Indian Journal of Experimental Biology
Vol. 51, January 2013, pp. 65-72

Pharmacological evaluation of hyperin for antihyperglycemic activity and effect on lipid profile in diabetic rats

Neeraj Verma[1], G Amresh[1], P K Sahu[2], Neelam Mishra[2], Ch V Rao[3] & Anil Pratap Singh[3*]

[1]Department of Pharmacology, Goel Institute of Pharmacy & Sciences,
Faizabad Road (Near Indira Canal), Lucknow 227 105, India
[2]Department of Pharmacology, School of Pharmaceutical Sciences, Siksha 'O' Anusandhan University,
Kalinga Nagar, Ghatikia, Bhubaneswar 751 003, India
[3]Pharmacognosy and Ethnopharmacology Division, CSIR-National Botanical Research Institute,
Rana Pratap Marg, P.O. Box No. 436, Lucknow 226 001, India

Received 5 March 2012; revised 3 September 2012

Antihyperglycemic potential of hyperin at 25 and 50 mg/kg doses for 30 days to streptozotocin induced diabetic rats has been reported. In oral glucose tolerance test, hyperin treated rats showed a significant reduction in blood glucose level after 120 min. It was found that hyperin exhibited dose dependent and significant antihyperglycemic activity in streptozotocin induced diabetic rats which were nearly similar with standard drug glybenclamide. Activities of glucose-6-phosphatase, fructose-1,6-bisphosphatase, glycogen phosphorylase, glycosylated haemoglobin and level of serum urea and creatinine were significantly decreased in hyperin supplemented diabetic rats, dose dependently. Activities of hexokinase and glycogen synthase were increased with augmentation in liver glycogen, insulin and haemoglobin content in hyperin treated diabetic rats. General hematological parameters did not show any significant change in hyperin treated diabetic rats hence it is safe at these doses. Histopathological studies showed significant morphological changes in pancreatic β-cells of streptozotocin induced diabetic rats. A decreased number of secretory granules of β- cells were observed in diabetic rats and these pathological abnormalities were normalized after treatment with hyperin and standard drug glybenclamide. Further, hyperin decreases significant in serum total cholesterol, triglyceride, low density lipoprotein, very low density lipoprotein levels coupled with elevation of high density lipoprotein in diabetic rats. These results suggest that hyperin has a pivotal role in blood glucose level in streptozotocin induced hyperglycemia by improving the function of pancreatic islets and increasing glycolysis and decreasing gluconeogenesis.

Keywords: Anti-hyperglycemic activity, Hyperin, Insulin, *Rhododendron arboreum*

Hyperin, a flavonoid is an active phytochemical constituent present in various plants including *Hypericum perforatum, Drosera rotundifolia*, *Stachys byzantina*, *Prunella vulgaris*, *Rumex acetosella*, *Abelmoschus manihot* and *Rhododendron arboreum*. Hyperin may have a protective effect on cultured PC12 cells against cytotoxicity induced by hydrogen peroxide and *tert*-butyl hydroperoxide[1]. Hyperin, isoquercitrin and quercetin isolated from ethyl acetate fraction of the root of *Acanthopanax chiisanensis* inhibit lipopolysaccharide-induced nitrite production in rat peritoneal macrophages[2]. As an important bioactive compound, hyperoside (hyperin) has been documented to possess antiviral activity[3,4], antinociceptive[5-7], anti-inflammatory[8], cardioprotective[9-11], hepatoprotective[12–14], and gastricmucosal-protective effect[15,16]. Flowers of *Rhododendron arboreum* showed anti-nociceptive and anti-inflammatory activity[17]. Aqueous methanolic extract of *Rhododendron arboreum* showed *in- vitro* α-glucosidase inhibitory and antidiabetic activity[18]. Ethyl acetate fraction of *Rhododendron arboreum* has hepatoprotective[19], antidiarrhoeal effect[20], and antihyperglycemic, antihyperlipidemic activity[21]. However, there is no experimental evidence presently available in the literature with regard to its effect on plasma glucose in streptozotocin-induced diabetes mellitus in rats. Hence, the present study has been carried out to investigate the possible antidiabetic activity of hyperin, isolated from the flowers of *Rhododendron arboreum* Smith in STZ-diabetic rats.

Materials and Methods

Hyperin—Hyperin was isolated as described earlier[17]. Briefly, air dried powdered flowers of *R. arboreum* were extracted with ethanol (50 %)

*Correspondent author
Telephone: + 91- 522 2205831
Fax: + 91- 522 2205836.
E-mail: anilneeraj@gmail.com

using soxhlet apparatus. The extract was then evaporated under vacuum and afford the ethanolic extract of flowers. Extract thus obtained was partitioned with organic solvents afford the *n*-hexane, chloroform, ethyl acetate and *n*-butanol fractions. Ethyl acetate fraction was applied to a Sephadex-LH 20 column (300 g) eluted with a gradient of ethyl acetate/methanol (70:30) to give 4 fractions. Fraction 2 was submitted to HPLC separation (RP-18; MeOH/H2O 72:28; flow 9 mL/min; UV detection at 280 nm) gave compound hyperin (Fig. 1).

Chemicals—Streptozotocin and glybenclamide were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Rat insulin ELISA kit was purchased from Crystal Chem Inc. (Downers Grove, US). All reagents were of analytical grade.

Experimental animals—Male Wistar rats (150−200 g) were kept in the departmental animal house of National Botanical Research Institute (NBRI), Lucknow at 25±3 °C and 50±5 % RH, 12:12 h light and dark cycles respectively for 1 week before and during the experiments. They were allowed free access to standard rat feed and water *ad libitum*. All the studies were performed in accordance with the guidelines for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee, CPCSEA, India (Reg. No.222/2000/CPCSEA).

Acute toxicity study—Healthy adult male Wistar rats starved overnight were divided into 4 groups of 6 rats each and were orally fed with increasing dose levels of 25, 50, 100 and 200 mg/kg body wt of hyperin[22]. Rats were observed continuously for 2 h for behavioural, neurological and autonomic profiles and after 72 h for any lethality[23].

Induction of diabetes—Diabetes was induced in overnight fasted rats by a single *intra peritoneal* injection of 60 mg/kg body wt. streptozotocin (STZ), dissolved in ice cold citrate buffer (0.1 M, *p*H 4.5)[24]. Hyperglycemia was confirmed by the elevated glucose level (> 300 mg/dL) in the blood. All the animals were allowed free access to water and pellet diet and maintained at room temperature (25±3 °C) in plastic cages.

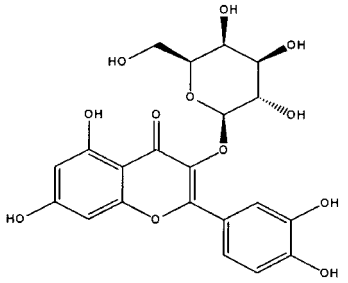

Fig. 1—Structure of hyperin.

Oral glucose tolerance test (OGTT)—Effective dose of hyperin was determined by oral glucose tolerance test performed in overnight fasted (18 h) normal Wistar rats. They were divided into 4 groups containing 6 animals each. Initial serum glucose was estimated by collecting the blood from tail vein. Normal control rats (Group 1) were given glucose (2 g/kg body wt) orally. Two different doses (25 and 50 mg/kg body wt) of hyperin in distilled water were administered orally 30 min before oral administration of 2 g/kg glucose solution to group 2 and 3 respectively. Group 4 received standard drug glybenclamide (20 mg /kg) before glucose load. Blood samples were collected from the tail vein at 0, 30, 60 and 120 min after the glucose loading and serum glucose levels were estimated[25].

Evaluation of antihyperglycemic activity of hyperin—Rats were divided into following 5 groups of 6 rats in each.

Group 1 (NC): normal control rats treated with distilled water
Group 2 (DC): diabetic control rats treated with distilled water
Group 3 (DH1): diabetic rats treated with 25 mg/kg of hyperin
Group 4 (DH2): diabetic rats treated with 50 mg/kg of hyperin
Group 5 (DG): diabetic rats treated with 20 mg/kg of glybenclamide

Blood samples from the experimental rats were collected by retro-orbital plexus technique using heparinised capillary glass tubes. Blood glucose was measured by GOD-POD method. Plasma insulin level was measured by using rat insulin ELISA kit. Body weights of all the animals were recorded prior and after the treatment.

Estimation of hematological and biochemical parameters—Rats were killed by cervical dislocation at the end of experiment. Blood was collected from the animal both in EDTA coated tubes and simple glass tubes (for separation of serum). Blood collected in EDTA coated tubes were analysed for white and red blood counts (WBC and RBC), haemoglobin (Hb), mean corpuscular volume (MCV) and haemocrit (HCT) on Hemato Analyser. Glycosylated haemoglobin (HbA1c) was estimated following the methods of Eross *et al*[26]. Serum was analysed for

urea, creatinine on a fully automatic clinical biochemical analyser. Serum total cholesterol, HDL cholesterol and TG were estimated according to the standard methods[27]. LDL cholesterol and VLDL cholesterol were calculated by using Friedewald formula[28].

Estimation of enzymes and glycogen content in liver—Liver homogenate (10 %) in hot ethanol (80 %) was centrifuged at 8000 rpm for 20 min. Residue was collected and allowed to dry over a water bath. To the residue, 5 mL of distilled water and 6 mL of 52 % perchloric acid were added. Extraction was done at 0 °C for 20 min, centrifuged at 8000 rpm for 15 min and supernatant was collected. To 1 mL of 5-fold diluted supernatant, 4 mL of freshly prepared anthrone reagent was added and heated on a boiling water bath for 20 min. Absorbance was recorded at 630 nm, a standard curve was also plotted with glucose solution[29]. Activities of hepatic hexokinase, glucose-6-phosphatase and fructose-1,6-bisphosphatase were assayed[30]. Glycogen synthetase and phosphorylase activities were assayed by the method of Leloir & Goldenberg[31] and Cornblath *et al.*[32] respectively.

Histological sample preparation—After sacrifice, the body of pancreas was dissected, collected and fixed in 10 % Neutral Buffered Formalin (NBF). Samples were processed in graded series of alcohol and embedded in paraffin wax, sectioned at 5 μm and stained with hematoxylin and eosin (H & E) for histological examination.

Statistical Analysis—Experimental results were expressed as mean ± SE and statistical comparison was done using one-way ANOVA followed by Duncan's multiple range test (DMRT). *P* values ≤ 0.05 were considered significant.

Results

Acute toxicity study—Acute toxicity studies revealed the non-toxic nature of hyperin from the ethyl acetate fraction of the ethanolic extract of *Rhododendron arboreum.* There were no lethality or toxic reactions found at any doses selected until the end of study.

Effect of hyperin on oral glucose tolerance test (OGTT) in non-diabetic rats—Peak blood glucose level reached at 30 min after glucose administration and greatest reduction in blood glucose was observed at 120 min in hyperin and glybenclamide treated rats (Fig. 2).

Antihyperglycemic Activity of hyperin—Blood glucose levels were significantly ($P < 0.05$) increased in diabetic rats as compare to normal rats. Treatment with hyperin at the dose of 25 and 50 mg/kg body wt. showed significant ($P < 0.01 - < 0.001$) reduction from 306.5 ± 5.4 to 195.3 ± 9.9 and 312.4 ± 3.2 to 108.6 ± 7.7 mg/dL respectively in fasting blood glucose level after 30 days treatment in STZ induced diabetic rats. Standard drug glybenclamide also showed significant ($P < 0.001$) reduction from 319.5 ± 7.8 to102.9 ± 6.9 mg/dL in fasting blood glucose level (Fig. 3).

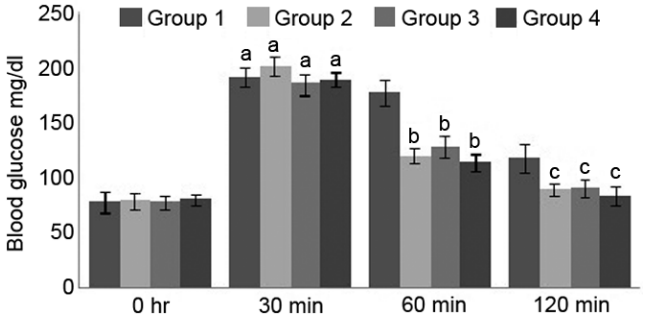

Fig. 2—Effect of different doses of hyperin on blood glucose level. Group 1: normal control rats, Group 2: normal rats administered with hyperin 25 mg/kg, Group 3: normal rats administered with hyperin 50 mg/kg Group 4: normal rats administered with glybenclamide 20 mg/kg. [a:*P*< 0.05; b:*P*< 0.01; c:*P*< 0.001 represents the significant change as compared to respective group. All values (n=6) represent mean ± SE; ANOVA followed by Duncan's multiple range test].

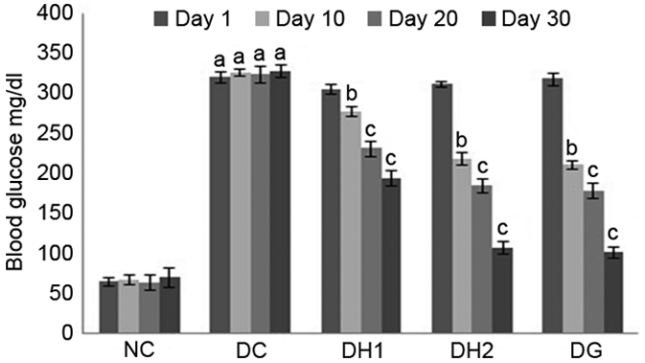

Fig. 3—Effect of different doses of hyperin on blood glucose level after 30 days treatment. NC: normal control rats; DC: diabetic control rats; DH1: diabetic rats treated with 25 mg/kg body weight of hyperin; DH2: diabetic rats treated with 50 mg/kg body weight of hyperin; DG: diabetic rats treated with 20 mg/kg body weight of glybenclamide.[a:*P*< 0.05 represents the significant change as compared to NC (normal control), b:*P*< 0.01; c*P*< 0.001 represents the significant change as compared to DC (diabetic control). All values (n=6) represent mean ± SE; ANOVA].

Antihyperlipidemic activity of hyperin—Dose dependent effect of the hyperin on the levels of serum total cholesterol, lipoproteins and triglycerides in normal and experimental diabetic rats is presented in Figure 4. Levels of total cholesterol, LDL-cholesterol, VLDL-cholesterol and triglycerides were significantly increased; whereas the level of HDL-cholesterol was significantly decreased in diabetic rats compared to those in normal. Administration of the hyperin at a dose of 50 mg/kg body wt. to diabetic rats for 30 days significantly reduced total cholesterol to 65.8 ± 6.5 mg/dL LDL-cholesterol to 13.2 ± 5.4 mg/dL and VLDL-cholesterol 8.7 ± 4.1 mg/dL compared with diabetic control rats. The same dose level in diabetic rats significantly increased the HDL cholesterol to 43.8 ± 1.9 mg/dL compared with diabetic control rats. Levels of triglycerides were significantly higher (77.1 ± 7.8 mg/dL) in diabetic rats compared to normal rats (48.7 ± 5.6 mg/dL). Treatment with the hyperin to diabetic rats at a dose of 50 mg/kg body wt. has resulted in a significant ($P < 0.0001$) decrease in the triglycerides levels 43.6 ± 7.9 mg/dL compared with diabetic control rats (Fig. 4).

Effect of hyperin on haematological and biochemical parameters to assess toxicity—Serum urea and creatinine are indicators of kidney functions. In the present study, serum urea and creatinine levels were found to be significantly higher in diabetic rats which decreased in hyperin treated rats. All hematological parameters were within the normal range throughout the study hence it was found to be safe at the studied doses (Table 1).

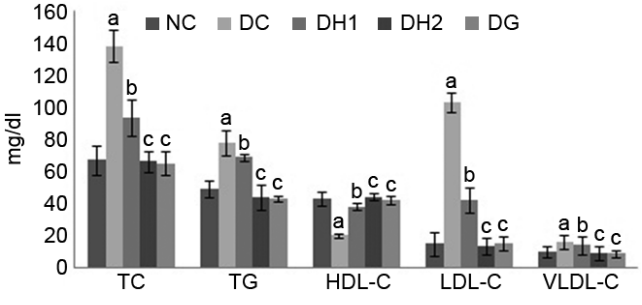

Fig. 4—Effect of different doses of hyperin on serum total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C). NC: Normal control rats, DC: Diabetic control rats, DH1: Diabetic rats treated with 25 mg/kg body wt of hyperin, DH2: Diabetic rats treated with 50 mg/kg body wt of hyperin, DG: Diabetic rats treated with 20 mg/kg body wt of glybenclamide [*P* values: a < 0.05 significant change as compared to NC (normal control), b < 0.01; c < 0.001 significant change as compared to DC (diabetic control). Values are mean±SE from 6 observations each; ANOVA].

Effect of hyperin on carbohydrate metabolizing enzymes—Hepatic glycogen content in diabetic rats was decreased which was restored to nearly control levels in hyperin supplemented diabetic rats. Decrease in glycogen synthase activity and concomitant increase in activity of glycogen phosphorylase was observed in diabetic rats and it was normalised after treatment. There was a significant decrease in the activity of hexokinase in diabetic rats compared to normal rats which were significantly increased in hyperin as well as glybenclamide treated diabetic rats. A significant increase in the activities of glucose-6-phosphatase and fructose-1,6-bisphosphatase was observed in diabetic rats compared to normal rats. Hyperin and glybenclamide treated diabetic rats were restored to near normal activities significantly (Table 2).

Histological study of pancreas in normal and diabetic treated rats—Pancreatic histology was observed in diabetic rats as shown in Fig. 5. The pancreas of diabetic rats receiving vehicle showed apparently irregular shaped islets of Langerhans crowded with small oval-shaped cells. All groups of diabetic rats treated with hyperin showed improvement of pancreatic histological features. In addition, there was a dose-dependent histological response in which increasing doses of the hyperin led to a rounder-shaped islets of Langerhans as well as larger and rounder cells within the islets of Langerhans than those of diabetic rats receiving vehicle.

Discussion

Prevalence of diabetes is currently increasing rapidly world-wide and it is the 16th leading cause of mortality[33]. Numerous studies demonstrated that a

Table 1—Effect of hyperin on hematological parameters to assess toxicity after 30 days treatment

[Values are mean ± SE from 6 observation each]

Groups	WBC (M/mm^3)	RBC (M/mm^3)	Hb (mg/dL)	MCV (Fl)	HCT (%)
NC	8.3 ± 2.1	8.3 ± 4.1	13.2 ± 2.9	61.3 ± 4.3	38.3 ± 3.5
DC	8.6 ± 3.3	6.7 ± 3.2	10.1± 3.7	67.2 ± 5.3	39.5 ± 3.2
DH1	9.3 ± 2.7	7.5 ± 2.9	13.6 ± 2.3	65.8 ± 6.1	37.2 ± 4.2
DH2	7.7 ± 4.5	7.9 ± 1.9	13.8 ± 4.1	70.1 ± 3.9	39.8 ± 2.1
DG	6.8 ± .4.1	7.4 ± 2.7	14.3 ± 2.1	69.5 ± 5.6	46.2 ± 5.7

NC= normal control; DC= diabetic control; DH1= diabetic rats treated with 25 mg/kg hyperin; DH2= diabetic rats treated with 50 mg/kg hyperin; DG= diabetic rats treated with 20 mg/kg of glybenclamide

variety of plant extracts had the hypoglycaemic and hypolipidemic effects[34]. Ethyl acetate fraction of *Rhododendron arboreum* flowers possess the antihyperglycemic and antihyperlipidemic activity[21]. Results showed in OGTT, administration of hyperin and standard drug glybenclamide cause significant reduction in blood glucose levels from 120 min onwards in glucose loaded normal rats compared to control. The hyperin enhanced glucose utilization, so the blood glucose level was significantly decreased in glucose-loaded rats. The results indicate that hyperin at a dose of 25 mg/kg and 50 mg/kg body wt possess significant glucose lowering activity after 30 days treatment in STZ- induced diabetic rats. Studies done to assess the safety profile of the hyperin had no adverse effect on haematological as well as kidney markers. Acute toxicity study also showed that there was no grass evidence of any abnormalities or mortality in rats up to the end of the observation period at the studied dose. Conversion of glucose to glycogen in the liver cells is dependent on the extracellular glucose concentration and on the availability of insulin which stimulates glycogen synthesis over a wide range of glucose concentration[35]. Regulation of glycogen metabolism *in-vivo* occurs by the multifunctional enzyme glycogen synthase and glycogen phosphorylase that play a major role in glycogen metabolism. Reduced glycogen store in the diabetic rats has been attributed to reduced activity of glycogen synthase[36] and increased activity of glycogen phosphorylase[37]. In the present study the experimental diabetic rats treated with hyperin at different doses and standard drug glybenclamide restored the levels of hepatic glycogen by means of decreasing activity of glycogen phosphorylase and increasing the activity of glycogen synthase.

One of the key enzymes in the catabolism of glucose is hexokinase, which phosphorylates glucose and converts it into glucose-6-phosphate[38]. Activity of this enzyme decreased in the liver of STZ-induced diabetic rats. Administration of hyperin to STZ-induced rats resulted in an increased activity of liver hexokinase. Increased activity of hexokinase can cause increased glycolysis and increased utilization of glucose for energy production. Decrease in the concentration of blood glucose in hyperin treated rats may be due to increased glycolysis (increased liver hexokinase activity). Activities of hepatic glucose-6-phosphatase and fructose-1,6-bisphosphatase were increased significantly in diabetic rats[39] , glucose-6-phosphatase and fructose-1,6-bisphosphatase are the regulatory enzymes in gluconeogenic pathway. Administration of hyperin and glybenclamide significantly decreased the activities of gluconeogenic enzymes in diabetic rats. Levels of plasma insulin was found to increase significantly in diabetic rats treated with hyperin, which may be a consequence for the significant reduction in the level of gluconeogenic enzymes. Reduction in the activities of gluconeogenic enzymes can result in the decreased concentration of glucose in blood. Histopathology results reveals that

Table 2—Effect of 25 and 50 mg/kg body wt. of hyperin on glycogen content, % HbA1c, insulin, kidney parameters and enzymes involved in regulation of carbohydrate metabolism after 30 days treatment.

[Values are mean ± SE from 6 observation each]

Group	Hepatic glycogen (mg glucose equiva-lents/g wet tissue)	Glycosylated haemoglobin (% HbA1c)	Insulin (µU/mL)	Urea (mg/dL)	Creatinine (mg/dL)	Glycogen synthetase (µ mole of UDP formed/hr/mg protein)	Glycogen phosphorylase (µ mole of phosphate liberate/hr/mg protein)	Glucose-6-Phosphatase (U/mg protein)	Hexokinase (U/g protein)	Fructose-1, 6-(bisphosphatase U/mg protein)
NC	6.7±2.1	7.9 ±2.4	25.6±4.3	30.1±2.8	0.5 ±0.03	830.1 ±42.8	651.3 ±23.2	0.19 ±0.09	167.1 ±6.7	0.45±0.04
DC	3.4±1.7[a]	14.7±2.1[a]	8.6±2.3[a]	59.5 ±2.1[a]	0.8 ±0.09[a]	559.5 ±32.1[a]	823.5 ±12.6[a]	0.28 ±0.04[a]	98.2 ±6.9[a]	0.76 ±0.05[a]
DH1	4.8±1.8[b]	10.9±2.5[b]	18.6±3.5[b]	46.2 ±3.4[b]	0.6 ±0.04[b]	646.2 ±27.4[b]	731.8 ±31.3[b]	0.22 ±0.05[b]	116.4 ±2.7[b]	0.54 ±0.05[b]
DH2	6.6±2.3[c]	7.7±1.7[c]	21.4±5.1[c]	36.3 ±4.5[c]	0.4 ±0.05[c]	836.3 ±64.5[c]	678.8 ±25.7[c]	0.15 ±0.06[c]	153.1 ±7.5[c]	0.41 ±0.07[c]
DG	6.4±2.7[c]	8.1±1.9[c]	22.3±4.9[c]	38.1±3.5[c]	0.5 ±0.07[c]	838.1 ±53.5[c]	717.9 ±27.7[c]	0.14 ±0.04[b]	161.2 ±6.6[b]	0.37 ±0.06[b]

NC= normal control; DC= diabetic control; DH1= diabetic rats treated with 25 mg/kg hyperin; DH2= diabetic rats treated with 50 mg/kg hyperin; DG= diabetic rats treated with 20 mg/kg of glybenclamide

P values: [a] < 0.01 as compared to NC; [b] < 0.001; [c] < 0.0001 as compared to DC

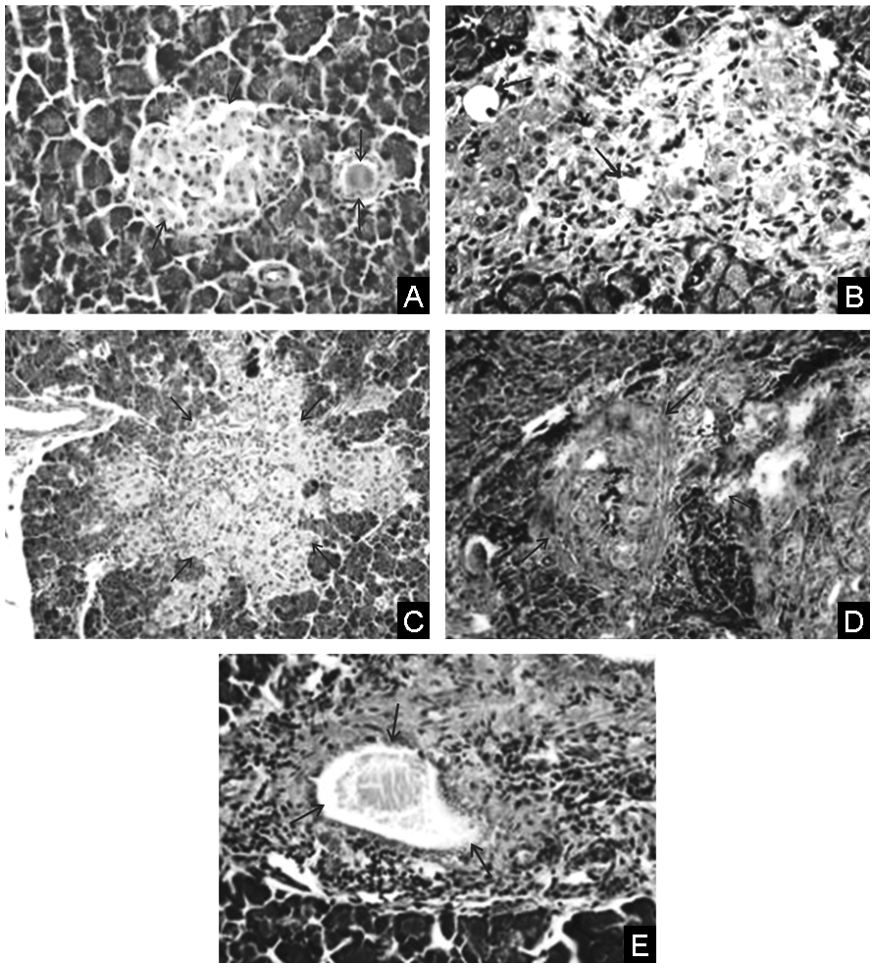

Fig. 5—Histopathology of pancreas:[A]-pancreas of NC animal showing normal histology; [B]-pancreas of DC animal showing severe congestion of pancreatic parenchymal cells, infiltration of inflammatory cells and hyperplasia of islets cell; [C]-pancreas of DH1 animal treated with 25 mg/kg of hyperin showing mild hyperplasia of islets cell and congestion of parenchymal cells; [D]-pancreas of DH2 animal treated with 50 mg/kg of hyperin showing moderate hyperplasia of islets cell and congestion of parenchymal cells; [E]-pancreas of DG animal treated with 20 mg/kg of glybenclamide showing normal histology.

diabetic group treated with hyperin at different doses, an increase in the number of β-cells in the islets shows that they were regenerated. Increase in secretory granules in the cells also indicates that the cells were stimulated for insulin synthesis.

Conclusion

It may be concluded that hyperin from ethyl acetate fraction of *Rhododendron arboreum* flowers possesses vital effect in diabetes mellitus. These effects are, at least in part mediated by increased glycolysis, insulin secretion and decreased gluconeogenesis stimulated by hyperin suggests the possible biochemical mechanism. Thus, hyperin may be the lead for the design and synthesis of more efficacious and safer analogues.

Acknowledgement

Thanks are due to DST, New Delhi for financial assistance.

References

1 Zhiyong L, Xinyi T, Chongwei Z, Yanhua L & Dongzhi W, Protective effects of hyperoside (quercetin-3-o-galactoside) to PC12 cells against cytotoxicity induced by hydrogen peroxide and *tert*-butyl hydroperoxide, *Biomed Pharmacother*, 59 (2005) 481.

2 Lee S, Park H S, Notsu Y, Ban H S, Kim Y P, Ishihara K, Hirasawa N, Jung S H, Lee Y S, Lim S S, Park E H, Shin K H, Seyama T, Hong J & Ohuchi K, Effects of hyperin, isoquercitrin and quercetin on lipopolysaccharide-induced nitrite production in rat peritoneal macrophages, *Phytother Res*, 22 (2008) 1552.

3 Shahat A A, Pieters L, Apers S, Nazeif N M, Abdel-Azim N S, Berghe D V & Vlietinck A J, Chemical and biological

investigations on *Zizyphus spina*-christi L, *Phytother Res*, 15 (2001) 593.

4 Shi Y, Shi R B & Liu B, Studies on antiviral flavonoids in Yinqiaosan Powder, *Chin J Chin Mat Med*, 26 (2001) 320.

5 Chen Z W, Ma C G & Fang M, Mechanism of analgesic action of hyperin, *Acta Pharm Sin*, 24 (1989) 226.

6 Ma C G, Xiang Z M & Xu S Y, Mechanism of analgesic action of hyperin on centrum, *Chin Pharmacol Bull*, 7 (1991) 345.

7 Zhang J S, Chen Z W, Wang Y, Song B W, Dong L Y& Cen D Y, Mechanism of analgesic action of hyperin on spinal cord, *Anhui Med*, 19 (1998) 3.

8 Lee S J, Son K H, Chang H W, Do J C, Jung K Y& Kang S S, Anti-inflammatory activity of naturally occurring flavone and flavonol glycosides, *Arch Pharm Res*, 16 (1992) 25.

9 Zhang Z, Chang Q, Zhu M, Huang Y, Ho W K & Chen Z, Characterization of antioxidants present in hawthorn fruits, *J Nutr Biochem*, 12 (2001) 144.

10 Li Q L, Yu G X, Chen Z & Ma C G, Inhibitory mechanism of hyperin of the apoptosis in myocardial ischemia/reperfusion in rats, *Acta Pharm Sin*, 37 (2002) 849.

11 Wang W Q, Ma C G & Xu S Y, Protective effect of hyperin against myocardial ischemia and reperfusion injury, *Acta Pharmacol Sin*, 17 (1996) 341.

12 Xiong Q, Fan W, Tezuka Y, Adnyana I K, Stampoulis P, Hattori M, Namba T & Kadota S, Hepatoprotective effect of *Apocynum venetum* and its active constituents, *Planta Medica*, 66 (2000) 127.

13 Carlo G D, Mascolo N, Izzo A A & Capasso F, Flavonoids: old and new aspects of a class of natural therapeutic drugs, *Life Sci*, 65(1999) 337.

14 Ito M, Shimura H, Watanaba N, Tamai M, Hanada K, Takahashi A,Tanaka Y, Arai K, Zhang P L & Chang R, Hepatoprotective compounds from *Canarium album* and *Euphorbia nematocypha*, *Chem Pharm Bull*, 38 (1990) 2201.

15 Zhao W Z, Chen Z W, Song B W, Wang Y L, Wang Q, Fang M, *et al*. The protective effect of hyperin on gastric mucosal injury in mice and its mechanism, *Acta Anhui Med Univ*, 3 (1999) 178.

16 Song B W, Chen Z W& Ma C G, The cell protective effect of hyperin on gastric mucosal injury in rats, *Chin Pharmacol Bull*, 11 (1995) 46.

17 Verma N, Singh A P, Gupta A, Sahu P K & Rao Ch V, Anti-inflammatory and anti-nociceptive activity of *Rhododendron arboreum*, *J Pharm Res*, 3 (2010) 1376.

18 Bhandary M R & Jun Kawabata, Antidiabetic activity of Laligurans (*Rhododendron arboreum* Sm.) flower, *J Food Sci Technol*, 4 (2008) 61.

19 Verma N, Singh A P, Gupta A, Sahu P K & Rao Ch V, Protective effect of ethyl acetate fraction of *Rhododendron arboreum* flowers against carbon tetrachloride-induced hepatotoxicity in experimental models, *Indian J Pharmacol,* 43 (2011) 291.

20 Verma N, Singh A P, Gupta A, Sahu P K & Rao Ch V. Antidiarrheal potential of standardized extract of *Rhododendron arboreum* Smith flowers in experimental animals, *Indian J Pharmacol*, 43 (2011) 689.

21 Verma N, Singh A P, Gupta A, Sahu P K & Rao Ch V, Antihyperglycemic and antihyperlipidemic activity of ethyl acetate fraction of *Rhododendron arboreum* Smith flowers in streptozotocin induced diabetic rats and its role in regulating carbohydrate metabolism, *Asian Pac J Trop Biomed*, 2 (2012) 1.

22 Ghosh M N, *Fundamentals of Experiment Pharmacology*, 2nd ed, (Scientific Book Agency, Calcutta) 1984, 192.

23 Turner R, Acute toxicity: The determination of LD50. In *Screening Methods in Pharmacology*, (Academic Press, New York) 1965, 300.

24 Erejuwa O O, Sulaiman S A, Mohd Wahab S A, Kuttulebbai Sirajudeen N S, Md Salzihan, Md Salleh & Sunil G, Antioxidant protective effect of glibenclamide and metformin in combination with honey in pancreas of streptozotocin-induced diabetic rats, *Int J Mol Sci*, 11 (2010) 2056.

25 Trinder P, Determinations of blood glucose using 4-amino-phenazone as oxygen acceptor, *J Clin Path,* 22 (1969b) 246.

26 Eross J, Kreutzmann D, Jimenez M, Keen R, Rogers S, Colwell C, Vines R & Sinik M, Colorimetric measurement of glycosylated protein in whole blood cells plasma and dried blood, *Ann Clin Biochem*, 21 (1984) 519.

27 Rao P V, Madhavi K & Naidu M D, Hypolipidemic properties of *Rhinacanthus nasutus* in streptozotocin-induced diabetic rats, *J Pharmaco Toxicol*, 6 (2011) 589.

28 Friedewald W T, Levy R I & Fradrickson D S, Estimation of concentration of low density lipoprotein cholesterol in plasma without the use of preparative ultracentrifuge, *Clin Chem*, 18 (1972) 499.

29 Sadasivam S & Manickam A, *Methods in Biochemistry*, (New Age International Pvt. Ltd, New Delhi) 1996, 11.

30 Guruvayoorappam C & Sudha G, Phytopharmacological evaluation of Byesukar for hypoglycaemic activity and its effect on lipid profile and hepatic enzymes of glucose metabolism in diabetic rats, *Ann hepatol*, 7 (2008) 358.

31 Leloir L F & Goldenberg S H, Glycogen synthetase from rat liver, In *Methods of Enzymology*, (Academic Press, New York) 1965,145.

32 Cornblath M, Randle P J, Parmeggiani A & Morgan H E, Regulation of glycogenolysis in muscle: Effects of glucagon and anoxia on lactate production, glycogen content and phosphorylase activity in perfused isolated rat heart, *J Biol Chem*, 238 (1963) 1592.

33 Qi X Y, Chen W J, Zhang L Q & Xie B J, Mogrosides extract from *Siraitia grosvenori* scavenges free radicals *in-vitro* and lowers oxidative stress, serum glucose and lipid levels in alloxan-induced diabetic mice, *Nutr Res*, 28 (2008) 278.

34 Sharma S B, Nasir A, Prabhu K M, Murthy P S & Dev G. Hypogyycemic and hypolipidemic effect of flavonoid rich extract from *Eugenia jambolana* seed on streptozotocin-induced diabetic rats, *Food Chem Toxicol*, 46 (2003) 2376.

35 Stalmans W, Cadefau J, Wera S & Bollen M, New insight into the regulation of liver glycogen metabolism by glucose, *Biochem Soc Trans*, 25 (1997) 19.

36 Akatsuka A, Singh T J & Huang K P, Comparision of liver glycogen synthase from normal and streptozotocin-induced diabetic rats, *Arch Biochem Biophys*, 220 (1983) 426.

37 Roesler W J & Khanderwal R L, Quantitation of glycogen synthase and phosphorylase protein mouse liver: Correlation between enzymatic protein and enzymatic acivity, *Arch Biochem Biophys*, 244 (1986) 397.

38 Laakso M, Malkki M & Deeb S S, Aminoacid substituents in hexokinase II among patients with NIDDM, *Diabetes* 44 (1995) 330.

39 Baquer N Z, Gupta D & Raju J, Regulation of metabolic pathways in liver and kidney during experimental diabetes: effects of antidiabetic compounds, *Indian J* Clin *Biochem*, 13 (1998) 63.