Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 2, 2012

Research Article

HYPOGLYCEMIC AND ANTIDIABETIC ACTIVITY OF FLAVONOIDS: BOSWELLIC ACID, ELLAGIC ACID, QUERCETIN, RUTIN ON STREPTOZOTOCIN-NICOTINAMIDE INDUCED TYPE 2 DIABETIC RATS

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Received: 1 Nov 2011, Revised and Accepted: 22 Dec 2011

ABSTRACT

The aim of the current study was to investigate the oral antidiabetic activity and antihyperlipidemic activity of four flavonoids: Boswellic acid (1), Ellagic acid (2), Quercetin (3), Rutin (4). Normoglycemic and STZ-nicotinamide induced diabetic rats were treated with these flavonoids with 50 mg/kg, 100mg/kg and the hypoglycemic and antidiabetic effects in acute experiments were determined. After 14 days of administration to STZ-nicotinamide induced diabetic rats, flavonoids induced a significantly diminishing of total cholesterol, Triglyceride compared with the control group ($p < 0.001$). To find out the probable mechanism of action of selected flavonoids as a antidiabetic agents i) glucose transport inhibition activity, and ii) glucose uptake by isolated rat hemi-diaphragm were estimated. Selected flavonoids increased the uptake of glucose by rat hemi-diaphragm significantly ($P<0.001$), There was a significant decrease in glucose transport activity ($P<0.05$. Compounds 4, 3, and 2 were found most active in both experiments in comparison with control group ($p < 0.001$).

Keywords: Diabetes mellitus (DM), Flavonoids, Hypoglycemic activity, Antidiabetic activity, OGTT, Hemidiaphragm, Glucose transporter.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from the defects in insulin secretion, insulin action or both. A chronic hyperglycemic condition in diabetes is associated with long term damage, dysfunction, and failure of various organs, such as eyes, kidneys, nerves, heart, and blood vessels. It is the most common serious metabolic disorder and is considered to be one of the five leading causes of death in the world[1]. DM is classified into two major categories: type 1 and type 2 diabetes[2]. Type 2 DM is a chronic and progressive syndrome characterized by metabolic abnormalities such as insulin resistance and decreased pancreatic b-cell function that modifies fuel-sensing processes in the body[3]. Although both types of diabetes have distinct pathogenesis, hyperglycemia, and various life threatening complications are common to both[4]. The plasma lipids are usually raised in diabetes and such an elevation represents a risk factor for coronary heart disease. Despite lifestyle modification as first-line approach for early stage diabetic patients, type 2 DM treatment through drug prescription remains to be the major successfully approach to improve this condition. Thus, there are several classes of approved oral antidiabetic drugs. However, most of them exert undesirable side effects, drug interactions and the treatment is expensive since antidiabetic therapies comprise polypharmacy regimen[5]. Natural products and their derivatives have been a successfully source of bioactive molecules in medicines much before the advancement of other modern therapeutics in the post-genomic era[6]. One of most studied natural products has been flavonoids which are found predominantly in several comestible fruits as part of daily food consumption[7]. These polyphenolic compounds had been widely investigated in recent years due to their beneficial properties in cardiovascular disease among their pharmacological effects as anti-inflammatory, antioxidant, antiviral and anticarcinogenic agents[8]. With these reports it was suggested that flavonoids are molecules capable to interact with more than one target, which allow defined them as privileged structures in accordance with Patchett's definition[9]. Moreover, recent investigations have demonstrated that flavonoids are very promising antidiabetic agents[10]. Currently, the National Institutes of Health Clinical Center is investigating the use of quercetin on glucose absorption in obesity, and obesity with type 2 diabetes patients on oral glucose tolerance test[11]. The aim of present study was to determine the acute hypoglycemic and antidiabetic activity of flavonoids in normoglycemic and STZ-nicotinamide induced diabetic rat models through blood glucose and serum lipid profile measurements.

MATERIALS AND METHODS

Chemicals

Glibenclamide, nicotinamide, streptozotocin (STZ), were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Flavonoids: Boswellic acid, Ellagic acid, Quercetin, Rutin were purchased from Yucca Enterprises-Mumbai. Test kits for enzymatic end point evaluation for glucose (GOD-POD), Total Cholesterol, were procured from M/s Excel Diagnostics Pvt. Ltd. Hyderabad, India. Triglycerides, SGOT (Serum glutamate oxaloacetate transaminase) and SGPT (Serum glutamate pyruvate transaminase) were purchased from Crest Biosystems (Dr.Antonio Do Rego Bagh, Goa, India).

Animals

Albino wistar rats (170-200) were purchased from Mahaveer Enterprises (CPCSEA Regd.No: 146/1999/CPCSEA), Medipalli Hyderabad. The animals were acclimatized to the conditions by maintaining them at the experimental conditions for about 7 days prior to dosing. The animals were fed under standard diet and water *ad libitum* maintained under standard laboratory conditions. The temperature 25±2˚C and relative humidity 55±10 at 12 hour each at dark and light cycle were maintained. All the protocols were performed in accordance with the Institutional Animal Ethical Committee (Regd.No: 1047/ac/07/CPCSEA) as per the directions of the CPCSEA.

Acute oral toxicity study

Acute oral toxicity of flavonoids (Boswellic acid, Ellagic acid, Quercetin, Rutin) was determined using Albino wistar rats (170-200). Rats were grouped (n=3) and fasted for 18 hours prior to the experiment and were administrated single dose of selected flavonoids, and observed with special attention during the first four hours for mortality and daily thereafter, for a total of 14 days. Based on the short term toxicity, the dose of the test animals were determined as per OECD guidelines 423.

Streptozotocin-nicotinamide induced diabetic rats

Streptozotocin (STZ) was dissolved in citrate buffer (pH 4.5) and nicotinamide in normal physiological saline solution (0.9% NaCl solution). Diabetes was induced in overnight fasted rats by a single intraperitoneal (i.p.) injection of 65 mg/kg of STZ, 15 min after the i.p. administration of 110 mg/kg of nicotinamide[12]. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined seven days after diabetes induction. Animals with blood glucose concentrations more than 150 mg/dL were used for the study.

Experimental protocol

In the experiment a total number of 42 animals belong to Wistar strain of albino rats (36 diabetes induced and 6 normal) were used. Animals were divided into 7 groups, each group comprised of 6 rats. Test samples were suspended in 5% of Acacia in distilled water (vehicle) and were administered orally by intragastric (i.g.) route using an i.g. tube. Treatment was started with 50mg/kg dose initially, after proper washing 100mg/kg dose were given. Rats were divided into the following groups[13].

Group I: Normoglycemic control. Received only vehicle at a dose of 1ml/100g

Group II: Diabetic Standard group. Glibenclamide was given at a dose of 10mg/kg

Group III: Diabetic Control. Received only 5% acacia (vehicle)

Group IV: Normoglycemic treated with Boswellic acid. Initially Boswellic acid was given at a dose of 50mg/kg, after proper washing 100mg/kg dose were given

Group V: Normoglycemic treated with Ellagic acid. Initially Ellagic acid was given at a dose of 50mg/kg, after proper washing 100mg/kg dose were given

Group VI: Normoglycemic treated with Quercetin. Initially Quercetin was given at a dose of 50mg/kg, after proper washing 100mg/kg dose were given

Group VII: Normoglycemic treated with Rutin. Initially Rutin was given at a dose of 50mg/kg, after proper washing 100mg/kg dose were given.

Acute experimental model

A protocol above described was used to evaluate hypoglycemic and antidiabetic effect. Sixteen hours before the experiments rats were fasted with free access to water. The fasting blood glucose levels of all rats were determined before the start of the experiment. Blood samples were collected from the retro-orbital plexus of each rat under mild anesthesia at 0h, 1st h, 2nd h, 4th h, 6th h, 8th h after test compound, Glibenclamide administration and serum glucose was estimated by GOD-POD method, and glucose levels (mg/dL) were calculated[14].

Evaluation of flavonoids in glucose tolerance test

Before induction of diabetes healthy rats were divided into five groups of six animals each: Group I served as control received only vehicle (5% acacia) and Groups II, III, IV and V received Boswellic acid, Ellagic acid, Quercetin and Rutin respectively at the dose of 100 mg/kg[15]. The animals were fasted overnight before commencing the experiment. All rats were loaded with 2 g/kg, p.o., d-glucose solution (S.D. Fine-Chem. Ltd, Mumbai) after 0.5 h of drug administration. Blood samples were collected by the retro orbital sinus puncture (ROP) method just prior to drug administration and 30, 60, and 120 min after glucose loading. Serum glucose level was measured immediately[16].

Biochemical Profile

Blood samples were centrifuged at 3000 rpm for 15 min at room temperature. Then, serum was collected for the respective analytical determinations. The serum Total Cholesterol, Triglyceride, SGOT and SGPT concentrations were determined using commercial kits by enzymatic photocolorimetric methods[17].

Study of glucose uptake mechanism using rat hemi-diaphragm technique

Glucose uptake by rat hemi-diaphragm was estimated by the methods described by Walaas and Chattopadhyay *et al.,* with some modification[18]. Twelve sets containing three numbers of graduated test tubes (n=3) each, were taken as follows:

Group 1: 2 ml of Tyrode solution with 2% glucose.

Group 2: 2 ml of Tyrode solution with 2% glucose and regular insulin (Nova Nordisk) 0.62 ml of 0.4 units per ml solution.

Group 3: 2 ml of Tyrode solution with 2% glucose and metformin (0.1%)

Group 4: 2 ml of Tyrode solution with 2% glucose and regular insulin 0.62 ml of 0.4 units per ml solution and metformin (0.1%)

Group 5: 2 ml of Tyrode solution with 2% glucose and 1.38 ml of Boswellic acid solution (0.1%)

Group 6: 2 ml of Tyrode solution with 2% glucose and regular insulin 0.62 ml of 0.4 units per ml solution and 1.38 ml of Boswellic acid solution (0.1%).

Group 7: 2 ml of Tyrode solution with 2% glucose and 1.38 ml of Ellagic acid solution (0.1%)

Group 8: 2 ml of Tyrode solution with 2% glucose and regular insulin 0.62 ml of 0.4 units per ml solution and 1.38 ml of Ellagic acid solution (0.1%).

Group 9: 2 ml of Tyrode solution with 2% glucose and 1.38 ml of Quercetin solution (0.1%)

Group 10: 2 ml of Tyrode solution with 2% glucose and regular insulin 0.62 ml of 0.4 units per ml solution and 1.38 ml of Quercetin solution (0.1%).

Group 11: 2 ml of Tyrode solution with 2% glucose and 1.38 ml of Rutin solution (0.1%)

Group 12: 2 ml of Tyrode solution with 2% glucose and regular insulin 0.62 ml of 0.4 units per ml solution and 1.38 ml of Rutin solution (0.1%).

The volumes of all the test tubes were made up to 4 ml with distilled water to match the volumes of the test tubes of Group 4. Rats were fasted overnight and killed by decapitation. The diaphragms were dissected out quickly with minimal trauma and divided into two halves. Two diaphragms from the same animal were not used for the same set of experiment. Three numbers of diaphragms were used for each group. The hemi-diaphragms were placed in test tubes and incubated for 30 min at 37^{o}C, with a gas phase of O_2+ CO_2 (95:5) with shaking at 140 cycles/min. Glucose uptake of tissue was calculated as the difference between the initial and final glucose content in the incubated medium[19].

Glucose Transport inhibitor activity

Healthy rats were divided into five groups of six animals each: Group I served as control received glucose (3.62mg/ml) and Groups II, III, IV and V received flavonoids: Boswellic acid, Ellagic acid, Quercetin, Rutin, at the dose of 3.62mg/ml. Before each experiment, the animals were starved for twelve hours but allowed for tap water use. Rats were sacrificed by cervical dislocation. The abdomen was opened by a midline incision. The entire small intestine was removed quickly by cutting across the upper end of the duodenum and the lower end of the ileum, and by stripping the mesentery manually. The small intestine was then washed out with normal saline solution (0.9% w/v Nacl) using a syringe equipped with blunt end. Intestinal segments (10±2 cm) were taken according to the method described by Wilson & Wiseman[20]. The sacs were filled with 0.5 ml of the incubation medium (serosal fluid) and were placed in 25 ml Erlenmeyer flasks with 5 ml of the same medium (mucosal fluid). After oxygenation of the flasks with 100% O_2 for 1 min, they were tightly stoppered and kept in a shaker (90-110 oscillations/min) for 1 h at room temperature. The incubation medium was a Krebs-Henseleit bicarbonate buffer (KHB). The composition of the buffer was (mM/L): $NaHCO_3$ 25; NaCl 118; KCl 4.7; $MgSO_4$ 1.2; $CaCl_2$ 1.2; and Na_2EDTA 9.7 mg/L. For studying the effect of the flavonoids on the transport of glucose (substrate), glucose (3.62) was added into mucosal compartment fluid. The flavonoids were also added in the same compartment (3.62 mg/ml).At the end of the incubation period (120 min); the sacs were removed from the flask. The fluid in these flasks was used for estimation of glucose. Glucose concentrations were measured using a commercially available glucose oxidase kit (M/s Excel Diagnostics Pvt. Ltd). The rise in glucose in serosal fluid was indicating that the glucose transported in the flask from mucosal fluid[21].

Statistical Analysis

Data are expressed as the Mean±SD for the number (n= 6) of animals in each group. Graphs were plotted and statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparisons. P-Values less than 0.05 were considered to be statistically significant.

RESULTS

Acute oral toxicity study

In acute toxicity study, Flavonoids: Boswellic acid, Ellagic acid, Quercetin and Rutin treated animals did not show any change in their behavioral pattern. There was no significant difference in the body weights and food consumption when compared to the vehicle treated group. Also, no gross pathological changes were seen. Thus, it was concluded these Flavonoids: Boswellic acid, Ellagic acid, Quercetin and Rutin was safe at 2000 mg/kg.

Acute hypoglycemic activity

Figure 1 shows that the oral administration of flavonoids at the dose of 100mg/kg induced a significant decrease in plasma glucose levels in normoglycemic rats ($p<0.001$) during acute time periods compared with control group. Rutin and Quercetin showed the best activity in the 2 h and 4 h respectively, compared with control group ($p<0.001$). 100mg/kg dose found to be more effective than 50mg/kg dose. Rutin, the most active flavonoid, showed sugar lowering activity profile comparable to standard drug glibenclamide.

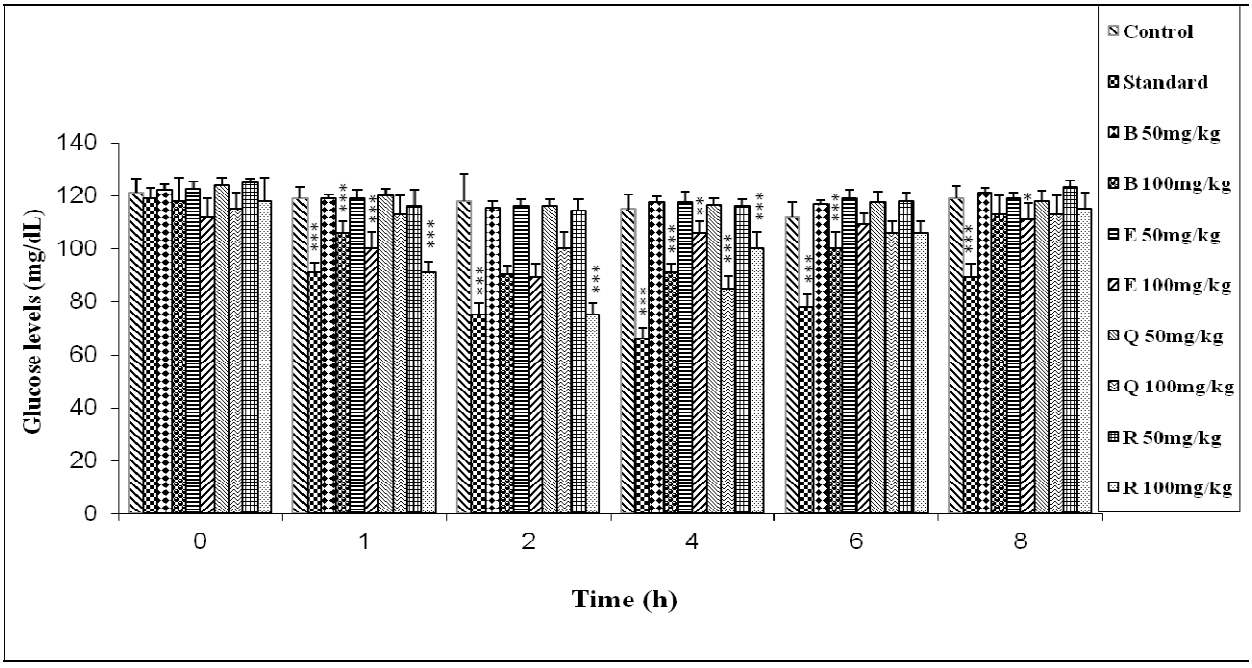

Fig. 1: shows acute *in vivo* hypoglycemic activity of flavonoids at 50mg and100mg/kg of dose

Values represent Mean±SD (n=6 in each group; *p<0.05 as compared to control group, **p<0.01 as compared to control group, ***p<0.001 as compared to control group).

Glucose tolerance test

As shown in figure 2 flavonoids: Boswellic acid, Ellagic acid, Quercetin, Rutin when administered 30 min. prior to glucose loading produced significant reduction ($P < 0.05$) in the rise in blood glucose levels at 30 min. after glucose administration. Flavonoids: Boswellic acid, Ellagic acid, Quercetin, Rutin at dose of 100 mg/kg produced 5.45%, 6.13%, 3.41% and 9.53% reduction in blood glucose respectively when compared to vehicle treated group at 30 min.

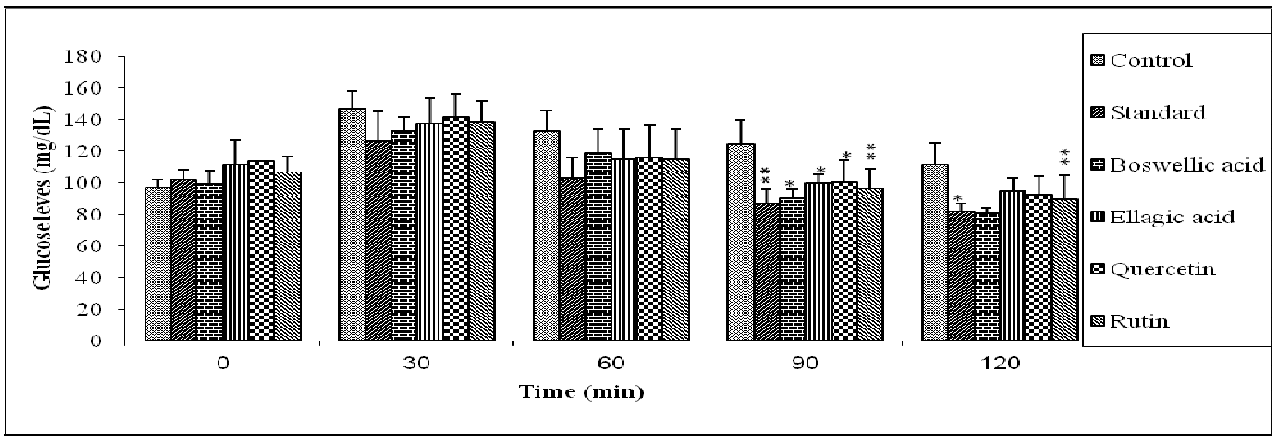

Fig. 2: showing glucose tolerance test with 100mg/kg dose of flavonoids

Values represent Mean±SD (n=3) in each group; *p<0.05 as compared to control group, **p<0.01as compared to control group.

Antihyperglycemic activity

In diabetic rats, figure 3 revealed that the acute oral administration of four flavonoids (50 mg/kg & 100mg/kg) significantly reduce in plasma glucose levels compared with diabetic control group ($p<0.001$). 100mg/kg dose found to be more effective than 50mg/kg dose. Again, Rutin was the most active compound compared with antidiabetic effect showed by glibenclamide (10mg/kg).

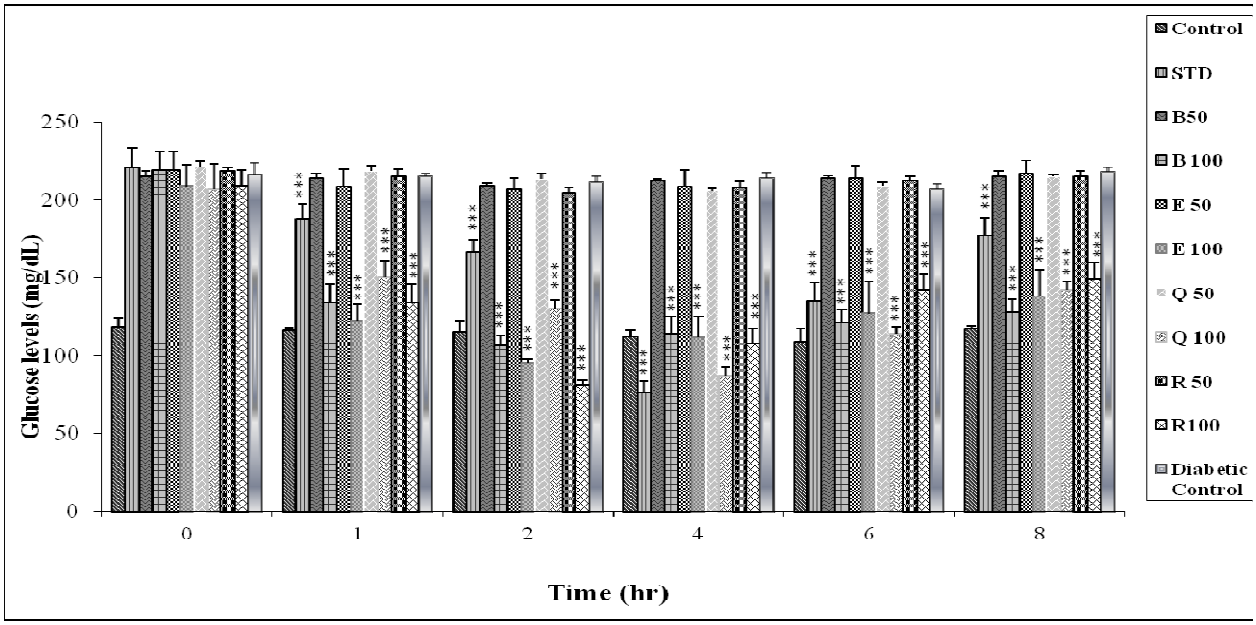

Fig. 3: shows antidiabetic activity with 50mg/kg and 100mg/kg dose of flavonoids

Values represent Mean±SD; ***p<0.001 as compared to diabetic control group.

Antihyperlipidemic activity

The concentrations of fasting serum triglyceride, cholesterol were analyzed in all groups. Serum triglyceride, cholesterol levels in diabetic control group (without treatment) were significantly higher than control group (normoglycemic untreated group). As shown in figure 4, serum levels of cholesterol tend to be reduced in all diabetic groups treated with flavonoids, On the other hand, all groups of STZ-nicotinamide induced diabetic rats treated with flavonoids significantly showed lowered triglycerides levels compared to the untreated diabetic rats, and also turn to the normal levels compared with normoglycemic group.

Estimation of SGPT and SGOT

Serum SGOT and SGPT levels in diabetic control group (without treatment) were significantly higher than control group (normoglycemic untreated group). Figure 5 shows SGOT and SGPT levels were significantly ($p<0.001$) decreased after oral dose with 100mg/kg dose of Boswellic acid, Ellagic acid, Quercetin, Rutin.

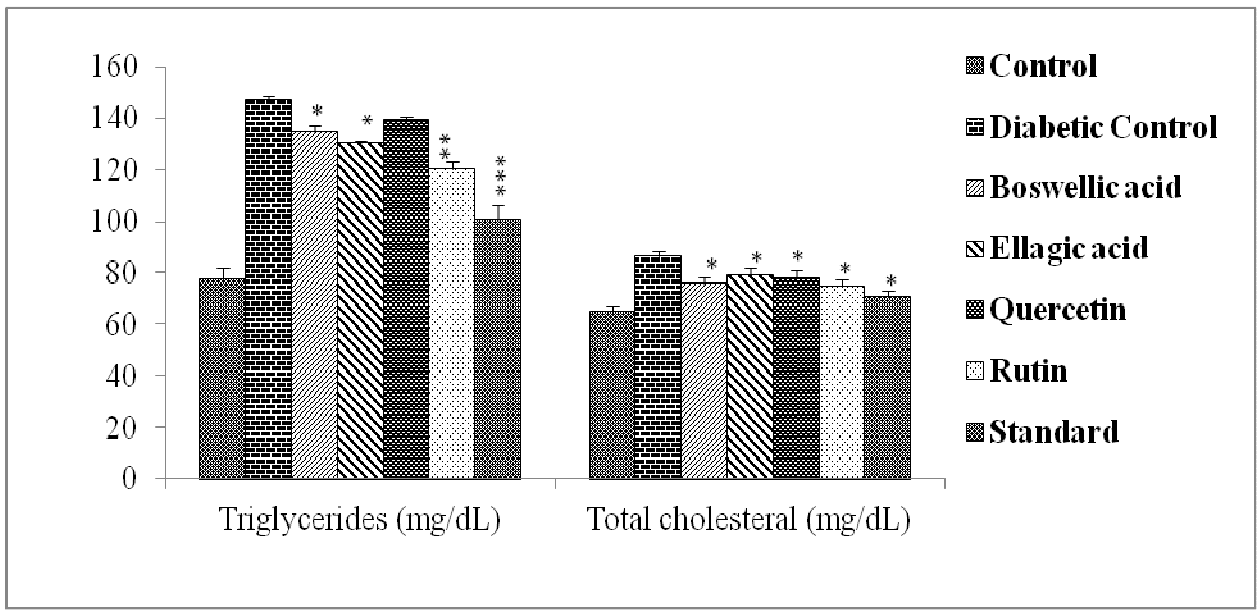

Fig. 4: Shows effect of flavonoids on Triglycerides, Total cholesterol in Streptozotocin-nicotinamide induced diabetic rats

Values represent Mean±SD (n=6 in each group); ***p<0.001 as compared to diabetic control group, **p<0.01 as compared to diabetic control group.

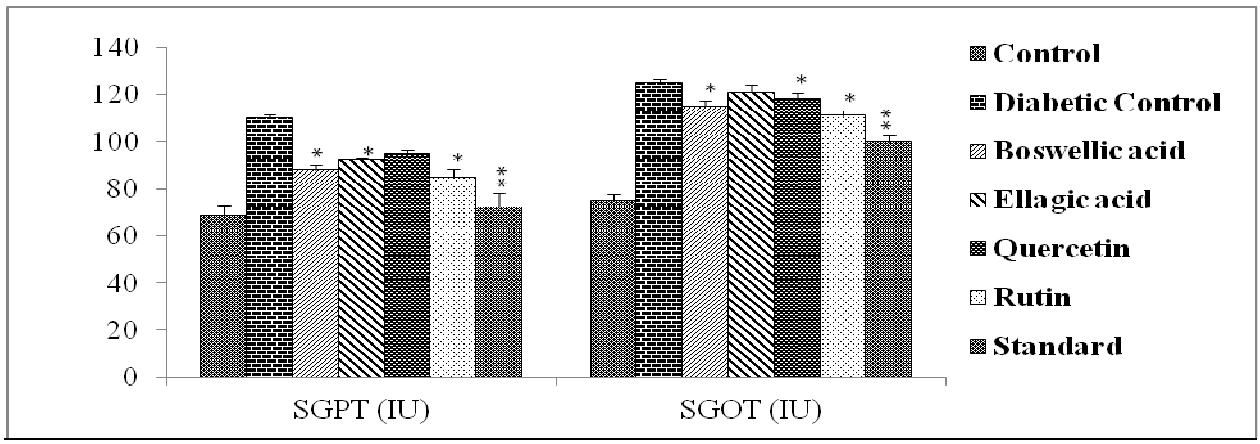

Fig. 5: Shows effect of flavonoids on SGOT and SGPT in Streptozotocin-nicotinamide induced diabetic rats

Values represent Mean±SD (n=6 in each group); **p<0.01 as compared to diabetic control group, *p<0.05 as compared to diabetic control group.

Glucose uptake mechanism using rat hemi-diaphragm technique

From the figure 6 it was evident that percentage glucose uptake by rat hemidiaphragm in the absence of insulin was found to be more for Rutin (27.28%) and Quercetin (17.65%) than Boswellic acid (12.5%) and Ellagic acid (4.35%), which indicates that these compounds showed glucose uptake activity comparable to that of standard metformin (28%).

Glucose Transporter inhibitor activity

As shown in figure 7, Incubation of the rat intestinal sacs with flavonoids resulted in the inhibition of transport of glucose. It was found that the percentage glucose transport inhibition by rat intestinal sacs was found to be more for Boswellic acid (50.28%) and Ellagic acid (43.14%) than Rutin (41.33%) and Quercetin (27.48%), which indicates that these compounds showed glucose transport inhibition activity comparable to that of control (glucose).

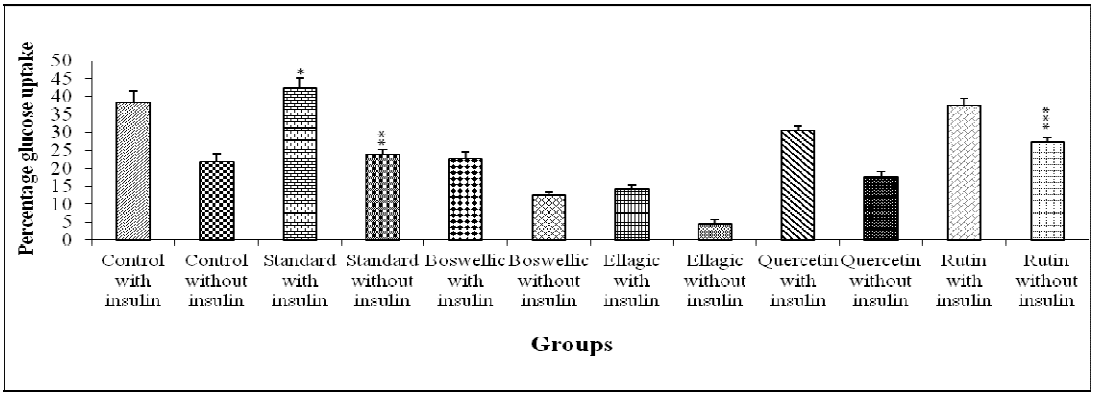

Fig. 6: Showing percentage glucose uptake of flavonoid compounds by rat hemidiaphragm

Values represent Mean±SD (n=6 in each group); *** p<0.001 as compared to diabetic control group, **p<0.01 as compared to diabetic control group, *p<0.05 as compared to diabetic control group.

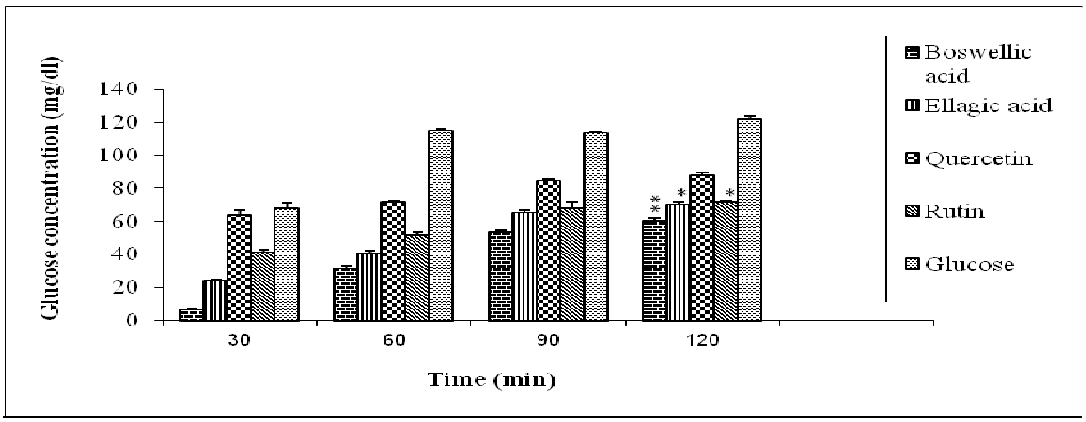

Fig. 7: Shows Glucose Transport inhibitor activity of flavonoid.

Values represent Mean±SD (n=6 in each group); *** p<0.001 as compared to diabetic control group, *p<0.05 as compared to diabetic control group.

DISCUSSION

Many traditional plant treatments for diabetes mellitus are used throughout the world. Management of diabetes without any side effect is still a challenge to the medical system. This has led to an increasing demand for natural products with antidiabetic activity and fever side effects. From literature review it was found that many herbs and plant products have been shown to have hypoglycemic action. Flavonoids are known to be bioactive antidiabetic principles. The results of the *in vivo* study that was undertaken in normal rats for hypoglycemic effect of flavonoid compounds: Boswellic acid, Ellagic acid, Quercetin, and Rutin reveals that maximum reduction in the blood glucose levels occurred at 2 h except for quercetin. For quercetin the maximum reduction in the blood glucose levels occurred at 4 h. The study results showed that Rutin has more activity when compared to other three flavonoids. The proposed mechanism of action may be by increasing the peripheral utilization of glucose and inhibiting the glucose transporter activity from intestine. The order of hypoglycemic activity observed was Rutin> Quercetin >Ellagic acid> Boswellic acid. An *in vivo* study in streptozotocin-nicotinamide induced diabetic rats and normal rats reveals that the flavonoid compounds: Boswellic acid, Ellagic acid, Quercetin, and Rutin, significantly reduced fasting blood glucose levels in both normal and diabetic rats as compared to control group. Rutin and Quercetin found to be more active than Boswellic acid and Ellagic acid. It is clear from the results that selected compounds increase glucose uptake by rat hemidiafragm suggesting that possible mechanism of action could be enhancing peripheral glucose utilization either by direct stimulation of glucose uptake or via the mediation of enhanced insulin secretion and inhibiting the glucose transporter activity.

CONCLUSION

The present investigation revealed that the blood glucose lowering activity of flavonoid compounds: Boswellic acid, Ellagic acid, Quercetin, and Rutin may be by stimulating β-cells to release more insulin. The pronounced activity of these compounds could be because of enhanced peripheral glucose utilization by skeletal muscle in addition to that of β-cell stimulation. The present experiment was conducted to evaluate comparative hypoglycemic, antidiabetic and antihyperlipidemic activity, in comparison Rutin and Quercetin found to be more effective antidiabetic compounds. However, other mechanisms of action related with the antidiabetic action cannot be described, and are the subject for future studies

ACKNOWLEDGEMENT

We are thankful to AICTE for providing stipend in time and management of Vaagdevi College of Pharmacy for lending its facilities.

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