Chapter 5:

Study on in vitro anti-diabetic properties
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5.1. INTRODUCTION

Diabetes mellitus is a leading metabolic disorder characterized by fasting and/or postprandial state hyperglycemia with altered carbohydrate, fat and protein metabolism resulting in absolute or relative insulin deficiency, resistance or both. Management of diabetes with minimal side-effect at a relatively low cost is still a challenging task.

The conventional available therapies for diabetes include stimulation of endogenous insulin secretion, enhancement of the action of insulin at the target tissues, oral hypoglycemic agents, such as biguanides and sulfonylureas and the inhibition of dietary starch degradation by glycosidases such as α-amylase and α-glucosidase (Rang et al., 2003). Despite the steady increase in the number of antidiabetic agents, the prevalence of the disorder remains stable may be due to the inconsistent efficacy of currently available drugs. Hence there is a requirement for new drugs to manage this metabolic disorder.

Over the last few decades the reputation of herbal remedies has increased globally due to its therapeutic efficacy and safety. 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.

Rotula aquatica, a member of family Boraginaceae has several medical applications especially for the treatment of many diseases like coughs, heart diseases, dysuria, blood disorders, fever, poisonings, ulcers and uterine diseases. The roots are bitter, astringent, cooling, diuretic and laxative (Warrier et al., 2002).
Inhibition of pancreatic α-amylase and α-glucosidase by plant extracts offer an attractive therapeutic approach for the treatment of postprandial hyperglycemia by decreasing glucose release from starch and delaying carbohydrate absorption in the small intestine (Kumavat et al., 2012; Lodha et al., 2010). Hence the study was carried out to analyze the pancreatic α-amylase and α-glucosidase inhibition ability of *R. aquatica* extract by *in-vitro* assays.

5.2. MATERIAL AND METHODS

5.2.1. Chemicals required

All chemicals and solvents used were of analytical grade. Pancreatic alpha amylase, dinitrosalicylic acid, acarabose were purchased from Sigma Chemical Co. (USA). Agappe Kit (Kerala). Starch from SRL.

5.2.2. Plant material and Preparation of Plant Extract

The plant, *Rotula aquatica*, collected from areas of Netravati river, during September (2010) dried extracted with methanol by cold maceration as described earlier.

5.2.3. Pancreatic α-amylase inhibition assay

The inhibition assay was performed by the chromogenic dinitrosalicylic acid (DNSA) method as described by Miller (1959), with slight modification. The assay mixture is composed of 500 μL of 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride), 0.25 units of porcine pancreatic alpha amylase (PPA) solution and extracts at 0.02 to 0.1 mg/mL (w/v) concentrations were pre-incubated at 37°C for 10 min. To the above buffer, 500 μL of 1% (w/v) starch solution was added to each tube and incubated at 37°C for 15 min. The reaction was terminated with 1.0 mL DNSA reagent, placed in boiling water bath for 5 min, cooled to room temperature, diluted and the absorbance measured at 540 nm. The control without plant extract represented 100% enzyme activity. The absorbance produced by plant extract was eliminated by including appropriate sample control.
with the extract in the reaction mixture except for the enzyme and starch. The known PPA inhibitor acarbose was used as positive control.

5.2.4. α-Glucosidase inhibition assay

The inhibition assay was performed according to the method of Andrade-Cetto with slight modifications (Andrade-Cetto et al., 2008). The assay was carried out by incubating a solution of starch substrate (2 % w/v maltose) 1 mL of 0.2 M Tris buffer pH 8.0 and various concentrations of plant extracts for 5 min at 37°C. The reaction was initiated by adding 1 mL of α-glucosidase enzyme (1U/mL) followed by incubation for 10 min at 37°C. The reaction mixture was heated for 2 min in boiling-water bath to stop the reaction. The quantity of glucose liberated was measured using GOD-POD kit. The control represented 100% enzyme activity without any plant extract. The absorbance produced by plant extract was eliminated by including appropriate sample controls with the extract in the reaction mixture except for the enzyme and substrate. The α-glucosidase inhibitor, acarbose was used as positive control. One unit of enzyme activity is defined as the amount of enzyme required to release one micromole of product from substrate per min under the assay conditions. The IC50 value is defined as that concentration of the extract containing the enzyme inhibitor which inhibits the activity by 50%.

The % inhibition was calculated as follows:

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\text{% Inhibition} = \frac{\text{EC} - (\text{ET} - \text{TC})}{\text{EC}}
\]

Where, EC is enzyme activity of control, ET is enzyme activity of test and TC is test control.

5.2.5. Statistical analysis

Data are expressed as the mean ± SD. The significance of the results was calculated using one-way ANOVA. The results were considered statistically significant at p<0.05.
5.3. RESULT AND DISCUSSION

Traditionally, herbal medicines are used to treat several disorders. However, inadequate knowledge of phytochemicals and their biochemical activities are lacking. In this regard, *Rotula aquatica* Lour, a medicinal herb used by ayurvedic practitioners was evaluated *in-vitro* inhibition of α-amylase and α-glucosidase, the two important enzymes that play a major role in diabetes.

5.3.1. Pancreatic alpha amylase and alpha-glucosidase inhibitors assay

Post-prandial hyperglycemia can be reduced via control of starch breakdown using pancreatic alpha amylase and alpha-glucosidase inhibitors. Hence to validate the hypoglycemic activity of *R. aquatica* extract, an *in-vitro* assay for alpha amylase and alpha glucosidase enzyme inhibition was performed.

According to the results obtained, the methanolic extract at a concentration of 800μg/mL is able to inhibit 50% of α-amylase activity and the extract displayed 50% inhibition of α-glucosidase at a concentration of 270 μg/mL (Figure 5.1). Absorption of glucose can be delayed by reducing the rate of starch digestion. Pancreatic α-amylase is a key enzyme in the digestive system and catalyses the initial step in hydrolysis of starch into mixture of smaller oligosaccharides. These are then acted on by α-glucosidases and are degraded to glucose that on absorption enters the blood stream. Dietary starch breakdown proceeds rapidly and leads to elevated postprandial hyperglycemia. Hence control of their activity is an important aspect in treatment of type-2 diabetes (Kim *et al.*, 2005; Matsui *et al.*, 2007). The main role of pancreatic α-amylase inhibitors is to delay carbohydrate digestion causing a reduction in the rate of glucose absorption and lowering the postprandial serum glucose levels. Glucosidase inhibitors are widely studied and isolated from different sources such as plants and microbes. Earlier investigators realized that inhibition of all or some of the intestinal disaccharidases and pancreatic α-amylase by inhibitors could regulate the absorption of carbohydrate and these inhibitors could be used therapeutically in the oral treatment of the noninsulin-dependent diabetes mellitus (type-2 diabetes) (Vijan *et al.*, 1997).
Inhibitors of α-glucosidase and pancreatic α-amylase can delay or prevent the complications of diabetes mellitus such as onset of renal, retinal, lens and neurological changes and the development of ischaemic myocardial lesions (Creutzfeldt, 1999). The present study reveals the potency of *R. aquatica* extract as inhibitors of α-amylase and α-glucosidase which offers an attractive therapeutic approach for the treatment of postprandial hyperglycemia by decreasing the release of glucose.

**Figure 5.1:** Inhibitory activity of *R. aquatica* on (a) Pancreatic α-amylase and (b) α-glucosidase. Values are mean ± SD
5.4. OUTCOME OF THE CHAPTER

- Herbal hypoglycemic agents are the major area of interest to provide better alternative option and avoid harmful side effects caused by prolonged intake of synthetic drugs.
- *In-vitro* analysis revealed that the methanolic extract of *R. aquatica* has significant inhibitory activity on α-amylase and α-glucosidase.
- The study revealed the potency of *R. aquatica* as a possible remedy for the treatment of hyperglycemia.