Chapter 6:

_In vivo antidiabetic activities of the extract on adult Wister rats_
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6.1. INTRODUCTION

Diabetes mellitus is a major endocrine disorder with microvascular and macrovascular complications associated with high mortality rate. It is predicted that the prevalence of diabetes is expected to increase by more than two-fold worldwide and approximately 57 million Indians would be affected by this disorder in the year 2025, illustrating the severity and impact of the disorder on the quality of life (King et al., 1998; Kameswara et al., 2003). The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels (American Diabetes Association, 2007). Diabetes has shadowed the modern lifestyle all over the world and has been linked to overweight and sedentary population. Hence new concepts and drugs are required to manage this epidemic disease.

Currently, conventionally available drugs include insulin and oral agents such as sulfonylureas, biguanides, alpha-glycosidase inhibitors and gliptins. According to the literature available, these drugs are known to produce serious side effects like hypoglycemic coma and hepatorenal failure (Ramirez et al., 2012). Hence it is a major and important task to identify a better lead molecule to cure diabetes which can avoid unwanted long-term adverse effects. In this regard, natural medicinal plants have been investigated for effective and safe alternative remedy.

Plants are exemplary source of drugs and according to ethnobotanical information around 800 plant species have recognized to possess antidiabetic potential (Grover et al., 2002). The medicinal values of plant lie in their component phytochemical such as alkaloids, flavonoid, phenolic compounds and other nutrients like as amino acid, proteins, which produce a definite physiological
action on the human body. India, represented by rich culture and natural biodiversity, offers a unique opportunity for drug discovery researchers (Jachak and Saklani, 2007) and is the largest producer of medicinal herbs and so called “Herbal Drug House” (Seth and Sharma, 2004).

The plant, *Rotula aquatica* contain Baunerol, steroid, alkaloid, and rhabdiol and allantoin. It is an important medicinal plant used in the preparation of various ayurvedic formulations for the treatment of many diseases like coughs, heart diseases, dysuria, blood disorders, fever, poisonings, ulcers and uterine diseases. Although *R aquatica* has been advocated in ayurvedic formulations for treating various diseases, however detailed preclinical studies to evaluate its safety and efficacy in managing type-2 diabetes is lacking. In this view, the present study was carried out to investigate the antidiabetic effect of *R aquatica* extract using diabetic induced rats model.

6.2. MATERIAL AND METHODS

6.2.1. Chemicals required

All chemicals and solvents used were of analytical grade. Streptozotocin and Glibenclamide were purchased from Sigma Chemical Co. (USA). Glucose oxidase-peroxidase reactive strips form Agappe Diagnostic Pvt. Ltd, Kerala, India. Insulin and C-peptide kit were purchased form BARC, Mumbai, India. Ferric chloride, acetic acid, HCl, isopropanol, triolein, phosphotungstic acid, EDTA, Tris-HCl, oxalate hydrochloride, H$_2$SO$_4$, KCl were procured from Sisco Research Laboratories, Mumbai, India.

6.2.2. Plant material and Preparation of Plant Extract

The plant, *Rotula aquatica* Lour, collected from areas of Netravati river, during September (2010) was dried and extracted with methanol by cold maceration as described earlier (chapter 3).
6.2.3. Animals care and handling

The study was carried out as per Organization for economic cooperation and development (OECD) guidelines revised draft guidelines 423 received from committee for the purpose of control and supervision of experiments of animals (CPCSEA), Ministry of Social Justices and Empowerment, Government of India. The study was approved by institutional animal ethics committee, University of Mysore, Mysore approval number: UOM/IAEC/1/2013. Experimental rats were obtained from animal house, Department of Zoology, University of Mysore, Mysore. Animals were housed individually in cages maintained under standard condition of temperature (23±2°C), humidity and dark–light cycle (lights on from 6:00 am to 6:00 pm). Tap water was available at libitum. All the animals were carefully monitored under the constant supervision of expertise.

6.2.4. Acute oral toxicity studies

Albino Wistar rats of either sex, weighing between 25-30 g were used for acute toxicity study. Animals were grouped into 3 groups (n=6). Group II and III were administered with methanolic extract of *Rotula aquatica* at a dose of 2000 mg/kg BW and 5000 mg /kg BW respectively. Group I served as control. Experimental animals were observed for behavioral changes like alertness, restlessness and irritability as compared to control. Defecation and urination were monitored for the next 72 h.

6.2.5. Induction of Experimental Diabetes

Rats were induced diabetes (experimental non-insulin dependent diabetes mellitus-NIDDM type 2) according to method described by Masiello *et al.*, (1998). In the present study male and female albino wistar rats aged four months weighing 140-170 g with no prior drug treatment were used. The animals were fed with standard pellet diet and had free access to water throughout the experiment. Initially, experimental rats were checked for fasting blood glucose level (tail vein puncture) and then administered with intra peritoneal injection of nicotinamide (NA; 230 mg/kg dissolved in saline), 15 min before streptozotocin (STZ; 65mg/Kg
dissolved in 0.1M citrate buffer PH 4.5). The next 24 h, rats were given 15% glucose solution to prevent hypoglycemia. The experimental animals were observed for evidence of allergic reactions, behavioural changes, and convulsions.

Five days after streptozotocin injection, fasting blood glucose level was recorded and screened for diabetes rats having glycosuria and hypoglycaemia. Animals having marked hyperglycemia (fasting blood glucose ≥250 mg/dL) were selected for the study. All animals were allowed free access to water and pellet diet.

**Experimental design**
A total 36 rats were used which are randomly divided into six groups as follows:

- **Group I (G1):** Normal untreated rats (control).
- **Group II (G2):** Normal rats treated with 400 mg/kg body weight of methanolic extract in water per day.
- **Group III (G3):** Diabetic untreated rats.
- **Group IV (G4):** Diabetic rats treated with 200 mg/kg body weight of methanolic extract in water per day.
- **Group V (G5):** Diabetic rats treated with 400 mg/kg body weight of methanolic extract in water per day.
- **Group VI (G6):** Diabetic rats treated with standard reference Glibenclamide (Anti-diabetic drug) 0.5 mg/kg body weight.

Methanolic extract of *Rotula aquatica*/Glibenclamide were intubated orally using a mouth gauge for a period of 21 days. Body weight and blood glucose were measured at weekly intervals during the experimental period. At the end of experiment animals were fasted overnight and sacrificed by cervical dislocation. Blood was drawn from cardiac puncture for serum and hematological studies. Vital organs like liver, kidney and pancreas were removed, washed with chilled saline and observed for any morphological changes or lesions. Further, a portion of these organs were collected in 10% formalin for histopathological studies.
6.2.6. Haematological studies

6.2.6.1. Blood collection

The blood samples were collected from tail vein puncture at the time of grouping of animals (basal reading) and at 1st, 7th, 14th and 21st day of treatment. Blood glucose was estimated by using dextro strip (glucose oxidize methods) with one touch glucometer (Johnson and Baker, 1998; American Diabetes Association, 2008).

6.2.6.2. Serum lipids and lipoprotein profiles

**Cholesterol**: Total cholesterol was estimated according to the method of Zlatkis et al. (1953). Initially ferric chloride-acetic acid reagent (9.9 ml) was added to serum (0.1 ml) for deproteinization. The mixture was centrifuged at 3000 rpm for 15 min. Five ml of the supernatant was taken and mixed with 3.0 ml of concentrated sulphuric acid and incubated at room temperature for 20 min. The pink color formed was read at 540 nm against reagent blank.

**Triglycerides**: Serum triglycerides were measured by the method described by Foster and Dunn (1973). Serum (0.1 ml) was taken in glass stoppered centrifuge tube and mixed with 4.0 ml of isopropanol and 400 mg of alumina. The tubes were tightly capped and shaken vigorously for 10 min and centrifuged at 3000 rpm for 15 min. Around 2 ml of supernatant was pipette out into clean test tubes and mixed with 0.5 ml of acetyl acetone reagent and 1.0 ml of meta-periodate reagent. The mixture was incubated at 50°C for 30 min. The color developed was read at 405 nm against reagent blank. Triolein was used as standard for comparison.

**HDL-Cholesterol (HDL-C)**: Determination of serum HDL-cholesterol was carried out by the method of Burstein et al. (1970). Briefly, Serum (0.5 mL) was taken in a centrifuge tube and mixed with 0.25 mL of phosphotungstic acid reagent, 0.25 mL of MgCl₂ and centrifuged at 3000 rpm for 30 min at 4°C. The amount of cholesterol in supernatant was determined by the method of Zlatkis et al. (1953) as described above.
VLDL and LDL Cholesterol (VLDL-C and LDL-C): The concentrations of VLDL and LDL cholesterol in serum were calculated by using Friedewald formula (Friedewald et al., 1972).

\[
\text{VLDL-C} = \frac{\text{Triglycerides}}{5} \\
\text{LDL-C} = (\text{total cholesterol} - \text{VLDL-c}) - (\text{HDL-c})
\]

6.2.6.3. Glycosylated hemoglobin (HbA1C)

Glycosylated hemoglobin in the blood was estimated by the method of Sudhakar nayak and Pattabiraman (1981). Blood were collected with EDTA and plasma was separated. Saline washed erythrocytes (0.5 mL) was lysed with 5 mL of water, mixed and incubated at 37°C for 15 min. The contents were centrifuged and the supernatant was discarded, then 0.5 mL of saline was added, mixed and processed for estimation. To 2 mL of aliquot, 4 mL of oxalate hydrochloride solution were added and mixed. The contents were heated at 100°C for 4 h, cooled and precipitated with 2 mL of 40% TCA. The mixture was centrifuged and to 0.5 mL of supernatant, 0.5 mL of 80% phenol and 3 mL of concentrated sulphuric acid were added. The color developed was read at 480 nm after 30 min. The concentration of glycosylated hemoglobin is expressed as mg/g of hemoglobin.

6.2.6.4. Blood haemoglobin

Haemoglobin percentage in anticoagulated blood was estimated using Sahli’s haemoglobinometer. HCl (0.1 N) was taken in diluting tube upto mark 20. Then, blood was taken on haemoglobin pipette upto 20 cubic mm mark and suspended into diluting tube and incubate for 5-7 min to form acid haematin. Later, distilled water was added until it has the exact color as the comparison standard. Reading indicates the percentage of haemoglobin.

6.2.6.5. Serum insulin and C-peptide

Chemiluminescence immunoassay kit were used for the estimation of insulin and c-peptide in serum.
6.2.6.6. Estimation of Serum Urea

DIASYS UREA-FS kit was used for determining serum urea by urease-GLDH enzymatic UV test (Talke and Schubert, 1965). Urease hydrolyse the sample urea and produced ammonia and carbon dioxide. The liberated ammonia reacts with 2 oxoglutarate to form L-glutamate simultaneously NADH is oxidized to NAD+. The resulting decrease in absorbance due to oxidation of NADH is measured bichromatically at 340/380 nm and the rate of decrease in absorbance is proportional to the concentration of urea (mg/dL) in the sample.

6.2.6.7. Estimation of Serum Creatinine:

Serum creatinine was determined by Alkaline picrate method using IDENTI kit (Bartels et al., 1972). A yellow complex is formed when creatinine reacts with picric acid in alkaline condition. Bichromatically at 520/800 was used to measure the rate of colour formation which is proportional to the creatinine concentration (mg/dL) in the sample.

6.2.7. Antioxidant Activity of Liver Homogenate

6.2.7.1 Preparation of Liver Tissue Homogenate

Homogenate of liver (10% w/v) were prepared in 150 mM KCl using homogenizer at 4°C. The homogenates were centrifuged at 3000g for 15 min at 4°C using REMI homogenizer fitted with a Teflon plunger (REMI laboratory instruments, Mumbai, India). The supernatants were frozen at –20°C until assayed for the determination of antioxidants liver enzymes namely catalase and superoxide dismutase.

6.2.7.2. Estimation of Lipid Peroxidation Activity

The level of Thiobarbituric Acid Reactive Substances (TBARS) in tissue was estimated by the method of Nichans and Samuelson (1968). Tissue homogenate (10%) was prepared in Tris-HCl buffer (pH 7.5). 1.0 mL of tissue homogenate was treated with 2.0 mL of TBA-TCA-HCl reagent and mixed. The mixture was kept in boiling water bath for 15 min. After cooling, the tubes were
centrifuged at 1000 g for 10 min. The absorbance of the chromophore (pink color) generated due to the reaction between malondialdehyde and other thiobarbituric acid reactive substances (TBARS) and thiobarbituric acid under acidic condition was read at 535 nm against the reagent blank.

6.2.7.3. Catalase activity

The activity of catalase was determined by the method of Sinha (1972). Briefly, 0.9 mL of phosphate buffer, 0.1 mL of tissue homogenate and 0.4 mL of hydrogen peroxide were added. After 60 sec, 2.0 mL of dichromate-acetic acid mixture was mixed. The tubes were kept in boiling water bath for 10 min and the color developed was read at 620 nm. Standards in the range of 2-10 μmol were taken and preceded as test with blank containing reagent alone. The activities are expressed as μmoles of H$_2$O$_2$ consumed/min/mg protein.

6.2.7.4. Superoxide dismutase

Superoxide dismutase was estimated by the method of Kakkar et al. (1984). Tissue homogenate (0.5 mL) was diluted to 1.0 mL with water. Then 2.5 mL of ethanol and 1.5 mL of chloroform (all reagents chilled) were added. This mixture was shaken for 1 min at 4°C and then centrifuged. The enzyme activity in the supernatant was determined. The assay mixture contained 1.2 mL of sodium pyrophosphate buffer (0.025 M, pH 8.3), 0.1 mL of 186 μM phenazine methosulphate, 0.3 mL of 300μM nitroblue tetrazolium, 0.2 mL of 780 μM NADH, appropriately diluted enzyme preparation and water in a total volume of 3.0 mL. Reaction was started by adding NADH. After incubation at 30°C for 90 sec, the reaction was stopped by adding 1.0 mL of glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4.0 mL of n-butanol. The intensity of the chromogen in the butanol layer was measured at 560 nm against butanol blank. A system devoid of enzyme served as control. One unit of enzyme activity is defined as the enzyme reaction which gives 50% inhibition of NBT reduction in one min under the assay condition and expressed as specific activity in units/mg protein.
6.2.8. Histopathology of vital organs

At the end of 21 days of experiment, all the animals were sacrificed which were fasted overnight, by cervical dislocation. The liver, kidney, pancreas were removed, washed with chilled saline and small weighed portion of the tissue were immediately processed for histopathological studies. Tissues stored in 10% formalin solution were embedded in paraffin and sections of 4mm thickness were cut and strained with Hematoxylin-Eosin stain. Slides were observed under light microscope for histopathological analysis.

6.2.9. Statistical analysis

Data are expressed as the mean ± SD. The significance of the results was calculated using one-way ANOVA and post hoc Duncan multiple range tests and the results were considered statistically significant at p<0.05.

6.3. RESULT AND DISCUSSION

Diabetes mellitus is a chronic disease characterized by elevated blood glucose levels, disturbance in carbohydrate, lipid, and protein metabolism. Traditionally herbal medicines are used to treat this disorder. However inadequate knowledge of phytochemicals and lack of information on their pharmaceutical toxicity has created an awareness among scientific community to carryout preclinical analysis to authenticate the medicinal value of these herbs or plants. In this regard, *Rotula aquatica* Lour, a medicinal herb used by ayurvedic practitioners is evaluated for management of diabetes

6.3.1. Acute toxicity assay

In acute toxicity assay, the methanolic extract of *R aquatica* was intubated at a dosage of 2000 mg/kg BW and 5000 mg/kg BW and observed for the subsequent 72 h for any oral toxicity changes. Results reveal no mortality or any sign of toxicity in experimental animals. Behavioral profile was normal as compared to control group. Neurological changes with respect to spontaneous reactivity, touch and pain response was comparable with control group. Defecation
and urination was normal. Hence $1/10^{th}$ and $1/5^{th}$ of the 2000 mg/kg BW was selected to evaluate the hypoglycemic activity in diabetes induced rats.

6.3.2. Hypoglycemic activity of *R aquatica* extract

6.3.2.1. Effect of on serum glucose level

Figure 6.1 summarizes the serum glucose levels in experimental rats. According to the results obtained, no pronounced change ($p>0.05$) in glucose level was observed in the normal rats fed with methanolic extract (G2) as compared to control (G1). However, in streptozotocin-nicotinamide induced diabetic rats (G3) a 3.75 fold increase ($p<0.05$) in fasting blood glucose levels was observed when compared control (G1). In diabetic induced rats, methanolic extract at a dose of 200 and 400mg/kg BW remarkably ($p<0.05$) decreased the serum glucose levels with increase in incubation time. However, no significant difference ($p>0.05$) was observed with varied dosage (200 or 400mg/kg BW). After 21 day treatment, the glucose level reduced by 24.8% in group fed with 200mg/kg BW. Similarly, the positive control Glibenclamide also significantly ($p<0.05$) decreased the serum glucose level by 27.9%.

In accordance to the present study, Ashwini *et al.* (2012) have reported the aqueous extract of *R aquatica* at a dose of 200 mg/kg BW reduced blood glucose upto 40.6%. Shyam *et al.* (2013) studied the effect of methanolic extract of *R aquatica* roots at three doses (100, 200 and 300 mg/kg BW) for a period of 24 h. They reported that the extract at a dose of 100 mg/kg BW significantly reduced (11.9%) within 8 h of administration. Priya *et al.*, (2014) observed 400 mg/kg BW of methanolic extract of *R aquatica* leaves had prominent effect of glucose level after 21 days of treatment.
Figure 6.1: Serum glucose level in the experimental rats during treatment for 21 days

6.3.3. Haematological analysis

6.3.3.1. Effect of methanolic extract on serum lipid profile

Table 6.1 illustrates the serum lipid profile of experimental rats. Control rats showed the normal range of HDL-C, LDL-C, TG and TC. On administration of methanolic extract slight decline in lipid profile was observed as compared to control. However, on induction of diabetes, significant (p<0.05) increase in cholesterol and triglyceride level was observed with simultaneous reduction in HDL level. Treatment of diabetic rats with methanolic extract (200 and 400mg/kg BW) produced a significant (p<0.05) reduction in serum levels of triglyceride, cholesterol, LDL and VLDL, which is explained by the increase in plasma insulin level after treatment (Table 6.2). The positive control group treated with Glibenclamide also significantly decreased the serum lipid level.

Ashwini et al. (2012) observed a reduction of 24% in total cholesterol, 59% in LDL and 41% in triglycerides after 14 days of treatment with 200 mg/kg BW of *R. aquatica* aqueous extract. Similarly, Priya (2014) observed significant decrease in serum lipids on administration of methanolic extract of *R. aquatica* leaves in
aloxan induced diabetic rats. Diabetes is known to be associated with hyperlipidemia as well as hypertriglyceridemia which are the major coronary risk factors (De sereday et al., 2004). Hyperlipidemia is the result of excess mobilization of fat from the adipose due to underutilization of glucose. In the present study, methanolic extract of *R. aquatica* has shown to exhibit anti-hyperlipidemic effect.
### Table 6.1: Lipid profile of experimental rats

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Cholesterol</th>
<th>Triglycerides</th>
<th>HDL</th>
<th>LDL-c</th>
<th>VLDL-c</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>44.62 ± 1.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>66.55 ± 0.57&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.28 ± 0.44&lt;sup&gt;d&lt;/sup&gt;</td>
<td>15.18 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.10 ± 0.56&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>G2</td>
<td>40.77 ± 3.99&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.13 ± 6.46&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.17 ± 0.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18.67 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.55 ± 0.47&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>G3</td>
<td>66.15 ± 3.08&lt;sup&gt;d&lt;/sup&gt;</td>
<td>85.60 ± 0.10&lt;sup&gt;i&lt;/sup&gt;</td>
<td>10.97 ± 0.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.45 ± 0.07&lt;sup&gt;d&lt;/sup&gt;</td>
<td>13.36 ± 1.75&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>G4</td>
<td>46.37 ± 3.88&lt;sup&gt;c&lt;/sup&gt;</td>
<td>39.27 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.82 ± 0.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.37 ± 0.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.42 ± 0.53&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>G5</td>
<td>40.58 ± 4.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.17 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.53 ± 0.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17.32 ± 0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.57 ± 1.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>G6</td>
<td>44.87 ± 2.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>61.50 ± 0.23&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10.75 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.56 ± 0.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.77 ± 2.28&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are Mean ± Standard Deviation (n=6); Mean values within the same column with different lowercase superscripts are significantly different (p<0.05) according to Duncan’s multiple range test.
Table 6.2: Effect of methanolic extract of *R* aquatica on serum glycosylated hemoglobin, hemoglobin, insulin and C-peptide level

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Glycosylated hemoglobin (g%)</th>
<th>Hemoglobin (g%)</th>
<th>Insulin (μl/mL)</th>
<th>C-peptide (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>3.68 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.43 ± 0.45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17.07 ± 1.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.75 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>G2</td>
<td>3.63 ± 0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.45 ± 0.31&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17.47 ± 2.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.47 ± 0.23&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>G3</td>
<td>8.95 ± 0.54&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.42 ± 0.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.82 ± 0.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.84 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>G4</td>
<td>5.83 ± 0.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.35 ± 0.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.73 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.18 ± 0.36&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>G5</td>
<td>5.83 ± 0.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.37 ± 0.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.40 ± 0.78&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.83 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>G6</td>
<td>5.70 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.48 ± 0.66&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.57 ± 1.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.53 ± 0.29&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are Mean ± Standard Deviation (n=6); Mean values within the same column with different lowercase superscripts are significantly different (p<0.05) according to Duncan’s multiple range test.

6.3.3.2. Effect on plasma insulin, glycosylated hemoglobin and hemoglobin and c-peptide levels

Table 6.2 depicts the plasma insulin, glycosylated hemoglobin, hemoglobin and c-peptide levels in experimental rats. There was a significant reduction in plasma insulin levels of diabetic rats (6.82 ± 0.56 μl/mL) as compared to control (17.07 ± 1.67 μl/mL). On treatment with methanolic extract, the insulin level raised slightly reaching 11.4 ± 0.78 μl/mL at a dose of 400mg/kg BW. Treatment of diabetic rats with Glibenclamide resulted in increase of plasma insulin levels to 11.57 ± 1.12 μl/mL. Insulin is secreted in response to high blood sugar levels. It balances glucose output from the liver and glucose uptake in skeletal muscle and adipose tissue. The raise in the insulin level on treatment with methanolic extract suggests that the extract might trigger the secretion of insulin from the existing β-cells of the pancreas and might regenerate the β-cells of the pancreas destructed by streptozotocin (Li *et al.*, 2004).
Similarly, glycosylated hemoglobin (HbA1c) level was significantly (p<0.05) elevated in diabetic rats (8.95 ± 0.54%) as compared to control (3.68 ± 0.16%). Treating diabetic rats with methanolic extract and Glibenclamide reduced HbA1c level noticeably as compared to diabetes rats. Extent of reduction in methanolic extract group and Glibenclamide administered group was statistically similar (p>0.05). In the diagnosis of diabetes, HbA1c has been suggested as the most reliable marker (Mohammadi and Naik, 2008). The increased level of HbA1c is directly proportional to the concentration of blood glucose. Hence the decreased HbA1c level, as observed after methanolic extract possibly signifies its normoglycemic control mechanisms.

According to the results obtained, c-peptide level significantly (p<0.05) reduced on induction of diabetes. Treatment with methanolic extract at a dose 200 mg/kg BW slightly increased the c-peptide level by 1.4 folds. However at higher dose of 400 mg/kg BW no change in the c-peptide level was observed. Administration of Glibenclamide significantly (p<0.05) increased the level by 4.2 folds.

6.3.3.3. Effect on superoxide dismutase (SOD), Lipid peroxidase (LPO) and catalase (CAT)

Figure 6.2 shows the activities of antioxidative enzymes in liver extract. The activity of SOD and CAT in liver was significantly (p<0.05) lower in diabetic rats as compared to control group. However the LPO activity increased noticeably in diabetic rats. Treatment with methanolic extract as well a Glibenclamide resulted in significant (p<0.05) increase in SOD and CAT activity with simultaneous reduction of LPO activity. Earlier studies reveal the lowering activity of antioxidant enzymes with enhanced peroxidase status in type-2 diabetes (Punitha et al., 2005). Peroxidase are destroyed by the enzymes, SOD and CAT. Further, they play a significant role in providing antioxidant defence to an organism (Punitha et al., 2005). SOD is an important antioxidant defence enzyme generated in response to oxidative stress and catalase enzymes are involved in
decomposition of H$_2$O$_2$ which is generated by the action of SOD on free oxygen radicals (Halliwell, 2006). In the present study comparative increase in SOD and CAT activity was observed on treating with methanolic extract. The study signifies the effect of *R aquatica* in repairing the complication in tissue function created on diabetic induction.

Lipid peroxidation is one of the most often used biomarker to investigate the oxidative damage. It leads to structural modification of free amino group of proteins, phospholipids, and nucleic acids (Pandey and Rizvi, 2010). Similar to the present study, increased levels of lipid peroxidation has been reported in STZ-diabetic rats (Hussein, 2008). Administration of methanolic extract reduced the LPO activity, indicating the effect of *R. aquatica* in protecting against oxidative stress.
Figure 6.2: Effect of methanolic extract of *R. aquatica* on antioxidative enzymes (a) superoxide dismutase (SOD); (b) Lipid peroxidase (LPO) and (c) Catalase (CAT). Values are mean ± SD (n=6)

6.3.3.4. Effect on serum urea and creatinine

Figure 6.3 depicts the effect of methanolic extract on serum urea and creatinine level. The results show a significant (p<0.05) increase in creatinine level on diabetic induction with simultaneous reduction in urea concentration. On administration of methanolic extract, decline in creatinine level was observed to a
level similar to control group. Percentage of reduction was higher in group fed with 200 mg/kg BW as compared to Glibenclamide. Similarly, urea concentration in group fed with 200 mg/kg BW was comparable with control group. Serum level of urea and creatinine indicate the kidney function.

![Graph showing serum creatinine and urea levels](image)

Figure 6.3: Effect of methanolic extract of *R aquatica* on serum (a) Creatinine and (b) Urea level. Values are mean ± SD (n=6)

6.3.4. Histopathology of vital organs

Histopathological studies are one of the best methods to understand the effect of administered drug. Pancreas, liver and kidney are the major organs affected during diabetes, hence in the present study these vital organs were studied.
for their morphological changes after HE staining. Pancreas, liver and kidney sections of normal healthy rats treated with methanolic extract showed no pathological changes and were comparable to normal control rats.

According to the results obtained pancreatic section of normal rats shows normal cell structure and number. However, in diabetic rats, minute and reduced number of islet cells could be seen (Figure 6.4). Pancreatic section of treated rats with methanolic extract and Glibenclamide showed the normal cellular population and size (Figure 6.4).

Liver section of normal rat showed normal central vein, sinusoids and the epithelium lining (Figure 6.5). In diabetic rat, liver section showed abnormal hepatic architecture and necrosis. In addition congestion of sinusoids and cell death was also evident. From earlier literature, it is evident that type-2 diabetes causes degenerative structural changes in hepatic tissues as a result of depletion in insulin levels (Can et al., 2005). Diabetic rats treated with Glibenclamide and methanolic extract showed less necrosis and almost normal hepatic architecture as compared to diabetic control rats.

Histopathology of kidney revealed a normal tubular epithelial cells and normal glomeruli in control group (Figure 6.5). In diabetic rats, necrotic tubular epithelial cells, dark pyknotic nuclei and atrophy of the glomeruli was observed. These structural changes in kidneys is due to metabolic variation in diabetic condition (Rasch, 1980). Similarly, Floretto et al. (1998) has reported that streptozotocin has significant nephrotoxic potential. Treated group with methanolic extract and glibenclamide showed normal tubular epithelial cells and normal intertubular vessels indicating the healing effect of *R. aquatica* extract.

The improvement in the cell structure and number of pancreas, hepatic architecture of liver and renal parameters of kidney of diabetic induced rats after treatment with methanolic extract could be attributed to the ability of *R. aquatica*
to combat diabetic condition by increasing the inhibition of α-amylase and α-glucosidase. Thereafter reducing the glucose absorption and increasing the plasma insulin levels.

Figure 6.4: Histopathological section of pancreas of representative rats from respective group (hematoxylin-eosin staining). (A) G-1; (B) G-2; (C) G-3; (D) G-4; (E) G-5; (F) G-6, Magnification 40X
Figure 6.4: Histopathological section of Liver and kidney of representative rats from respective group (hematoxylin-eosin staining). Magnification 40X.
6.4. OUTCOME OF THE CHAPTER

- The aim of the present work was to evaluate the hypoglycemic activity and diabetic control efficiency of *Rotula aquatica* Lour.

- The preclinical studies showed the non-toxicity nature of methanolic extract and its remarkable effect on blood glucose level and marked improvement in lipid profile of diabetic rats.

- Improvement in superoxide dismutase, catalase and lipid peroxidase justifies the protective ability of the extract against oxidative stress.

- The histopathological studies revealed that in islets of langerhans, the structural deformities were observed in diabetic uncontrolled experimental rats.

- However, after treatment with *R. aquatica* extract, there was a change in the architecture of the islets of langerhans and β-cells were regenerated and restored and is documented with more number of β-cells than in the negative control without methanolic extract of *R. aquatica*.

- This is the significant observation made in the present investigation by documenting the results by histopathological studies.

- Overall the study justifies its use in ethnomedical potential for the treatment of diabetes.