greater than 200 mg/dL. With good glycemic control, several long-term, life-threatening complications of diabetes can be prevented.

In this present study the normal animals have, 129.0mg/dl as the blood level of glucose. After the induction with alloxan (40mg/kg) in diabetic animals, it was found that there was an increase by 152mg/dl than the normal level. After the treatment with BMC crude powder at the dose of 100mg/dl, the blood glucose level was increased to 130mg/dl, 136mg/dl and 130mg/dl respectively.

Uncontrolled diabetes leads to increased hepatic glucose output. The possible mechanism is that liver glycogen stores are mobilized and then hepatic gluconeogenesis is used to produce glucose (Duzguner and Kaya, 2007). Simultaneously, insulin deficiency also impairs non-hepatic tissue utilization of glucose. Therefore, alloxan induced diabetes is characterized by the drastic loss in body, liver, liver glycogen and in insulin as was seen in our results. Also, the increase in glucose was induced by alloxan (Abbasi et al., 2010; Hussain et al., 1990)

Administration of alloxan causes decrease to 16mg/g in glycogen content due to enhanced glycogenolysis when compared to the normal 20.5mg/g, which is due to insulin deficiency. So the normal capacity of the liver to synthesize glycogen is impaired. A significant increase in the liver glycogen by administration of (BMC) may be due to an increase level of insulin
by it, In the BMC induced diabetic rats the level of glycogen are 180mg/g, 18.3mg/g and 18.0mg/g respectively.

The abnormally high concentration of plasma and hepatic lipids in diabetes is mainly due to an increase in the mobilization of free fatty acids from the peripheral depots, since insulin inhibits the hormone sensitive lipase. The marked hyperlipidemia that characterizes the diabetic state is regarded as a consequence of the uninhibited action of lipolytic hormones (glucagon and catecholamines) on the fat depots (Ravi et al., 2005).

This study evaluate the serum level of cholesterol, the normal animals have 110mg/dl, cholesterol. After the induction with alloxan in diabetic animals, it was found that there was an increase by 200mg/dl than the normal level. After the treatment with BMC crude powder at the dose of 100mg/dl, the serum cholesterol level was reduced to 137mg/dl from the untreated control animal and the difference was found as 27.27%.

The result of this study shows that alloxan produced diabetic was evidenced by increasing in lipid per oxidation products suggesting the involvement of oxidative stress and suggestive of cellular damage. The drug treated groups exert a protection against oxidative stress and liver damage against alloxan induced diabetes.

Lipid peroxidation was induced by glucose through activation of lipoxygenase enzymes (Kaimal, 2010). Free radical induced lipid peroxidation has gained much importance because of its involvement in several pathologies such as ageing, wound healing, oxygen toxicity, liver disorders, inflammation, etc (Gupta, 2010). The increased level of lipid peroxidation induced tissue injury was observed 4.96 nM/ml MDA in diabetic rats than the normal level 2.05 nM/ml MDA. Whereas the free radical scavenging activity of BMC 2.01 nM/ml MDA, 2.17 nM/ml MDA and 2.01 nM/ml MDA respectively inhibited the lipid peroxidation.

Administration of alloxan produces a variety of metabolic alterations in peripheral tissues. The changes can be attributed to the decrease in insulin levels. There was an increase in glucose, cholesterol, LPO and decrease in the liver glycogen levels showing the impairment of liver function probably as the result of hepatic damage.

The oral administration of crude powder of BMC for 30 days was able to protect the liver damage induced of lipid per oxidation and activation of antioxidant enzymes. There is evidence that alloxan interferes with cellular oxidative mechanisms. Islet cells possess very
low antioxidant enzyme activity and therefore, vulnerable to oxidative stress. The free radical scavenging activities of many herbal drugs have been attributed to their polyphenolic and flavonoidal constituents.

Table 1: Anti-diabetic effect of Boerhavia diffusa, Momordica charantia and Carica papaya leaves in experimental male albino rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Glucose</th>
<th>Glycogen</th>
<th>Cholesterol</th>
<th>LPO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dose</td>
<td>mg/dl</td>
<td>mg/g</td>
<td>mg/dl</td>
<td>nM/ml MDA</td>
</tr>
<tr>
<td>Normal</td>
<td>Saline</td>
<td>129.0±9.03</td>
<td>20.5±1.23</td>
<td>110.5±7.29</td>
<td>2.05±0.164</td>
</tr>
<tr>
<td>Alloxan treated</td>
<td>40mg/kg</td>
<td>152.5±12.2</td>
<td>16.0±0.96</td>
<td>200.0±19.0</td>
<td>4.96±0.43</td>
</tr>
<tr>
<td>Boerhavia diffusa Treated</td>
<td>100mg/kg</td>
<td>130.8±1.12ᵇ</td>
<td>18.0±1.02ᵇ</td>
<td>137.5±7.42ᵇ</td>
<td>2.01±0.156ᵃ</td>
</tr>
<tr>
<td>Momordica charantia Treated</td>
<td>100mg/kg</td>
<td>136.3±10.88ᵇ</td>
<td>18.3±1.02ᵇ</td>
<td>133.3±8.79ᵇ</td>
<td>2.17±0.108ᶜ</td>
</tr>
<tr>
<td>Carica papaya Treated</td>
<td>100mg/kg</td>
<td>130.8±1.12ᵇ</td>
<td>18.0±1.02ᵇ</td>
<td>137.5±7.42ᵇ</td>
<td>2.01±0.156ᵃ</td>
</tr>
</tbody>
</table>

Each value is the mean ± values are significant from control,
Whereᵃ is P < 0.005,ᵇ is P < 0.001 andᶜ is P > 0.005

CONCLUSION
The study also shows the significant efficacy of Boerhavia diffusa, Momordica charantia and Carica papaya leaves in the treatment of diabetes was also evidenced by decrease in serum glucose, cholesterol, LPO and increase in liver glycogen. The diabetic effect of BMC crude powder may be due to the activity of the phyto-constituents present which might have exerted the protection against the liver damage and the subsequent enzyme activities as observed.

REFERENCE


