Evaluation of Effect of Rutin in Diabetes Rat Gastropathy
*S.K.Bais, Sanjay Shrirao, N.I.Kochar, #Avinash Jiddewar, R.L.Bakal

Department of Pharmacology, P.Wadhwani College of Pharmacy, Yavatmal-445001,India
# NSPM, College of Pharmacy,Darwha,Dist-Yavatmal,India

Received on: 25/04/2012 Accepted on: 14/06/2012

ABSTRACT
The antioxidant effect of Rutin was investigated in diabetic rats. The Alloxan monohydrate was used to induce the diabetes in normal rats. The animals were divided into two groups. The I\textsuperscript{st} group comprising control group treated with vehicle while in II\textsuperscript{nd} group Diabetes was induced. The Gastric emptying was evaluated by Phenol red assay method and Intestinal transit by Charcoal meal Method. The results indicated that 20 mg/kg and 40 mg/kg of Rutin improved gastric emptying and intestinal transit significantly and in dose dependent manner. The result was analyzed statistically. The present study revealed the usefulness and beneficial value of rutin in the treatment of disorder of gastric emptying and intestinal transit in diabetes gastropathy.

Key Words: Diabetes gastropathy, Gastric emptying, Intestinal transit time, Rutin

INTRODUCTION
The number of people with diabetes is increasing day by day; the main cause of this problem is aging, urbanisation and increasing privilege of obesity and physical inactivity. Quantifying the prevalence of diabetes and the number of people affected by diabetes, now and in the future it is important to have rational planning and allocation of resources towards treatment and prevention of this disease\cite{1,2}. Diabetes mellitus is a chronic metabolic disorder caused by an absolute or relative lack of insulin and or reduced insulin activity which results in hyperglycemia and abnormalities in carbohydrate, fat and protein metabolism\cite{3,4}. Diabetic gastropathy is a term that encompasses a number of neuromuscular dysfunctions of the stomach, including abnormalities of gastric contractility, tone, and myoelectrical activity in patients with diabetes. Gastrokinetic agents such as metoclopramide, cisapride, domperidone, and erythromycin increase fundic or antral contractions and/or eradicate gastric dysrhythmias. Diet and glucose control also are important in the management of diabetic gastropathy. As the pathophysiology of diabetic gastropathy is better understood, more specific and improved treatments will evolve.\cite{5}

Rutin\cite{6}\{2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[\alpha-L-rhamnopyranosyl-(1→6)-\beta-D-glucopyranosyloxy]-4H-chromen-4-one\}(Fig.1) is a potent antioxidant and anti-inflammatory agent that has the potential to provide far-reaching health benefits\cite{7}. It has been shown to be helpful in rheumatoid arthritis, inflammatory bowel disease, pancreatitis, Alzheimer’s disease, heart disease, diabetic retinopathy, and cancer\cite{8}. Rutin can protect against colon, intestinal, oral, and skin cancer\cite{9}. It has protective action against vascular dementia by exerting antioxidant activity.\cite{9} In addition, Rutin prevents diabetes-induced oxidative stress, reduces blood glucose and increase plasma insulin and act as gastroprotectant against irritants.

Rutin, also called rutoside, quercetin-3-O-rutinoside and sophorin, is the glycoside between the flavonol quercetin and the disaccharide rutinose \{\alpha-L-Rhamnopyranosyl-(1→6)-\beta-D-glucopyranosyloxy\}. In fava d'anta, the synthesis is done via a rutin synthase activity.\cite{10} Rutin is a citrus flavonoid glycoside found in buckwheat, the leaves and petioles of Rheum species and asparagus. Rutin is also found in the fruit of the fava d'anta tree (from Brazil), fruits and flowers of the pagoda tree, fruits and fruit rinds (especially citrus fruits (orange, grapefruit, lemon, lime) and berries such as mulberry, ash tree fruits and cranberries. Its name comes from the name of Ruta graveolens, a plant that also contains rutin. It is sometimes referred to as vitamin P, although not
strictly a vitamin. Rutin is one of the primary flavonols found in Clingstone peaches. Literature survey reveals that David A. Droppleman 1980 determined the effects of experimental compounds on gastric emptying in rats. The technique, using a methylcellulose-based test meal, is simple, versatile and reliable. Data are presented to show the utility of the method in demonstrating both enhancement and inhibition of gastric emptying. Shu-Chi Wang et al 2001, assessed gastric emptying and gastrointestinal transit in a rat uremia model. Chronic uremia was induced by five-sixths nephrectomy in the rats. After 20-hour fasting, the rats were loaded with 70 glass beads as solid markers through a gastric catheter. Narasimhan audi Kamalakannan et al 2006 studied Oral administration of rutin to diabetic rats significantly decreased fasting plasma glucose, glycosylated haemoglobin and increased insulin, C-peptide, haemoglobin and protein levels. The present study was undertaken to investigate the therapeutic potential of Rutin, with antioxidant activity.

MATERIALS AND METHODS

Animals
Sprague Dawley rats of either sex were used. They were maintained at 25 ± 2°C and relative humidity of 45 to 55% and under standard environmental conditions (12 h light: 12 h dark cycle). Animals were allowed to take specified amount of standard laboratory feed and water ad libitum. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) constituted as per the guidelines of CPCSEA

Drugs and Chemicals
Rutin crystalline was obtained from Viresh chemical, Mumbai. Alloxan was supplied by Loba Chemie,Mumbai and Diagnostic kits were supplied by Ambika Diagnostic.

Induction of Diabetes using Alloxan Rat Model
Rats were made diabetic by intraperitoneal injection of alloxan (hydrate) at a dose of 120 mg/kg. Alloxan was first weighed individually for each animal according to the body weight and solubilized with 0.5 ml of normal saline. It was then injected to 18 hr. fasted diabetic group rats to induce hyperglycemia and was maintained during the time of the study by the reinforcement of 100 mg/kg alloxan (ip) at day 12 and 21 after the first administration.

Collection of Blood Samples
All rats fasted for 18 h prior to the determination of blood glucose levels on day three, 15 and 57. During this time interval, 1.0 ml of blood was withdrawn from the retroorbital plexus under light ether anesthesia and centrifuged at 3000 rpm to separate plasma and cells. The plasma was used to estimate glucose levels.

Determination of Blood Glucose
The plasma glucose levels were estimated using the glucose oxidase-peroxidase (GOD-POD) method with the glucose GOD-POD kit.

Assessment of Gastrointestinal Parameters
Gastrointestinal Emptying
Gastric emptying was assessed by Phenol red Assay. The meal consisted of 1.5 or 2.25 ml of a solution of 50 mg phenol red in 100 ml 1.5% methylcellulose. It was constantly stirred and held at 37°C. The meal was administered by gavage and the animals were killed by a blow on the neck immediately or 20 min after administration of the meal. During these 20 min the animals were housed in small cages (length × width × height: 410 × 215 x 140 mm). After laparotomy, the stomach was quickly ligated at the lower esophageal and pyloric sphincter region and removed. The stomach and its content were homogenized in 100 ml 0.1 M NaOH. In short, 50 ml of the homogenized mixture was centrifuged (700 × g) for 20 min and 5 ml of the supernatant was added to 0.5 ml 20% trichloroacetic acid to precipitate the proteins. After vortexing and centrifugation (20 min, 2600 × g) 4 ml of the supernatant will added to 2 ml 1 M NaOH to develop the maximum intensity of the color. The solutions were colorimetrically assayed with an UVikon 930 spectrophotometer (Kontron Instruments) at 560 nm. Gastric emptying (%) will be calculated according to the following formula:

\[ \text{Gastric emptying} = \left( \frac{\text{Amount of phenol red recorded after 20 min}}{\text{Amount of phenol red administered}} \right) \times 100 \]

Intestinal Transit (By Charcoal meal Method)
The meal consisted of 1 ml of 10% charcoal in a 5% Arabic gum aqueous suspension and was constantly stirred and held at 37°C. The meal was administered by gavage and after 15 or 30 min the rats were killed by a blow on the neck. The small intestine was removed rapidly after laparotomy and the distance traveled by the charcoal front was measured and expressed as percentage of the total length of the small intestine.

\[ \% \text{ Intestinal Transit} = \left( \frac{\text{Distance travelled by charcoal meal \times 100}}{\text{Total length of small intestine}} \right) \]

RESULTS AND DISCUSSION

Blood Glucose Level
Blood glucose levels were expressed as mg/dl. The rats showing a fasting glucose of more than 170 mg/dl three days after the first administration of alloxan were considered diabetic.

Table 1 and figure 1 shows that significant increase in the blood glucose levels in the diabetic group at all-time intervals (i.e., day 3, 15 and 57; p < 0.0001) as compare to normal. After treatment of Rutin at both doses in diabetic rats it was significantly decreased (p < 0.0001) as compare to alloxan alone rat.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>0 day</th>
<th>3 day</th>
<th>15 day</th>
<th>57 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Normal</td>
<td>116.5±13.36</td>
<td>99.10±10.49</td>
<td>115.87±11.06</td>
<td>111.76±11.34</td>
</tr>
<tr>
<td>Normal+d1</td>
<td>118.02±14.07</td>
<td>104.04±10.35</td>
<td>111.81±13.35</td>
<td>108.38±9.79</td>
<td></td>
</tr>
<tr>
<td>Normal+d2</td>
<td>110.01±13.01</td>
<td>93.60±9.62</td>
<td>105.65±14.31</td>
<td>125.09±19.01</td>
<td></td>
</tr>
<tr>
<td>Diabetic</td>
<td>Alloxan</td>
<td>107.4±6.06</td>
<td>338.2±25.74</td>
<td>289.39±35.55</td>
<td>287.15±70.02***</td>
</tr>
<tr>
<td>Alloxan+d1</td>
<td>95.47±11.06</td>
<td>271.2±45.04</td>
<td>253.49±28.01</td>
<td>149.55±10.00***</td>
<td></td>
</tr>
<tr>
<td>Alloxan+d2</td>
<td>101.06±12.51</td>
<td>296.83±51.52</td>
<td>274.1±25.83</td>
<td>140.50±7.44***</td>
<td></td>
</tr>
</tbody>
</table>

n=6

Values are expressed as Mean±SEM.

** p<0.001 when compared with corresponding control group

***p<0.0001 when compared with corresponding control group

364
Gastric Emptying
Results of gastric emptying are summarised in Table-2. Figure 2 and 3 represents effect of Rutin on gastric emptying of Nondiabetic rat and diabetic rats respectively. Figure 4 represents effect of alloxan on gastric emptying of rat.
It was observed that Alloxan decreased the gastric emptying time (Fig.4) which was significantly increased in fig.3 in which 20 mg and 40 mg Rutin was administered. The comparative study clearly indicates in fig.3 that the gastric emptying was increased significantly.

Table 2: Gastric emptying and Intestinal Transit

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>% gastric emptying</th>
<th>% Intestinal transit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetic</td>
<td>Normal</td>
<td>83.05±1.214</td>
<td>59.57±0.5982</td>
</tr>
<tr>
<td></td>
<td>Normal+d1</td>
<td>98.25±0.4787</td>
<td>72.50±0.6455</td>
</tr>
<tr>
<td></td>
<td>Normal+d2</td>
<td>119.8±0.8539</td>
<td>78.50±0.6455</td>
</tr>
<tr>
<td>Diabetic</td>
<td>Alloxan alone</td>
<td>42.50±1.427***</td>
<td>19.16±0.8117*</td>
</tr>
<tr>
<td></td>
<td>Alloxan+d1</td>
<td>69.57±0.3460**</td>
<td>41.42±0.7377**</td>
</tr>
<tr>
<td></td>
<td>Alloxan+d2</td>
<td>81.96±0.8539***</td>
<td>49.08±0.6861***</td>
</tr>
</tbody>
</table>

n=6
Values are expressed as Mean±SEM
** p<0.001, when compared with corresponding control group
***p<0.0001, when compared with corresponding control group

Intestinal Transit
Results of intestinal transit are summarised in Table-2. Figure 7 represents significantly delayed intestinal transit in diabetic rat. In fig. 6, intestinal transit was significantly decreased in diabetic rat compared to normal rat, (***)p<0.0001).
Upon treatment with Rutin, it was increased significantly (***)p<0.0001).
Rutin improved intestinal transit time in diabetic rat with gastropathy when compared with the normal rat treated with vehicle. This effect may be mediated through potentiation of the peripheral cholinergic pathways in the enteric nervous system. The results indicated that 20 mg/kg and 40 mg/kg of Rutin improved gastric emptying time and intestinal transit significantly and in dose dependent manner.

CONCLUSION
In the present study, Rutin improved gastric emptying time and intestinal transit in diabetic rat with gastropathy when compared with the normal rat treated with vehicle. This effect may be mediated through potentiation of the peripheral cholinergic pathways in the enteric nervous system. It is well known that many gastrointestinal peptides participate in the regulation of gastrointestinal functions.

ACKNOWLEDGEMENTS
I am thankful to Prof.Dr.A.V.Chandewar, Principal, P.Wadhwani College of Pharmacy, Yavatmal-445001 for providing me kind support and academic environment which resulted in completion of this project.

REFERENCES
12) http://www.herbalextractplus.com/rutin.cfm
14) Sha-Chi Wang1, Kuo-Yun Lu1, Shu-Ming Chen2 and Tze-Kong Young, “Gastric Emptying and Intestinal Transit of Liquid and Solid Markers in Rats with Chronic Uremia” Chinese Journal of Physiology, 44(2): 81-87, 2001
15) Narasimhanandai Kamalakkannan and Ponnaian Stanley Mainzen Prince Antihyperglycaemic and Antioxidant Effect of Rutina Polyphenolic Flavonoid, in Streptozotocin-Induced DiabeticWistar Rats, Basic & Clinical Pharmacology & Toxicology, 2006, 98:97–103