SYNOPSIS

Thesis submitted to Acharya Nagarjuna University, Nagarjuna nagar, Guntur Dist, A.P.,
Entitled “STUDIES ON THE ANTIDIABETIC POTENTIAL OF COCCINIA GRANDIS IN ALLOXAN TREATED ALBINO RATS”. Submitted by S.SRINIVASA RAO, M.Pharm. under the Guidance of Dr. P.V.V.Satyanarayana, M.Sc., Ph.D., Research Director, Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar.

The thesis contains 5 chapters

Chapter- I  --- Introduction.
Chapter- II  --- Materials and Methods
Chapter- III  --- Results
Chapter- IV  --- Discussion
Chapter- V  --- Summary and Conclusions

CHAPTER-I : INTRODUCTION

Diabetes mellitus is a metabolic disorder related to carbohydrates and lipids.

Diabetes has been classified in to 2 types

Type - I : Insulin Dependent Diabetes Mellitus
Type – II : Non Insulin Dependent Diabetes Mellitus

In Type – I : Auto destruction of pancreatic β – cells
In Type – II : Deficiency of Insulin secretion / action
Treatment of Diabetes:

1. Oral hypoglycemic agents control the glucose levels in the blood
   i) Sulphonyl ureas
   ii) Biguanides
   iii) Troglitazones
   iv) inhibitors of α – glucosidase

   The above agents are having side effects.

2. Other alternative method is insulin therapy.

   The disadvantage with insulin therapy is weight gain, therefore there is a need for the search of alternative chemical agents to control diabetes which are not having side effects.

   AYURVEDA is a branch of science which has been originated and practice from 2500 B.C.

   In medicinal plants glycosides, polysaccharides, oils, alkaloids are having hypoglycemic activity according to the literature.

   Therefore in this investigation an attempt has been made to study the hypoglycemic action of leaf extract of COCCINIA GRANDIS with the following objectives

Aims and objectives of present study:

1. To prepare the aqueous extract from the leaves of Coccinia grandis by using distilled water as solvent.

2. To investigate the changes in enzyme activities related to the carbohydrate metabolism in diabetic albino rats on long term treatment with plant extracts.
3. To investigate the effect of plant extract on the lipid peroxidation and its role in the antioxidant defense mechanism.

4. To characterize the histopathological changes in the tissues of liver and kidney.

Chapter II: Materials and Methods

For the above study *COCCINIA GRANDIS* leaves were selected and aqueous (With distilled water) fractions were prepared and the dry powder was made by using freeze-drying.

Finally the freeze dried powders were dissolved in saline and they are administered to the control as well as diabetic rats.

**INDUCTION OF DIABETES:**

Male albino weister rats of 4 months age (Body weight approximately 150 gm) were used for present study

Diabetes was induced in the rats by intraperitonal injection with cold aqueous alloxan monohydrate (160 mg/ Kg body weight)

Experimental rats were grouped in the following manner after administration of leaf extract.

**EXPERIMENTAL DESIGN:**

The rats were divided into 6 groups

Group – I : Normal

Group – II : Normal rats treated with low concentration of CG extract (70 mg/ Kg body weight)
Group–III : Normal rats treated with high concentration of plant extract (130 mg/ Kg body weight)

Group–IV : Diabetic untreated rats

Group–V : Diabetic treated rats with low concentration of plant extract (70 mg/ Kg body weight)

Group–VI : Diabetic treated rats with high concentration of plant extract (130 mg/ Kg body weight)

The estimations were made using serum, liver and kidney.

1. Fasting blood glucose
2. Lipid profile
3. Carbohydrate metabolizing enzymes
4. Lipid peroxidation
5. Antioxidant enzymes and antioxidants
6. Glycoprotein analysis
7. Membrane bound enzymes
8. Histopathological study

After 15 days of treatment, the 12 hr fasted animals were anaesthetized between 7 am to 8 am, using ketamine (24 mg/kg bw, intramuscular injection) and sacrificed by decapitation. Blood was collected in two different tubes (i.e.,) one with whole blood for serum separation and another with anticoagulant-potassium oxalate and sodium fluoride for plasma insulin assay. Tissues (liver and kidney) were surgically removed, washed with cold physiological saline, cleared off the adherent lipids- and immediately transferred to ice-cold containers and weighed.
Erythrocytes were also prepared for the estimation of various biochemical preparations.

**Parameters analyzed to evaluate antidiabetic potential:**

**Blood parameters:**

**Whole blood**

Hemoglobin (Hb) and Glycosylated haemoglobin (HbA$_{1c}$).

**Plasma**

1. **Fasting plasma glucose insulin**

2. **Lipid Peroxidative markers:** Thiobarbituric acid reactive substances (TBARS) and Lipid hydroperoxides (HP).

3. **Nonenzymic antioxidants:** Ascorbic acid, $\alpha$-tocopherol, Reduced glutathione (GSH) and ceruloplasmin

4. **Lipid profile:** Total cholesterol (TC), Free fatty acids (FFA), Triglycerides (TG), Phospholipids (PL), High density lipoprotein (HDL), Low density lipoprotein (LDL) and very low density lipoprotein (VLDL) cholesterol.

5. **Glycoprotein component analysis:** Total hexoses, Hexosamine, Sialic acid, and Fucose.

**Serum:**

1. **Carbohydrate metabolizing enzymes:** Glucokinase, Glucose 6-phosphatase dehydrogenase, Glucose 6-phosphatase, Fructose 1,6-bisphosphatase, Glycogen phosphorylase and Glycogen synthatase.
2. **Hepatic marker enzymes:** Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Acid phosphatase (ACP), Alkaline phosphatase (ALP) and γ-glutamyl transferase (GGT).

**Erythrocyte:**

1. Lipid peroxidation markers.

2. **Antioxidants:** Enzymic: Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx) and Non-enzymatic antioxidants (as mentioned above).

3. **Membrane bound enzymes:** Total ATPase.

**Tissue parameters:**

**Liver and Kidney:**

1. Carbohydrate metabolizing enzymes

2. Glycoprotein component analysis

**Liver and Kidney:** Lipid peroxidative markers, Enzymatic and nonenzymatic antioxidant markers

**Liver and Kidney:** Lipid profiles

**Liver and Kidney:** Membrane bound enzymes
CHAPTER-III : RESULTS

After induction of diabetes, the rats were given treatment with or without aqueous leaf extract of CG and the effect and protection was studied mainly on the carbohydrate and lipid metabolism and antioxidant defense by following the methods already described. To determine the effective dose of CG on glycemic control in alloxan – diabetic rats oral administration of selected doses of CG (70 and 130 mg/kg bw) was given and after 15 days and plasma blood glucose levels were studied in normal, normal treated, diabetic and diabetic treated rats (Table 4). The concentration of glucose in control sample was 89 mg/dl. The induction of diabetes raised the glucose level to 252 mg/dl. Treatment with two different concentrations (70 mg and 130 mg of plant extract with control samples) is able to maintain almost equal concentrations of glucose levels as observed in control. But in the case of diabetic rats treatment with low concentrations as well as high concentrations of leaf extract brought the glucose level to 115 and 117 mg/dl. Glycosylated Hb levels are good indicators of progression of diabetes and the levels are around 0.61 mg/g. Decrease in the plasma insulin, total Hb and hepatic glycogen was noticed in diabetic rats. Oral administration of CG-leaf extract for 15 days maintained the above parameters are same levels like that of normal status in diabetic rats.
Table 4: Levels of glucose in different experimental animals.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>89 ± 7.2</td>
</tr>
<tr>
<td>Normal + CG (70 mg/kg bw)</td>
<td>81 ± 7.5</td>
</tr>
<tr>
<td>Normal + CG (130 mg/kg bw)</td>
<td>85 ± 7.1</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>252 ± 16.7</td>
</tr>
<tr>
<td>Diabetic + CG (70 mg/kg bw)</td>
<td>115 ± 9.2</td>
</tr>
<tr>
<td>Diabetic + CG (130 mg/kg bw)</td>
<td>117 ± 8.1</td>
</tr>
</tbody>
</table>

CHAPTER- V: SUMMARY AND CONCLUSIONS

The management of diabetes mellitus is considered as a global problem and successful treatment is yet to be discovered. The modern drugs including insulin and other oral hypoglycemic agents such as biguanides, sulphonyl ureas, α-glucosidase inhibitors control the glucose as long as they administered regularly and they have undesirable side effects. These side effects, lack of curative treatment for several chronic diseases have led to the discovery of complementary and alternative medicines. The use of medicinal plants in modern medicine refers from the fact that thousands of plants are used in world to prevent or cure diabetes, specific evidence in terms of modern medicine are lacking. Therefore in this investigation an attempt has been made by taking aqueous leaf extract of CG and its protective role has been studied by using the albino rat as experimental
material. To study the protective role the effect on enzymes related to carbohydrate metabolism and antioxidant defense in control, diabetic and diabetic treated rats was compared.

After studying the protective effect of CG, the observations are summarized below:

- The treatment of leaf aqueous extract of CG decreased the plasma glucose by increasing the plasma insulin levels.
- During the dose determination 70 and 130 mg/kg bw dose of leaf extract showed better glucose lowering affect.
- Decreased levels of total Hb, liver glycogen and high levels of HbA₁/c were restored after oral administration of CG extract.
- Serum and tissue carbohydrate metabolizing enzymes were altered in diabetic rats. After treatment with CG extract the HK activity was decreased and Glu-6-P and Fru-1,6-bis phosphatase levels were increased.
- Oral administration of CG extract significantly reactivated the glycogen synthase due to increased insulin secretion.
- LDL, VLDL and HDL cholesterol levels were significantly altered in diabetic rats. Serum and tissue concentrations of lipid parameters were significantly restored by the treatment of CG leaf extract.
- The CG leaf extract increased the non-enzymatic (ascorbic acid, α-tocopherol and GSH) and enzymatic (SOD, CAT and GPx) anti oxidant levels during diabetes.
Lipid peroxidation and glycosylation of proteins caused reduction in the activities of enzymes and alterations in the structure and functions of membranes.

Administration of CG leaf extract to diabetic rats showed a significant elevation of activities of total ATPase in erythrocyte membranes.

Oral administration of CG-leaf extract improved the liver function by decreasing the serum ALT, AST and ALP levels in diabetic rats.

Oral administration of CG-leaf extract significantly improved the glycoprotein levels in diabetic rats.

Histopathological studies clearly demonstrated the restoration of β-cell biomass by the administration of CG-leaf extract.

Thus, the leaf extract of CG did not produce any toxic effects on liver and kidney during acute or chronic conditions. The principle was not lethal in the range of 70 to 130mg/kg bw and exerts antidiabetic effects and control the several biological parameters during diabetes in albino rats.