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(Research Article)

Evaluation of Effect of Rutin in Diabetes Rat Gastropathy

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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 Ist group comprising control group treated with vehicle while in IInd 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[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[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.[5]

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

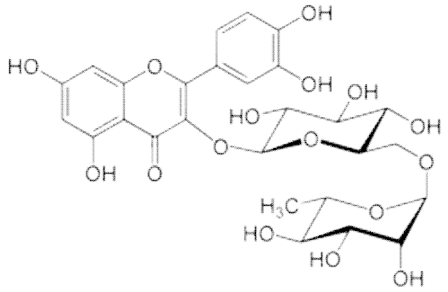

Fig.1: Structure of Rutin

Rutin, also called rutoside, quercetin-3-O-rutinoside and sophorin, is the glycoside between the flavonol quercetin and the disaccharide rutinose {α-L-Rhamnopyranosyl-(1→6))-β-D-glucopyranose}. In fava d'anta, the synthesis is done via a rutin synthase activity.[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[11,12]

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.[13] 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.[14] Narasimhanaidu Kamalakkannan et al 2006 studied Oral administration of rutin to diabetic rats significantly decreased fasting plasma glucose, glycosylated haemoglobin and increased insulin, C-peptide, haemoglobin andprotein levels.[15]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.[16]

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 retrorbital plexus under light ether anesthesia and centrifuged at 3000 rpm to separate plasma and cells. The plasma was used to estimate glucose levels.[17]

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 assesed by Phenol red Assay[18]. 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 ram). 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:

$$1-\left(\frac{\text{Amount of phenol red recovered after 20 mn}}{\text{Amount of phenol red recovered after 0 mn}}\right) X100$$

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.[19]

$$\% \text{ Intestinal Transit} = \frac{\text{Distance traveled by charcoal meal}}{\text{Total length of small intestine}} x100$$

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.[20]

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 1: Blood glucose level of Non-diabetic and diabetic rat

Groups	Treatment	0 day	3 day	15 day	57 day
Non-diabetic	Normal	116.5±13.36	99.10±10.49	115.87±11.06	111.76±11.34
	Normal+d1	118.02±14.07	104.04±10.35	111.81±13.51	108.38±9.79
	Normal+d2	110.01±13.01	93.40±9.62	105.65±14.31	123.09±19.01
Diabetic	Alloxan alone	107.4±6.06	338.2±25.74	289.39±43.55	287.15±70.02***
	Alloxan+d1	95.47±11.06	271.2±45.04	253.49±28.01	149.55±9.00**
	Alloxan+d2	101.06±12.51	296.83±51.52	274.1±25.83	140.50±7.44***

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

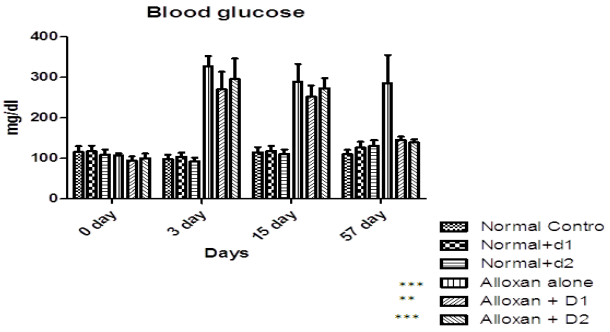

Figure 1: Blood glucose of Nondiabetic & diabetic rat

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

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

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

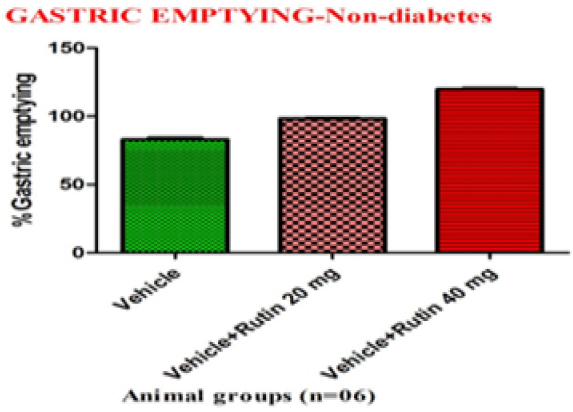

Figure 2: Effect of Rutin on gastric emptying of Nondiabetic rat.

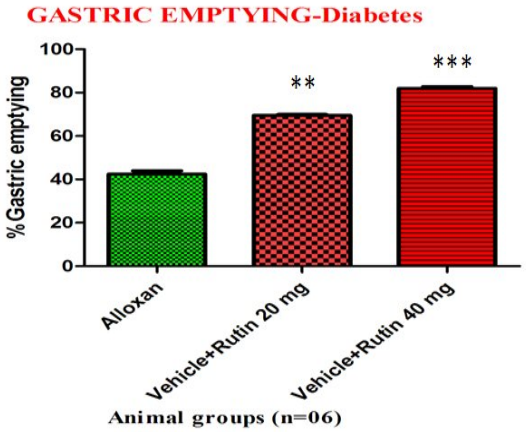

Figure 3: Effect of Rutin on gastric emptying of diabetic rat.

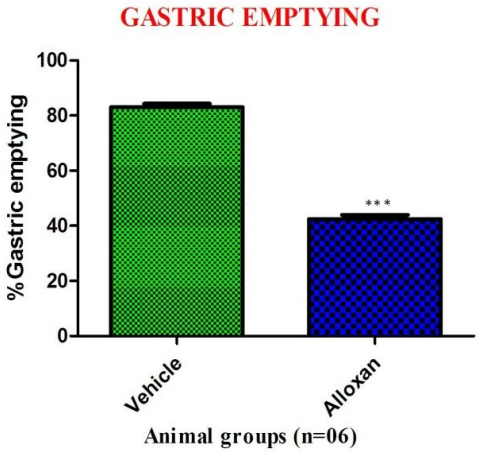

Figure 4: Effect of alloxan on gastric emptying of rat.

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).

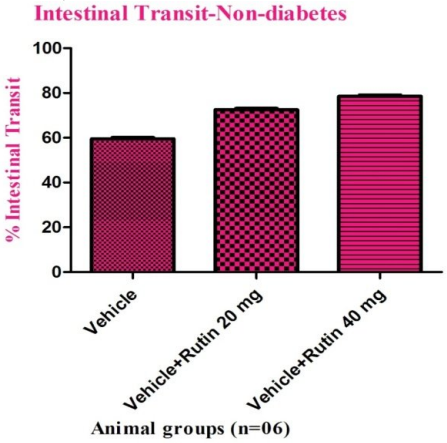

Figure 5: Effect of Rutin on intestinal transit of Nondiabetic rat.

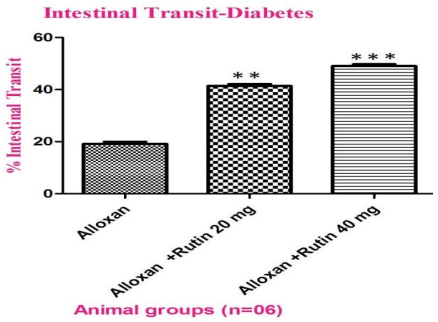

Figure 6: Effect of Rutin on intestinal transit of diabetic rat.

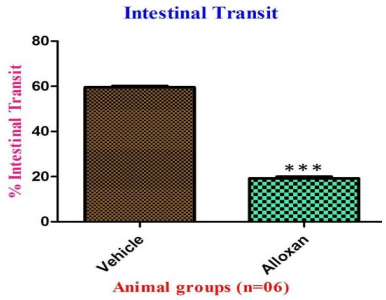

Figure 7: Effect of alloxan on intestinal transit of normal rat

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 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.

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REFERENCES

1) Champion M, "Gastroparesis" Med North A, 1991; 4:2524-31.
2) Feldman. M, Schiller L.R, "Disorders of gastrointestinal motility associated with diabetes mellitus"AnnIntern Med,1983,98:378-84.
3) Horowitz M, Harding. P.E, et al, "Gastric and oesophageal emptying in patients with type 2 (non-insulin-dependent) diabetes mellitus" Diabetologia, 1989, 32:151-59.
4) Weisberg SP, Leibel R, Tortoriello DV. "Dietary curcumin significantly improves obesity-associated inflammation and diabetes in mouse models of diabesity" Endocrinology, 2008: 3549-58.
5) Weiss JS, Sumpio BE. "Review of prevalence and outcome of vascular disease in patients with diabetes mellitus". European Journal of Vascular and Endovascular Surgery, February 2006, 31(2):143- 50.
6) Navarro-Núñez, Palomo, et al "Inhibits Platelet Adhesion and Thrombus Formation and Synergizes with Aspirin in the Suppression of the Arachidonic Acid Pathway", J. Agric. Food Chem, 2008, 56 (9): 2970–6.
7) Guardia, Juarez AO, Pelzer LE, "Anti-inflammatory properties of plant flavonoids. Effects of rutin, quercetin and hesperidin on adjuvant arthritis in rat". Il Farmaco 2001, 56 (9) : 683–7.
8) Chan Hun Jung, Cho, Chul Hyung; Kim, Chang Jong, "Anti-asthmatic action of quercetin and rutin in conscious guinea-pigs challenged with aerosolized ovalbumin" Arch. Pharmacal Research, 2007, 30 (12): 1599–1607.
9) Ihme N, Kiesewetter H, et al., Leg oedema protection from a buckwheat herb tea in patients with chronic venous insufficiency: a single-centre, randomised, double-blind, placebo-controlled clinical trial, European Journal of Clinical Pharmacology, 2008, 50(6):443-447.
10) Kreft S, Knapp M, Kreft I, "Extraction of rutin from buckwheat (Fagopyrum esculentum Moench) seeds and determination by capillary electrophoresis". J. Agric. Food Chem. November 1999, 47 (11) : 4649–52.
11) Susan Chang, Christine Tan, et al "Low-Density Lipoprotein Antioxidant Activity of Phenolic Compounds and Polyphenol Oxidase Activity in Selected Clingstone Peach Cultivars". J. Agric. Food Chem., 2000, 48:147−151
12) http://www.herbalextractsplus.com/rutin.cfm
13) David A. Droppleman, Robert L. Gregory, Reevis S. Alphin, "A simplified method for assessing drug effects on gastricemptying in rats" Journal of Pharmacological Methods,1980, 4, (3): 227–230
14) Shu-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) Narasimhanaidu Kamalakkannan and Ponnaian Stanely Mainzen Prince ,Antihyperglycaemic and Antioxidant Effect of Rutin,a Polyphenolic Flavonoid, in Streptozotocin-Induced DiabeticWistar Rats, Basic & Clinical Pharmacology & Toxicology, 2006, 98:97–103
16) Watkins CC, Sawa A, Jaffrey S, et al, Insulin restores neuronal nitric oxide synthase expression and function that is lost in diabetic gastropathy, J Clin Invest, 2000, 106: 373–384.
17) Winter BY, Bredenoord AJ, et al, Effect of inhibition of inducible nitric oxide synthase and guanylyl cyclase on endotoxin-induced delay in gastric emptying and intestinal transit in mice, Shock, 2002, 18:125–131
18) Sorg DA, Buckner B., A simple method of obtaining venous blood from small laboratory animals, Proc Soc expbiol Med, 1964, 115:1131–1132.
19) Trinder P, "Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen" J Clin Pathol, 1969, 22:158–161.
20) Sharma SS, Gupta YK, Effects of antioxidants on cisplatin-induced delay in gastric emptying in rats, Environ Pharmacol Toxicol 1997, 3:41-46.

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