Hypoglycemic effect of ethanolic extract of *Hedychium coronarium* linn., rhizome in alloxan induced diabetes in rat model.


Department of Pharmacology, Bharat Institute of Technology (Pharmacy), Mangalpally, Ibrahimpatnam – 501510, Andhra Pradesh, India.

*Corresponding author: Pranitha.k,*
E-mail id: pranithak.16@gmail.com

**ABSTRACT**

*Purpose*

To study the Hypoglycemic effect of ethanolic extract of *Hedychium coronarium* linn., rhizome in Alloxan induced diabetes in rat model.

*Methods*

The preliminary photochemical studies were carried out on the crude extract which was prepared and the various chemical constituents present in the crude extract were evaluated. Acute Toxicity studies as per OECD-23 guidelines to determine the safety doses of the root extract was carried out on male wistar strain of Albino rats. The various factors such as body weight, feed intake, blood glucose, serum insulin etc were regularly studied and evaluated.

*Results*

The hypoglycemic effect of the ethanolic extract of *Hedychium coronarium* linn. Rhizome was studied on the various factors such as body weight, blood glucose levels etc of the alloxan induced diabetic rat model.

*Conclusion*

In present study administration of *Hedychium coronarium* rhizome extract produced a significant reduction in blood glucose, serum insulin, serum catalase and haemoglobin in alloxan induced rats. However, it seems promising that if these data will be validated in the future clinical trials, *Hedychium coronarium* rhizome extract may offer an alternative treatment for diabetes.

**Keywords** - *Hedychium coronarium* linn., Diabetis, Albumin rats, blood glucose.

**INTRODUCTION**

“Diabetes Mellitus (DM) is a disorder caused by the total (or relative) absence of insulin, which manifests clinically as an elevated blood glucose” (Wareham N, O’Rahilly et al., 1998). DM is a heterogeneous clinical syndrome, in which chronic elevation of
sugar (glucose) in blood leads to spillage of the glucose in the urine. Increased glucose levels due to disturbances in the metabolism of carbohydrates, fats, proteins resulting from absolute defect in insulin secretion/action (or) different levels of relative peripheral resistance to insulin (Palanisamy malini et al., 2011). Normally blood glucose is tightly controlled by insulin of human pancreatic b- cells. Insulin lowers the blood glucose levels when the blood glucose levels are elevated after a meal. Insulin is released to a variety of stimuli such as carbohydrates (glucose, mannose), Amino acids (leucine, arginine) ketone bodies and vagal activity, and also by the stimulation of b- receptors in pancreas. Hyperglycemia is closely associated with increased production of free radical species and increased oxidative stress (pooja et al., 2011). Persistent hyperglycemic status in diabetes and increased oxidative stress is associated with altered glucose and lipid metabolism. Lipid peroxide mediated tissue damage has been observed in the development of both the types of diabetes (Ramachandran vadivelan et al., 2011).

Increased concentration of TBARS (Thiobarbituric acid reactive substances) and the simultaneous decline in anti-oxidative defensive mechanisms observed in diabetic patients promotes the development of late complications.

Insulin is essential to process carbohydrates, fats, proteins that blood glucose levels by allowing glucose to enter muscle cells and by stimulating the conversion of glucose to glycogen (glycogenesis) as a carbohydrate store. It can also inhibit the release of stored glucose from glycogen (glycogenolysis) in both liver and kidney. Therefore hyperglycemia is promoted by increasing glicogenolysis, glycogenesis and reduction in glucose utilization.

**Classification of diabetes**

In 1998, a new classification system based upon the etiological factors at work in diabetes was proposed by the WHO and we have listed it below: this has now become the accepted system for classifying diabetes mellitus [1].

**Type 1 diabetes**

It is an immune mediated and idiopathic forms of b cell dysfunction, which lead to absolute insulin deficiency. This is an autoimmune mediated disease process which gives rise to absolute deficiency of insulin and therefore total dependency upon insulin for survival.

**Type 2 diabetes**

This is a disease of adult onset, which may originate from insulin resistance and relative insulin deficiency or from a secretory defect. This is a disease, which appears to have a very strong genetic predisposition and is caused by a combination of inadequate insulin secretion and an insensitivity of the body tissues to insulin so leaving patients with this condition relatively deficient in insulin.

**Type 3 diabetes** is gestational diabetes.

**The complications of diabetes**

The complications of diabetes are classified as follows:

- Diabetic Retinopathy
- Diabetic Neuropathy,
- Diabetic Nephropathy,
- Atherosclerosis.

**AIM AND OBJECTIVE**

In a modern medicine no satisfactory effective therapy is still available to eradicate diabetes. Insulin and oral anti diabetic agents are used as a monotherapy or in combination to achieve better glycogenic control. Each of the oral agents suffers from a number of serious adverse effects. As a consequence there is a consequence there is high demand for new oral anti diabetic agents. Plants have played a major role in the introduction of new therapeutic agents and the beneficial multiple activities like, manipulating carbohydrate metabolism by various mechanisms, preventing, restoring integrity and function of b- cells, insulin releasing activity, improving glucose uptake and utilization and anti oxidant properties in medicinal plants.

**Plant profile:**

**Botanical classification:**

- **Kingdom:** Plantae
- **Subkingdom:** Tracheobionta
- **Superdivision:** Spermatophyta
- **Division:** Magnoliophyta
- **Class:** Monocotyledons
- **Subclass:** Zingiberidae
Order: Zingiberales  
Family: Zingiberaceae  
Genus: Hedychium Koenig-Garland-Lily  
Species: Hedychium coronarium Koenig-White Garland-Lily (National Plant Database. 2004.)

Common names  
White ginger lily, white garland lily, Butterfly Lily, Garland Flower.

Parts used: Flowers, Rhizome, Leaves

Habitat: Grows in moist tropical evergreen forest. Mostly confined to north eastern part of India.

Description  
Erect herb with stems 1 m tall. The leaves are narrowly oblong or lanceolate, acuminate, about 50-60 cm long, 10 cm wide with short petiole. Pure white, showy flowers emerge from one large bud sometime in late spring through summer at the tip of each unbranched stem. Each flower lasts about one day. Several hundred flowers can appear from each bud during a 6 week period. These herbaceous perennials spread by underground rhizomes, often forming dense clumps of multiple stems. (Wagner.1990.)

Chemical constituents  
The essential oil from fresh and dried rhizomes of Hedychium coronarium on GC-MS analysis resulted in the identification of 44 and 38 constituents representing 93.91% and 95.41%, respectively. The major components of the essential oil from fresh and dried Hedychium coronarium rhizome were 1,8-cineole (41.42%, 37.44%), beta-pinene (10.39%, 17.4%) and alpha-terpineol (8.8%, 6.7%).

MATERIALS AND METHODS  
Preparation of crude extract  
The roots of Hedychium Coronarium were collected, shade dried at room temperature the roots were subjected to size reduction to a coarse powder by using dry grinder mixer, passed through the sieve (50 mesh). The power (80 gram) was extracted with 5 liters’ of ethanol overly for 7 days by method of cold maceration at room temperature occasionally mixing the contents every 6 hrs. The container was closed with lid to prevent the evaporation of ethanol. After 7 days the contents are filtered and the filtrate is evaporated, air-dried and kept in a desiccators. The yield of ethanolic extract obtained was about 15 grams i.e. 18.75%. The concentrated residue was stored in air tight container in refrigerator (2-8 degrees C) for use in further experimentations. The suspension of the extract was prepared freshly by using 1% CMC.

Preliminary phytochemical studies  
The ethanolic extract of the roots of Hedychium coronarium was subjected to preliminary phytochemical screening.

Test for Carbohydrate and glycosides  
A small quantity of the extract was dissolved separately in 4ml of distilled water and filtered. The filtrate was subjected to Molisch’s test to detect the presence of carbohydrates.

Molisch’s Test  
The filtrate was treated with 2-3 drops of 1% alcoholic –alpha napthol solution and 2ml of conc.sulphuric acid was added along the sides of the tube. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrates.

Another portion of extract was hydrolyzed with hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to Legal’s and Borntrager’s test to detect the presence of different glycoside.

Legal’s test  
To hydrolysate 1ml of pyridine and few drops of sodium nitropruside solution were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red color shows the presence of glycosides.

Borntrager’s Test  
Hydrolysate was treated with chloroform and then the chloroform and then the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Ammonia layer acquires pink color showing the presence of glycosides.
Test for Alkaloids
A small portion of extract was stirred separately with few drops of dil. Hydrochloric acid and filtered. The filtrate was tested with various reagents for presence of alkaloids.
- Mayer’s reagent - Cream precipitate
- Dragendorff’s reagent - Orange brown precipitate
- Hager’s reagent - Yellow precipitate
- Wagner’s reagent - Reddish brown precipitate

Test for Phytosterols
The extract was refluxed with solution of alcoholic potassium hydroxide till complete saponification has taken place. The mixture was diluted and extracted with ether. The ether layer evaporated and the residue was tested for the presence of phytosterols.

Libermann Bucaard Test
The residue was dissolved in few drops of dil. Acetic acid; 3ml of acetic anhydride was added followed by few drops of conc.sulphuric acid. Appearance of bluish green color shows the presence of phytosterols.

Test for fixed oils
Spot test
Small quantity of extract was separately pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil. Few drops of 0.5N alcoholic potassium hydroxide were added to small quantity of extract along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

Test for Gums and mucilage
Small quantity of extract was added separately to 25ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties for the presence of gums and mucilage.

Test for Saponins
The extract was diluted with 20ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam shows the presence of saponins.

Test for Proteins and Free Amino acids
Small quantity of extract was dissolved in few ml of water and treated with following reagents.
- Million’s reagent - Appearance of red color shows the presence of protein and free amino acids.
- Ninhydrin reagent – Appearance of purple color shows the presence of proteins and free amino acids.
- Biuret test – Equal volumes of 5% sodium hydroxide solution and 1% copper sulphate solution was added. Appearance of pink or purple color shows the presence of proteins and free amino acids.

Test for Phenolic compounds and Tannins
Small quantity of extract was taken separately in water and tested for the presence of phenolic compounds and tannins out with the following reagents.
- Dil.Ferric chloride solution (5%) – violet color
- 1% solution of gelatin containing 10% sodium chloride -- white precipitate
- 10% lead acetate solution – white precipitate

Test for Flavonoids
With aqueous sodium hydroxide solution
- Blue to violet color (anthocyanins),
- Yellow color (flavones),
- Yellow to orange (flavonones).

Shinoda’s test
Small quantity of extract was dissolved in alcohol, to it a piece of magnesium followed by
- Conc. Hydrochloric acid drop wise added and heated. Appearance of magenta color shows the presence of flavonoids.

Acute oral toxicity studies
Acute Toxicity studies were performed as per OECD-23 guidelines to determine the safety doses. Acute Toxicity studies of the root extract was carried out on mail wistar strain of albino rats bearing were fasted over night and weight of each animal was recorded just before use. Animals are divided into five groups
they were fed orally with the ethanolic extract of Hedychium coronarium in increasing dose levels of 100, 500, 1000 and 2000 mg/kg body weight. The animals were observed continuously for 2 hr for the following:

- Behavioral profile: Alertness, Restlessness, irritability, and fearfulness
- Neurological profile: spontaneous activity, reactivity, touches response, and gait
- Autonomic profile: defecation and urination
The number of deaths, if any was recorded after 24 and 72 h (Rakesh Barik, et al., 2008)

**Experimental design**

Male wister albino rats were divided into six groups of six animals in each group as follows:

- **Group 1**: Vehicle controlled received 1% CMC daily for 15 days.
- **Group 2**: Metformin (10mg/kg) dissolved in normal saline given orally for 15 days.
- **Group 3**: Extract (100mg/kg) suspended in 1% CMC given orally for induced diabetic rats.
- **Group 4**: Extract (200mg/kg) suspended in 1% CMC given orally for induced diabetic rats.
- **Group 5**: Diabetic control.

**Preparation of Alloxan solution:**

Alloxan was dissolved in normal saline solution and injected.

**Metformin suspension**

Standard Metformin was suspended in 1% CMC solution. This suspension was administered at a dose of 10 mg/kg body weight using clean and dry oral feeding needle for 15 days.

**Preparation of extract suspension**

Extract was suspended in 1% CMC solution. This suspension was administered using clean and dry oral feeding needle for 15 days.

**Animal procurement and maintenance**

Male albino (wistar strain) rats (200-250g) were procured from the National Institute of Nutrition (NIN), Tarnaka, Hyderabad. The animals were housed in poly propylene cages with not more than 3 animals per cage, at an ambient temperature of 18 ± 20 C with 12-h-light /dark cycle. Rats have free access to standard rodent feed (Nutrilab) and water and libitum. The maintenance and the handling of animals were performed according to the rules of Institutional animal Ethical Committee.

**Experimental induction of Diabetes**

Diabetes was induced in overnight faster experimental groups by a single intra-peritoneal (i.p) injection of freshly prepared STZ (60mg/kg b.w) dissolved in 0.1 M citrate buffer (pH 4.4), 15 min after the i.p administration of NIC (120 mg/kg b.w). Hyperglycemia was confirmed by the elevated glucose levels. After 72 h, blood glucose was determined and those rats with fasting glucose levels greater than 126mg-dl were used in the present study (Rakesh Barik et al., 2008).

**Estimation of parameters**

**Body weight**

The body weight of each animal was recorded on the days 0, 7, and 14.

**Feed intake**

Food weight was recorded daily for 15 days

**Blood glucose levels**

The blood was collected from retro-orbital plexus, glucose levels of each animal were recorded on the days 0, 7, 14 by using glucometer

**Estimation of enzyme catalase**

**Reagents**

6M sodium phosphate buffer (ph 7.4)  
Hydrogen peroxide-369ml of H₂O₂ was added to 50ml of buffer.  
Ammonium molybdate – was prepared by adding 1gm ammonium molybdate in 50 ml of buffer.

**Principle**

Catalase activity in sample was detected by using ammonium molybdate metho by measuring the intensity of yellow complex formed by molbdate and hydrogen peroxide at 405nm, after ammonium molybdate was added to terminate the degredation reaction catalyzed by catalase enzyme.
Procedure
1. Blank 1: buffer 1ml-this was used for blanking the instrument.
2. Blank 2:1ml H2O2 + 100 ml buffer +1ml ammonium molybdate- Read at 405nm
3. Blank 3:1.1 ml buffer + 1ml ammonium molybdate- Read at 405nm
Calculation: The enzyme catalase was calculated by using the formula
Sample- blank 2/ blank 2- blank 3 ×271 =KU/L

Hemoglobin (Hb) value
Hemoglobin gives RBC’s their color. Hb carries oxygen from the lungs to the tissues and takes carbon dioxide from the tissues to the lungs. Hemoglobin is measured grams per deciliter (g/dl) of blood.

Reagents: Drabkin reagent contains potassium ferricyanide. Potassium cyanide, monopotassium phosphate.

Procedure
Drabkin reagent 1/50 was diluted with 4.5ml distilled water +2 drops of conc. Reagent. The working reagent was stable for 1 month at 2-8°C away from direct light. The contents are mixed well and after 5mins incubation, the optical density (OD) was measured. The color was stable upto 30mins.

RESULTS AND DISCUSSION
Preliminary phytochemical screening
The preliminary phytochemical analysis of ethanolic extract of Hedychium coronarium rhizomes revealed that the presence of various phytoconstituents.

Table 1 – Presence of Various Phytoconstituents in the ethanolic extract

<table>
<thead>
<tr>
<th>S.No</th>
<th>CHEMICAL TESTS</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Test for carbohydrates</td>
<td>Molisch’s test  -ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fehling’s test   -ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Barfoed’s test   -ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Benedict’s test  -ve</td>
</tr>
<tr>
<td>2</td>
<td>Test for alkaloids</td>
<td>Hager’s test     -ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mayer’s test     -ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dragendorff’s test -ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wagner’s test    -ve</td>
</tr>
<tr>
<td>3</td>
<td>Test for phytosterols</td>
<td>Libermann- Burchard -ve</td>
</tr>
<tr>
<td>4</td>
<td>Test for fixed oils</td>
<td>Spot test        -ve</td>
</tr>
<tr>
<td>5</td>
<td>Test for saponin glycosides</td>
<td>Foam test        -ve</td>
</tr>
<tr>
<td>6</td>
<td>Test for Cardiac glycosides</td>
<td>Baljet test      -ve</td>
</tr>
<tr>
<td></td>
<td>Test for anthraquinone glycosides</td>
<td>Modified Borntrager’s test  -ve</td>
</tr>
<tr>
<td>7</td>
<td>Test for Proteins &amp; Amino Acids</td>
<td>Millon’s Test    -ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Biuret test      -ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ninhydrin test   -ve</td>
</tr>
<tr>
<td>8</td>
<td>Test for Terpenoids</td>
<td>Salkawoski test  +ve</td>
</tr>
<tr>
<td>9</td>
<td>Test for Phenolic Compounds</td>
<td>Lead acetate solution -ve</td>
</tr>
</tbody>
</table>

Acute oral toxicity studies
Acute oral toxicity studies of Hedychium coronarium extract did not produce any mortality or signs of toxicity at the dose of 2000 mg/kg b.w /p.o in experimental rats

Body weight
Body weight of all animals in different groups are taken on 0.7 & 14 days. They are mentioned in following table.
Table 2 – Body weights of the experimental animals

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>0 Days</th>
<th>7 Days</th>
<th>14 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>125±5</td>
<td>130±5</td>
<td>130±5</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>125±5</td>
<td>120±5</td>
<td>120±5</td>
</tr>
<tr>
<td>Metformin</td>
<td>115±4</td>
<td>110±0</td>
<td>115±5</td>
</tr>
<tr>
<td>Extract (100mg/kg)</td>
<td>125±5</td>
<td>130±5</td>
<td>135±5</td>
</tr>
<tr>
<td>Extract (200mg/kg)</td>
<td>125±5</td>
<td>130±5</td>
<td>135±5</td>
</tr>
</tbody>
</table>

As per the data presented in the table decreased body weight observed in diabetic control group from 0 to 14th day (125-120) and gradual increase in body weight observed in extract – 1 (125-135), extract- 2 (125-135) treated groups & metformin treated group (115 to 115).

The values are expressed as mean ± SEM. Statistical significance test for comparison was done by ANOVA.

Table 3 – Feed intake consumed by individual animal daily

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>FEED INTAKE ( gm/animal) (mean SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>14.66±1.25</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>15.6±1.3</td>
</tr>
<tr>
<td>Metformin</td>
<td>12.3±3.95</td>
</tr>
<tr>
<td>Extract I (100mg/kg)</td>
<td>12.6±4.4</td>
</tr>
<tr>
<td>Extract II (200mg/kg)</td>
<td>12.5±3.4</td>
</tr>
</tbody>
</table>

As per the data presented in the table the diabetic group consumed more feed when compared to other groups.

The values are expressed as mean ± SEM. Statistical significance test for comparison was done by ANOVA.

Table 4 The blood glucose levels of the experimental animals

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>0 days</th>
<th>7 days</th>
<th>14 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>139</td>
<td>118</td>
<td>105</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>271</td>
<td>286</td>
<td>295</td>
</tr>
<tr>
<td>Metformin</td>
<td>181</td>
<td>150</td>
<td>133</td>
</tr>
<tr>
<td>Extract I(100mg/kg)</td>
<td>271</td>
<td>120</td>
<td>93</td>
</tr>
<tr>
<td>Extract II(200mg/kg)</td>
<td>279</td>
<td>94</td>
<td>86</td>
</tr>
</tbody>
</table>

As per the data presented in the table on 0 day, blood glucose levels in all groups are high indicating induction of diabetes after administration of alloxan compared to vehicle control group. On 7th day, blood glucose levels increased in untreated diabetic group (271 to 286) & slightly decreased in metformin (181 to 150), extract- I (271 to 120) & extract II (279 to 94) treated groups. On 14th day, blood glucose levels further increases in untreated diabetic group (286 to 295), and further decreased in metformin (150 to 133), Extract I(120 to 93) & Extract II (94 to 86) treated groups. 200mg/kg
extract treated groups lowers glucose levels more when compared to 100mg/kg extract treated groups. The values are expressed as mean ± SEM. Statistical significance test for comparison was done by ANOVA.

SERUM INSULIN
Serum insulin levels (µU/ml) are mentioned in the table as mean ± SD.

Table - 5 The serum insulin levels administered to the animals

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>SERUM INSULIN (µU/ml) (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>19.77±0.5</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>7.80±0.49</td>
</tr>
<tr>
<td>Metformin</td>
<td>16.06±0.72</td>
</tr>
<tr>
<td>Extract I (100mg/kg)</td>
<td>16.44±0.26</td>
</tr>
<tr>
<td>Extract II (200mg/kg)</td>
<td>17.99±0.15</td>
</tr>
</tbody>
</table>

Insulin level decreased in diabetic control group (7.8±0.49) compared with normal control group (19.77±0.5). Extract I (16.44±0.26), Extract II (17.99±0.15) Metformin treated groups (16.06±0.72) were slightly increased compared to diabetic group. The values are expressed as mean ±SEM. Statistical significance test for comparison was done by ANOVA,

Hemoglobin
Hemoglobin levels of different group of animals have been measured.

Table – 6 The Hemoglobin levels of the various experimental animals

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>HAEMOGLOBIN (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>13±1.31</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>5.6±0.4</td>
</tr>
<tr>
<td>Metformin</td>
<td>11.33±0.5</td>
</tr>
<tr>
<td>Extract I (100mg/kg)</td>
<td>10.2±0.5</td>
</tr>
<tr>
<td>Extract II (200mg/kg)</td>
<td>11.13±0.3</td>
</tr>
</tbody>
</table>

Hemoglobin levels was decreased in diabetic control group (5.6±0.4) compared with normal control group (13±1.31). Extract I (10.25±0.5), Extract II (11.13±0.5) were slightly increased compared to diabetic group. The values are expressed as mean ± SEM. Statistical significance comparison test was done by ANOVA.

Catalase
Serum catalase levels of different groups of animals have been measured which is shown in the table.

Table – 7 The serum catalase levels observed in the experimental animals

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>CATALASE(µU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>7106.2±88.5</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>3304±36.9</td>
</tr>
<tr>
<td>Metformin</td>
<td>5845±82.4</td>
</tr>
<tr>
<td>Extract I (100mg/kg)</td>
<td>6259±152.2</td>
</tr>
<tr>
<td>Extract II (200mg/kg)</td>
<td>6992.2±85.9</td>
</tr>
</tbody>
</table>

Catalase levels decreased in diabetic control group (3304±36.9) compared with normal control group (7106.2±88.5). Extract I (6259±152.2), Extract II (6992.2±85.9) and Metformin treated groups (5845±82.4) were slightly increased compared to diabetic group.
The values are expressed as mean ± SEM. Statistical significance test for comparison was done by ANOVA.

The present study we have investigated the anti-diabetic effect of ethanolic extract of Hedychium coronarium Rhizome in alloxan induced diabetic rats in dose dependent manner. Previously it was reported that different parts of H.coronarium plant have anti-diabetic efficacy by alloxan induced method ( F Lukmanul Hakkim et al., 2007). This has been further supported by Latha and Pari using aqueous extract of H.coronarium rhizome several authors reported that flavonoids,sterols/tepenoids, phenolic acid are know to be bioactive anti-diabetic principles (Atta-Ur-Rhemann, Khurshid Zaman, 1989). Flavonoids are known to regulate the damaged beta cells in diabetic rats (Chakravarthy BK et al., 1980). Phenolics are found to be effective antihyperglycemic agents (Manickam M, et al., 1997).

Alloxan produces very similar action as that of type 2 diabetes in animal experiments. The type 2 diabetes mellitus characterised by two metabolic defects such as (1). decreased insulin secretion that is delayed or insufficient relative to glucose load (2). Inability to peripheral tissues to responds to insulin-called insulin resistance. Beta-cells defect and insulin resistance are major features of non insulin dependent diabetes mellitus, and both features are the focus of intensive investigation. Major characteristics of type 2 diabetes include impaired utilization of glucose and resistance to the ability of insulin to stimulate glucose uptake and disposal in tissues (chia-Wen Chen* and Hsing-Hsien Cheng 2011).

Medicinal property of various plants has been screened for its anti-diabetes property. As I mentioned in the aim and objectives the crude ethanolic extract of Hedychium coronarium rhizome was subjected to preliminary phytochemical analysis which shows the presence of flavonoids, sterols, alkaloids, glycosides, saponins.

Acute oral toxicity study of extract of Hedychium coronarium rhizome did not exhibit any lethality or any profound toxic reactions at a dose of 200mg/kg/p.o. According to the (OECD) 423 guidelines for acute oral toxicity study LD50 dose of 200mg/kg/p.o of Hedychium coronarium rhizome is found to be safe.

In the diabetic state, the body weight is reduced where as food intake is increased and this recovers during the exposure of hypoglycemic treatment (F Lukmanul Hakkim et al., 2007). In the present study the group of diabetic rats showed progressive and significant (P<0.05) loss in body weight throughout the study period as compared to the body weight gain of normal group of rats. This might be due to increased muscle wasting in diabetes and loss of tissue proteins. For this reason, weight reduction is being used as a marker of DM. Administration of ethanolic extract and Metformin to diabetic rats resulted in an increase in body weight compared to diabetic rats (Group II). Results suggested that Hedychium coronarium treatment has positive effect on maintaining body weight in diabetic rats.

Untreated diabetic rats consume more food when compared to normal rats and extract (100 & 200mg/kg b.w) and Metformin treated groups as diabetic rats suffer from polyphasia. This was supported by Ganesan kanchana et al. 2011.)

Generally in diabetic condition liver weight is decreased and kidney weight is increased (Ganesan Kanchana et al., 2011). A decrease in the liver weight observed in diabetic animals might be due to an increased breakdown of glycogen or pronounced gluconeogenesis. Significant increase in liver weight was observed in ethanolic extract (100 & 200mg/kg) and Metformin treated groups this may be due to the accumulation of glycogen in liver. A significant increase in kidney weight was observed in diabetic animals when compared with the normal control animals. The kidney enlargement is an early feature in both experiment and human diabetes due to an increase in the capillary length and diameter. This might be due to the glomerular cell proliferation and glomerular enlargement in diabetic rats. Significant decrease in kidney weight was observed in ethanolic extract (100 & 200mg/kg) and Metformin treated groups.

Blood glucose level of untreated diabetic rats were significantly increased than those in normal rats(A. Kumari et al., 2008). This might be due to over production of glucose by means of excessive hepatic glycoegenolysis and gluconeogenesis and decreased utilization by the tissues. Oral administration of the extract (100 & 200mg/kg b.w) and Metformin (10mg/kg b.w) for 15 days significantly (P<0.05)
lowered the hyperglycaemia of the experimental groups in respect to diabetic rats. Among the two doses of extract, 400mg/kg dose (group-V) showed significant anti-hyperglycemia effect. As it is evident from the results that maximum reduction in the blood glucose levels were observed after 15 days treatment. The proposed mechanism of action may be by protecting the cells in pancreas from destruction, by restricting glucose load as well as by promoting unrestricted endogenous insulin action and further effect, beta-cells to release insulin and activate the insulin receptors to absorb the blood sugar.

In present study administration of Hedychium coronarium rhizome extract produced a significant reduction in blood glucose, serum insulin, serum catalase and haemoglobin in alloxan induced rats. However, comprehensive chemical and pharmacological researches are required to find out the exact mechanism of this extract for its anti-diabetic effect and to identify the active constituents(s) responsible for this effect. However, it seems promising that if these data will be validated in the future clinical trials, Hedychium coronarium rhizome extract may offer an alternative treatment for diabetes.

REFERENCES


