**Anti-diabetic effect of *Curculigo orchioides* Gaertn extracts in alloxan induced diabetic rats**

P. Susindran¹, N. Ramesh²

¹ Bharathidasan University, Department of Biotechnology, J.J. College of Arts and Science, Pudukkottai- 622 001, Tamil Nadu, India.

Received on:25-04-2014; Revised on: 17-05-2014; Accepted on:29-06-2014

**ABSTRACT**

In the present study, the aqueous and ethanolic extracts of *Curculigo orchioides* Gaertn rhizome was used to screen the antidiabetic and antioxidant activity in alloxan induced diabetic rats. Hyperglycemia induced in rats by alloxan (150mg/kg i.p). Two weeks after alloxan induction, diabetic rats received aqueous and ethanolic extracts of *Curculigo orchioides* (CO) orally 100, 200 and 300 mg/kg body weight respectively for 90 days. In this study, protective effects of CO rhizome on glucose tolerance test and an alloxan induced diabetes were evaluated. Serum biochemical parameters glucose, urea, total cholesterol and total protein were measured. The activity of tissue antioxidant enzymes like superoxide dismutase (SOD), reduced glutathione (GSH), catalase (CAT), and lipid peroxides (LPO) and also liver enzymes like SGPT, SGOT,ALP were also done. The observation confirm that glucose tolerance were significantly (P<0.01) maximum with ethanolic extract of CO at 120 minutes (min).Serum glucose, urea, total cholesterol were significantly (P<0.05) decreased with aqueous extract, reverse in the protein concentration. Elevated liver enzymes (SGOT, SGPT, and ALP) activity in alloxan induced rats were regained with ethanolic extract of CO rhizome administration at 300mg/kg. All the three doses of aqueous and ethanolic extract administration increased the enzyme activity (GSH, SOD and CAT) maximum effect found on 300mg/kg of ethanolic extract administration and reverse was recorded in lipid peroxidase enzyme system. The observation confirm that aqueous and alcoholic extract of *Curculigo orchioides* thizome has remarkable antidiabetic activity and, the hepatoprotective activity of the extract may be due to its antioxidant property exerted by glycosides and flavonoids respectively. It also warrants further investigation to isolate and identify the compound.

**KEY WORDS:** Alloxan, Diabetes mellitus, *Curculigo orchioides*, antioxidant, hepatoprotection.

**1. INTRODUCTION**

Diabetes is defined as a state in which the homeostasis of carbohydrate and lipid metabolism is improperly regulated by the pancreatic hormone, insulin, ultimately resulting in increased blood glucose level. It is the world’s largest endocrine disorder and is one of the major killers in recent times¹. According to world health organization (WHO), the world wide global population is in the midst of a diabetes epidemic with people in southeast Asia and western Pacific being mostly at risk. The number of cases for diabetes which is currently at 171 million is predicted to reach 366 million by the end of 2030². Therefore, it is necessary to search for new drugs and interventions that can be used to manage this metabolic disorder. The most prevalent form of diabetes is non-insulin dependent diabetes mellitus (type 2).³

Several investigations have been conducted and many plants have shown a positive activity⁴. Although many drugs are available in modern medicine to treat diabetes mellitus, they produce various systemic side effects or exhibit tolerance upon chronic use⁵. In Ayurveda, many plant products have been claimed to be free from side effects and less toxic than synthetic drugs⁶.

Oxidative stress is imbalance between the generation of reactive oxygen species (ROS) and the body defence mechanisms. Environmental pollutants, toxic habits (drug, smoking and/or alcohol), inadequate nutrition, excess solar radiation, large exposure to toxic substances, drug metabolism(side effect), and a high physical or psychologocal stress are the most common exogenous factors originating ROS in human body⁷. Oxidative stress has also been implicated in the pathogenesis of diabetes, liver damage, nephrotoxicity, inflammation, cancer, cardiovascular disorder, as well as in process of aging⁸.

*Curculigo orchioides* grows as forest herb used in traditional medicine system. It belongs to the family Amaryllidaceae. It is also known as Nilappanaikkilanku in tamil; black musli and Golden Eye-grass in English, found throughout India. In Ayurveda the roots are used for treatment of general debility, jaundice, colic and are good aphrodi-
The acute toxicity of Rhizome of Curculigo orchioides is used for the treatment of adaptogenic, androgenic, antioxidant, asthma, cancer, convulsant, diabiotic, diarrhea, gonorrhoea, hepatoprotective agent, immunostimulant, inflammation, jaundice, male sterility, piles, pimples, sedative, spasmyotic, stop bleeding and dry up wounds, tonic, strength, vigour. Therefore, a proper scientific evaluation a screening of plant by pharmacological tests followed by chemical investigations is necessary (Wadkar et al., 2008).

Venukumar et al. (2002) noticed that methanolic extract of CO rhizome exhibit a liver protective effect against CCL induced hepatotoxicity and possessed anti-lipid peroxidative and antioxidant activities. In the present investigation, the aqueous and ethanolic extract of CO rhizome was used to evaluate the antidiabetic and antioxidant in normal and alloxan induced diabetic rats.

2. MATERIALS AND METHODS

COLLECTION AND AUTHENTICATION OF PLANT MATERIALS:
Rhizome of Curculigo orchioides Gaertn. Were obtained from local plant supplier of Madurai district Tamil Nadu, India. The plants were botanically identified and authenticated by Dr. P. Brindha, Associate Dean, Department of CARISM, SASTRA University, Thanjavur. Voucher specimen was deposited in the Department of CARISM, SASTRA University, Thanjavur, Tamil Nadu, India for future reference.

PREPARATION OF EXTRACTS
Rhizome of Curculigo orchioides Gaertn. was cut into pieces, shade dried and powdered. The powder was passed through the mesh No. 60 and stored in an air tight container for further use. Dry powder (350g) was extracted with ethyl alcohol (COEE) and Water (COWE) by cold maceration method for 72 hours. The ethyl alcohol and water extracts were filtered and concentrated using vacuum desiccator.

PREPARATION OF DIABETIC ANIMALS
Inbred Albino rats (Wistar strain) of either sex weighing (150-180 g) were procured from the Tamil Nadu Veterinary and Animal Science University. The animals were kept in polypropylene cages (6 in each cage) under standard laboratory conditions (12:12 h dark/light cycle) and had a free access to commercial pellet diet (Hindustan Lever LTD, India) and water *ad libitum*. The animal house was maintained at temperature at 25 ± 2°C and relative humidity at 50± 15%. All the animal experiments were carried out in accordance with the guidelines of CPCSEA (Reg. No.: 790/03/ae/CPCSEA) and the study was approved by the Institutional Animal Ethics Committee (IAEC).

ACUTE TOXICITY
The acute toxicity of Rhizome of Curculigo orchioides Gaertn. in albino rats was studied as per OECD guideline 425. The median lethal dose (LD50) value was determined using the method of maximum livelihood.

ORAL GLUCOSE TOLERANCE TEST
The oral glucose tolerance test was performed in overnight fasted normal rats. Rats were divided into Eight groups (n=6). Group I served as normal control and received distilled water(5ml/kg bw p.o), and group II served as diabetic control and received glucose only(3gm/kg bw p.o). Group III to VIII received aqueous and ethanolic extract of Curculigo orchioides at the rate of 100, 200 and 300 mg/kg bw respectively. The rats of group III to VIII were loaded with glucose (3gm/kg, p.o), 30 min after drug administration. Blood samples were collected by puncturing the retro orbital sinus under light ether anesthesia just prior to drug administration 0min, 30min, 60min, 90min, 120min after loading glucose. Blood glucose level were measured immediately by using Ortho toludine methods.

ALLOXAN INDUCED DIABETES:
In this method albino rats were fasted in individual cage for 24hr. Diabetes was induced in the rat by injecting alloxan monohydrates intraperitoneally in single dose of 150mg/kg bw, to the over night fasted rat. The rat were kept for next 24 hr on 10% glucose solution contain in a bottle kept in their cages, to prevent hypoglycemia. After 72hrs of injection, fasting blood glucose level was measured. A animal whose glucose level with the range of 200 to 260mg/100ml for 72hrs of injection, fasting blood glucose level was measured. A animal whose glucose level with the range of 200 to 260mg/100ml for 72hrs of injection, fasting blood glucose level was measured.

WORK PLAN:
Wistar strains of albino rats of either sex weighing 150–180gms were divided in to eight groups of six animal each and grouped as follows.

- **Group I** Normal Rats
- **Group II** Disease control (Diabetes will be induced with alloxan (IP injection of 150mg/Kgbw).
- **Group III** Treatment of alloxan induced diabetic rat with Aqueous extract (100mg/kg body weight) for 90 days
- **Group IV** Treatment of alloxan induced diabetic rat with Aqueous extract (200mg/kg body weight) for 90 days
- **Group V** Treatment of alloxan induced diabetic rat with Aqueous extract (300mg/kg body weight) for 90 days
- **Group VI** Treatment of alloxan induced diabetic rat with ethanolic extract (100mg/kg body weight) for 90 days
- **Group VII** Treatment of alloxan induced diabetic rat with ethanolic extract (200mg/kg body weight) for 90 days
- **Group VIII** Treatment of alloxan induced diabetic rat with ethanolic extract (300mg/kg body weight) for 90 days
- **Group IX** Treatment of alloxan induced diabetic rat with standard drug Glibenclamide 250mg for 90 days

After 90 days treatment, blood was collected from overnight fasted
rats from each group by cardiac puncture for estimation of serum biochemical parameters. Then the rats were sacrificed by cervical dislocation for the study of liver enzymes.

**SERUM BIOCHEMICAL PARAMETERS**

Collected Blood was used for estimation serum Biochemical parameters viz. glucose (Sasaki et al., 1972)\(^{15}\), Urea (Natelson et al 1951)\(^{16}\), Cholesterol (Parekh et al 1970)\(^{17}\), Total protein (Lowry et al., 1951)\(^{18}\).

**ESTIMATION OF LIVER AND ANTIOXIDANT ENZYMES:**

Estimation of Liver enzymes like Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT) (Bergmeyer et al 1978)\(^{19}\), Serum Alkaline Phosphatase (SALP) (King, 1965)\(^{20}\) and activites of antioxidant enzymes such as SOD (Misra et al 1979)\(^{21}\), Reduced glutathione (GSH)(Ellman et al 1959)\(^{22}\), Catalase (CAT) (Bergmeyer et al 1974)\(^{23}\) and LPO(Ohkawa et al 1979)\(^{24}\) were assayed.

**STATISTICAL ANALYSIS**

The values are expressed as Mean ± SEM. Student’s-T test was used to analyze statistical significance.

**3. RESULTS:**

**ACUTE TOXICITY:**

The oral LD\(_{50}\) Value of *Curculigo Orchioides* in rats was 3000mg/kg body weight.

**ORAL GLUCOSE TOLERANCE:**

In alloxan induced diabetic rats, the blood glucose levels were in the range of 215mg/dl to 335mg/dl, which were considered as severe diabetes. The serum glucose level was lowered significantly (P<0.05) with all the three aqueous extracts at different time intervals. The activity shown by ethanolic extract were highly significant (P<0.01) compared to diabetic control and maximum tolerance were found at the dose rate of 300mg/kgbw at 120min (Table -1). Therefore, antidiabetic activity of ethanolic extract was maximum when compared to aqueous extract of CO.

**HEPATOPROTECTION:**

The effect of rhizome of CO on alloxan induced damage in rats with reference to biochemical changes in serum is shown in table -2. The single intra peritoneal injection of alloxan monohydrate (150mg/kg bw) let to elevation of serum glucose, urea, cholesterol and decrease in protein concentration was observed. The antihyperglycemic effect of all the three doses of aqueous and ethanolic extracts of CO significantly reduced the serum glucose, urea, cholesterol profile and reverse Phenomena in protein concentration was recorded. Animal treated with high dose of ethanolic extract of CO (300mg/kg) shown a significant (P<0.01) decrease in serum glucose, urea, cholesterol compare to diabetic control rats. Therefore compared to aqueous extract, ethanolic extract restored the all serum Biochemical parameter to near normal level.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glucose (mg/dl)</th>
<th>Urea (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>Protein (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>083.2±3.1</td>
<td>32.1±0.8</td>
<td>127.1±0.9</td>
<td>7.3±0.10</td>
</tr>
<tr>
<td>II</td>
<td>205.5±3.2</td>
<td>82.1±0.7</td>
<td>337.3±1.3</td>
<td>3.6±0.60</td>
</tr>
<tr>
<td>III</td>
<td>179.3±1.7</td>
<td>69.3±1.0</td>
<td>289.1±1.4</td>
<td>4.2±0.10</td>
</tr>
<tr>
<td>IV</td>
<td>149.6±1.5</td>
<td>57.6±0.4</td>
<td>264.7±1.0</td>
<td>5.1±1.00</td>
</tr>
<tr>
<td>V</td>
<td>109.3±1.2</td>
<td>41.2±0.8</td>
<td>159.3±1.0</td>
<td>6.8±0.05*</td>
</tr>
<tr>
<td>VI</td>
<td>172.6±3.5</td>
<td>67.4±0.8</td>
<td>262.8±1.4</td>
<td>3.9±0.07</td>
</tr>
<tr>
<td>VII</td>
<td>139.4±0.8</td>
<td>56.1±0.8</td>
<td>222.5±1.8</td>
<td>4.4±0.04</td>
</tr>
<tr>
<td>VIII</td>
<td>099.4±0.8</td>
<td>39.0±0.7**</td>
<td>149.0±1.5**</td>
<td>7.0±0.07**</td>
</tr>
<tr>
<td>IX</td>
<td>084.2±1.3</td>
<td>31.7±1.0</td>
<td>127.2±1.2</td>
<td>7.4±0.04</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=6)

*P < 0.05 Statistically Significant When compared to Group -II
**P<0.01 Statistically Significant When compared to Group –II

All the liver marker enzymes viz, SGPT, SGOT and ALP registered enhanced activity in alloxan induced diabetic rats as compared to normal control group (table-3). The aqueous extract shown decreased these enzymes level in dose dependent manner and maximum significant (P<0.005) was found at the dose (300mg/kg), compare to diabetic rats. The effect of ethanolic extract of CO rhizome is nearly equal to that of Glibenclamide treated rats. Therefore the diabetes induced
liver damage was evident by elevated liver enzymes, were regained normalcy on ethanolic extract of CO rhizome administration (300mg/kg).

Table 3. Effect of Plant extracts of Curculigo Orchiodes on liver enzyme level in alloxan induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (U/g tissue)</th>
<th>GSH (g/g tissue)</th>
<th>Catalase (H₂O₂ oxidised/ min/g tissue)</th>
<th>LPO (nM MDA/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>75.40±1.1</td>
<td>446.4±1.2</td>
<td>137.1±0.8</td>
<td>363.3±2.2</td>
</tr>
<tr>
<td>II</td>
<td>29.00±0.8</td>
<td>186.5±1.1</td>
<td>070.1±0.9</td>
<td>796.4±1.7</td>
</tr>
<tr>
<td>III</td>
<td>36.22±0.8</td>
<td>235.6±1.3</td>
<td>085.9±0.8</td>
<td>686.1±1.8</td>
</tr>
<tr>
<td>IV</td>
<td>53.22±0.8</td>
<td>313.1±3.0</td>
<td>102.1±0.9</td>
<td>592.8±2.2</td>
</tr>
<tr>
<td>V</td>
<td>69.05±0.9*</td>
<td>426.4±1.2*</td>
<td>128.0±0.6*</td>
<td>390.3±2.5*</td>
</tr>
<tr>
<td>VI</td>
<td>69.18±1.0</td>
<td>456.3±1.0</td>
<td>135.2±0.9</td>
<td>661.0±2.4</td>
</tr>
<tr>
<td>VII</td>
<td>54.36±1.0</td>
<td>322.0±1.6</td>
<td>090.4±0.9</td>
<td>571.6±2.0</td>
</tr>
<tr>
<td>VIII</td>
<td>69.94±0.7**</td>
<td>431.7±1.6**</td>
<td>129.5±1.1**</td>
<td>360.8±1.6**</td>
</tr>
<tr>
<td>IX</td>
<td>72.98±0.7</td>
<td>441.6±1.0</td>
<td>136.1±0.7</td>
<td>362.2±1.4</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=6)
*P < 0.05 Statistically Significant When compared to Group -II
**P<0.01 Statistically Significant When compared to Group –II

All the antioxidant enzymes viz, SOD, GSH, catalase and LPO exhibited decreased activity in alloxan induced diabetic rats as compared to control group (table-4). All the three doses of aqueous and ethanolic extract shown increased enzyme activity in dose dependent manner on diabetic rats and it was found maximum significant (P<0.01) at the dose of 300mg/kg of ethanolic extract administration on diabetic rats. On the other hand, reverse phenomenon was found in LPS enzyme. Therefore ethanolic extract administered group, level of these enzyme were found retrieving towards normal by that of Glibenclamide.

Table 4. Effect of Plant extracts of Curculigo orchiodes on alloxan induced diabetic rats with reference to antioxidant enzymes.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGOT (IUL)</th>
<th>SGPT (IUL)</th>
<th>ALP (IUL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>070.1±01.2</td>
<td>043.5±03.3</td>
<td>094.50±3.4</td>
</tr>
<tr>
<td>II</td>
<td>202.6±43.9</td>
<td>105.1±17.7</td>
<td>285.00±5.8</td>
</tr>
<tr>
<td>III</td>
<td>159.0±04.4</td>
<td>090.8±02.8</td>
<td>245.83±4.3</td>
</tr>
<tr>
<td>IV</td>
<td>128.3±05.3</td>
<td>071.6±02.0</td>
<td>178.33±2.8</td>
</tr>
<tr>
<td>V</td>
<td>080.1±02.9</td>
<td>058.6±01.2</td>
<td>170.17±2.9</td>
</tr>
<tr>
<td>VI</td>
<td>075.6±03.5</td>
<td>047.1±02.5</td>
<td>102.67±2.1</td>
</tr>
<tr>
<td>VII</td>
<td>115.4±03.8</td>
<td>069.0±02.0</td>
<td>172.20±2.5</td>
</tr>
<tr>
<td>VIII</td>
<td>078.6±02.3**</td>
<td>051.2±02.3**</td>
<td>097.00±2.3**</td>
</tr>
<tr>
<td>IX</td>
<td>078.0±03.1</td>
<td>044.8±02.5</td>
<td>089.60±1.6</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=6)
*P < 0.05 Statistically Significant When compared to Group -II
**P<0.01 Statistically Significant When compared to Group –II

4. DISCUSSIONS:
Management of diabetes with the agents devoid of any side effects is still a challenge to the medical system. This concern has led to an increased demand for natural products with antihyperglycaemic activity, having fewer side effects. Alloxan causes diabetes through its ability to destroy the insulin-producing beta cells of the pancreas11-19. In vitro studies have shown that alloxan is selectively toxic to pancreatic beta cells, leading to the induction of cell necrosis25. The cytotoxic action of alloxan is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration, leading to a rapid destruction of beta cells25. According to earlier studies, plant extracts cause antihyperglycemic effect by promoting regeneration of β-cells or by protecting these cells from destruction, by restricting glucose load as well as by promoting unrestricted endogenous insulin action. Antihyperglycemic effect may also be caused by the effect of plant extract on β-cells to release insulin or activate the insulin receptors to absorb the blood sugar and stimulate the peripheral glucose consumption10.

Antihyperglycemic activities of most effective medicinal plants were in part explained by the ability of the phytoconstituents to increase glucose transport and metabolism in muscle and/or to stimulate insulin secretion by repairing damaged beta cells by their antioxidant power. It has been reported in the literature that the plant extracts have antioxidant potential. Presence of flavonoids and tannins in the extracts is known to possess antidiabetic activity26. In the present investigation, the observed antidiabetic potential of test extracts might be due to presence of similar phytoconstituents which were evident by preliminary phytochemical screening, and also due to the antioxidant potential of the plant.

The present experimental study reveals that the aqueous and ethanolic extracts from CO rhizome at three different dose level were administered orally for 90 days produced a significant decreased in the blood glucose, urea, cholesterol in the mode of alloxan induced diabetes and glucose tolerance in the rats.

In diabetic mellitus there is an increase in glucose, total cholesterol and total urea in blood which may contributes to various illness (like Coronary Artery disease), in the present study this elevated glucose, total cholesterol and total urea level in diabetic rats were significantly brought down by ethanolic extract. It also shows that the total protein content were significantly decreased in alloxan induced diabetic rats. The increase in the level of transaminase reflects a clear indication of cellular leakage and loss of functional integrity of cell membrane27. Assessment of liver function can be made by estimating the activities of serum SGOT, SGPT, SALP, which are originally present in higher
concentration in cytoplasm. In hepatopathy, these enzymes leak into blood stream in conformity with the extent of damage. The elevated levels of marker enzymes such as SGOT, SGPT, and ALP in alloxan induced diabetic rats corresponded to the impaired liver function. Diabetic complications such as increased gluconeogenesis and ketogenesis may be due to elevated transaminase activity. Treatment with 300 mg/kg Ethanolic extract of rhizome significantly reduced the elevated liver enzymes, indicating hepatoprotective action. Diabetic induced liver damage might be due to oxidative damage by free radical generation and antioxidant property is claimed to be one of the mechanisms of hepatoprotective drug.

Free radicals mediated tissue damage occurs in the generation and progression of diabetes mellitus. Insulin secretion is impaired during diabetes and this may evoke lipid peroxidation in biological systems and similar finding were observed in our present study. Lipid peroxidase enzyme level were increased in diabetic rats with 90days treatment of curculigo orchioides plant extract restored towards normalcy at the dose rate 300mg/kg bw.

Reduced glutathione (GSH) is essential to maintain structural and functional integrity of cells. Apart from its direct free radical scavenging properties and abilities to conjugate with several electrophilic intermediates that are capable of initiating lipid peroxidation, GSH acts as the physiological co-substrate of the conjugating enzyme system. The distinct diminution in GSH content of tissues in diabetic rats and its subsequent attainment of near normalcy on Curculigo orchioides extract administration reveals that the protection offered by Curculigo orchioides in combating oxidative insult due to diabetes. This observation is in agreement with the findings of prince et al. Decline in the activities of antioxidant enzymes, such as SOD, GSH, and CAT observed in diabetic rats indicate the extent of free radical induced damage due to hyperglycemia. It is now known that, when there is an imbalance between free radical production and antioxidant defenses, ‘oxidative stress’ occurs resulting in deregulation of cellular functions. An antioxidant drug is expected to bring an alleviation of this type of cellular dysfunctions. The profound increment in the activities of the antioxidant enzymes in Curculigo orchioides co – administered rats unravels the efficacy of the drug in resisting oxidative insult due to diabetes.

CONCLUSION

In order to provide a better understanding of possible role of extract of rhizome of Curculigo orchioides in the antidiabetic, hepatoprotective and antioxidant effect observed in this study, we carried out a preliminary phytochemical screening of the extract of the rhizome and found it to contain phenolic glycosides, flavonoids, saponin, and tannins. Pioneer study reveals that the phenolic glycosides exhibit antidiabetic activity by increased peripheral utilisation of glucose. Antioxidant effect evident by the flavonoids are phenolic compounds exert multiple biological effect, including antioxidant properties and free radical scavenging abilities. Therefore, the antidiabetic and the hepatoprotective activity of the extract may be due to its antioxidant property exerted by presence of glycosides and flavonoids in the rhizome.

5. REFERENCES:

16. Natelson S, Scott ML, Beffa D, A rapid method for the esti-
mation of urea in biological fluids by means of the reaction between diacetyl and urea, Am J Chem Patol, 1951; 21: 275-81

Source of support: Nil, Conflict of interest: None Declared