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RESEARCH ARTICLE

Bioflavonoids Inhibits Dipeptidyl Peptidase-IV Expressions in Diabetic Rats

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ABSTRACT

The study aim is to investigate the Dipeptidyl peptidase-iv inhibitory activity, antidyslipidemic and anti diabetic effects of the flavonoids (Quercetin, Chrysin & Hesperdin) in alloxan-induced diabetic rats. The effects of orally administered flavonoids (Quercetin, Chrysin & Hesperdin) on serum glucose and antidyslipidemic activity were examined in diabetic control and flavonoids treated diabetic rats. While the activities of the Dipeptidyl peptidase-iv levels, Lipid profiles in the serum were assessed. The flavonoids were administered over a period of 21 days. Results involves the quercetin, chrysin & Hesperdins were significantly ($P<0.05$) reduced serum glucose, Dipeptidyl peptidase-iv activity and dyslipidemic status in all the flavonoids and pioglitazone treated groups. Conclusions of the present investigation suggests that flavonoids and pioglitazone combination with flavonoids was inhibits Dipeptidyl peptidase-iv activity, dyslipidemic status and hypoglycemic effect in diabetes rats.

KEYWORDS

DPP-IV, Flavonoids, Blood Glucose, Lipid Profiles

INTRODUCTION

Dipeptidyl peptidase-IV (DPP-IV) is serine amino peptidase that inactivates in cretins, especially Glcogon like peptide (GLP-1) and glucose induced peptide (GIP), which are gut hormones released in response to food absorption. GLP-1 has several gluco-regulatory activities as outlined. This spectrum of gluco-regulatory actions of GLP-1 underscores its importance in T2DM therapy. As soon as released from the gut during meals, the incretin hormones (GLP-1 and GIP) serve as enhancers of glucose-dependent insulin release from pancreatic B-cells.[1] GLP-1 is hydrolysed by DPP-IV so therefore inhibition of DPP-IV as new drug target for Type 2 Diabetes Mellitus.[2,3].

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As new drug therapy for DPP-IV inhibitors were enhance the in cretins effect.[4] and increasing the in cretin activity is plays a important role in blood glucose control and insulin secretion.[5]

Flavonoids were important polyphenolic compounds in plants; they are widely distributed in many frequently consumed beverages and food products of plant origin such as fruit, vegetables, wine, tea and cocoa.[6] The polyphenols are well accepted for protecting against many chronic diseases like cancer, cardiovascular and diabetes mellits.[7] Flavonoid their mechanism of action exerts antioxidant activity by scavenging or quenching of free radicals or chelating of metal ions or by inhibiting enzymatic activity.[8] Alloxan administration cases necrosis of pancreatic Beta-cell.[9] Alloxan induces the production of hydrogen peroxides and some free radicals such

as speroxides and hydroxyl radicals which can damage and leads to death of the beta cells.[10]

The aim of this study was to evaluate the effect of the flavonoids quercetin, hesperidin and chrysin on Dipeptidyl peptidase activity in alloxan-induced diabetes mellitus.

MATERIALS AND METHOD

Experimental Animals

White male Wister rats weighing about 150-180 g were used. They were purchased from the mahaveer enterprises hyderabad. They were kept under observation for about 15 days before the onset of the experiment to exclude any intercurrent infection. The chosen animals were housed in plastic well aerated cages at normal atmospheric temperature (25±5 °C) and normal 12- hour light/dark cycle. Moreover, they had free access to water and were supplied daily with standard diet of known composition *ad libitum*. All animal procedures were in accordance with the recommendations of the ethical Committee guidelines for Care and Use of Animals.

Chemical Agents

Alloxan(A) as well as quercetin(Q), chrysin(Ch), Hesperidin (Hesp) were purchased from Sigma Chemical Company (St. Louis, MO). Pioglitazone (Natco, Hyderabad).

Induction and Treatment of Diabetes

Diabetes was induced by a single injection of alloxan 120 mg/kg bodyweight to rats fasting for at least 16 hours, through the intraperitonial route in freshly prepared 1% sodium carboxy methyl cellulose, Blood glucose levels were measured after 48 hours and 21 days after alloxan administration. Development of diabetes mellitus was proven by sustained hypergycemia and glycosuria (diabetic rats had glucose levels > 16 mmol/l).The diabetes developed rats were treated with the flavonoids quercetin (Q), chrysin (Ch), Hesperidin (Hesp) and pioglitazone for 21 consecutive days (after alloxan administration).

The rats were randomly divided into 9 groups (n =6) as follows:

Group I: control animals (sod. carboxymethyl cellulose-1%, orally).

Group II: diabetic animals

Group III: diabetic animals + Quercetin (Q).

Group IV: diabetic animals + Chrysin (Ch)

Group V: diabetic animals + Hesperidin (Hsp)

Group VI: diabetic animals + Pioglitazone(P)

A flavonoids plus pioglitazone was administered in diabetic animals (groups IIIa-Va) which was received Q or Ch or Hesp at the same doses and schedule as groups III – V.

The flavonoids were administered orally (by gavage) in sod carboxymethyl cellulose as a vehicle. Doses of flavonoids were assigned on the basis of experience from literature.[11, 12]

Biochemical Evaluation

Blood samples were drawn from the retro orbital puncture and centrifuged at 1000 *g* for 10 min, In order to determine the blood glucose levels[13] lipid profiles[14] and Serum DPP-4 activity.[15]

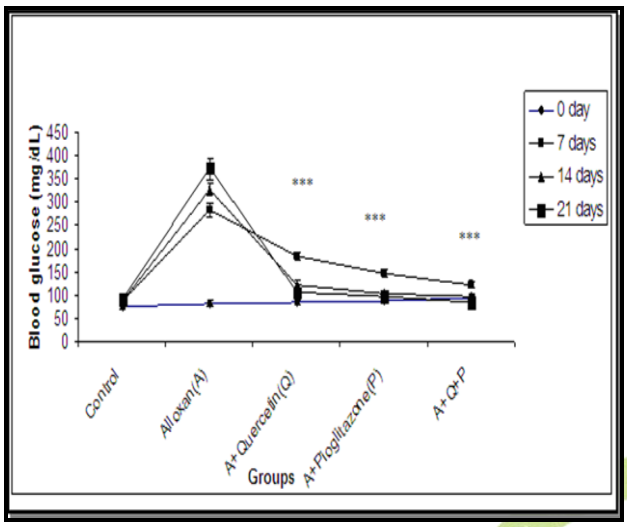
Statistical Analysis

The data are presented as mean ± S.D Statistical comparisons were made by one-way analysis of variance (ANOVA) and followed by Student-Neuman-Keuls as the *post hoc* test. Data were considered significant when *p* values were lower than 0.05.

RESULTS

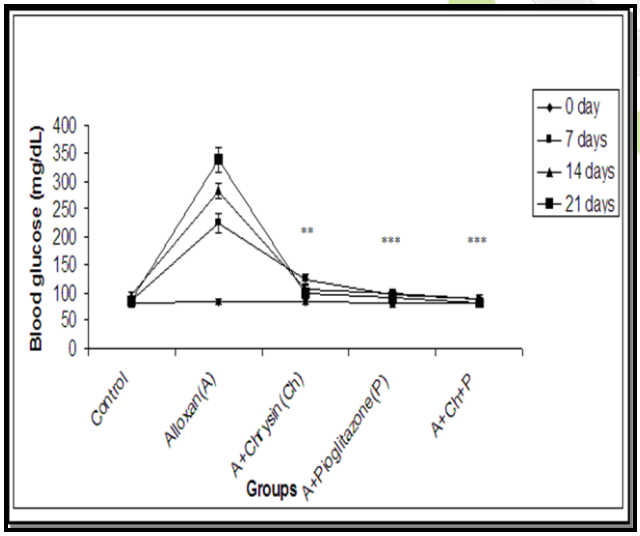
The present study results were the effect of flavonoids in normal control, alloxan induced and treatment group blood glucose levels were represented in figure 1, 2, 3. The data of lipid profile levels (21days after flavonoids treatment) were represented in Table.1; Dipeptidyl peptidase inhibitory activity (0, 7, 14 and 21days) is represented in figs 4, 5 and 6 respectively. The treatment with flavonoids (quercetin or Chrysin or hesperidin) and flavonoids with pioglitazone combination was significantly reduced in the blood glucose concentration in diabetics ($p < 0.001$) and also all the flavonoids (quercetin or Chrysin or

hesperidin) were significantly reduce the Dipeptidyl peptidase activity, total cholesterol, triglycerides, and low density lipoprotein (LDL), very low density lipoprotein (VLDL) and but increased the High density lipoprotein (HDL) levels.



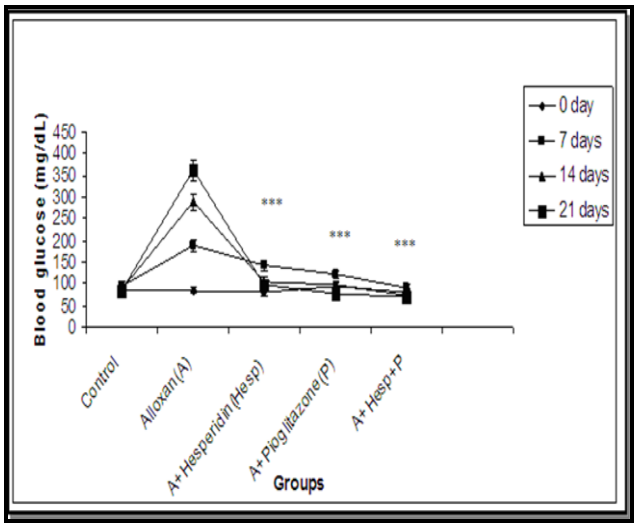
Values were Mean ± SD; n=6. *** p<0.001 Vs treated groups, diabetic group

Figure 1: Blood glucose of the control, diabetic, Quercetin treated and Quercetin with pioglitazone combination treated groups



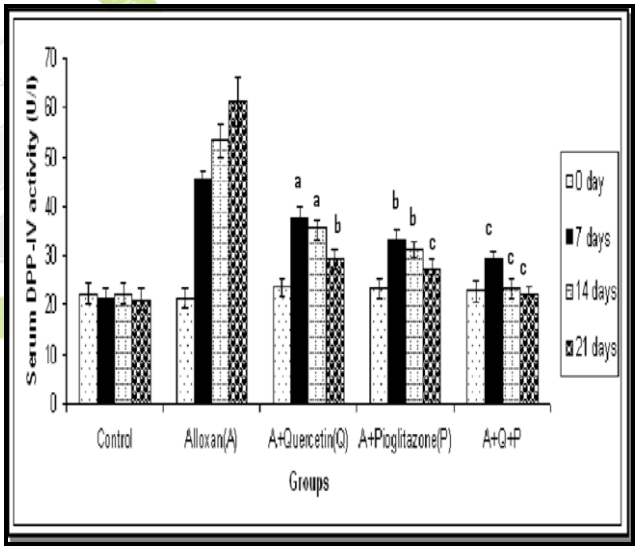
Values were Mean ± SD; n=6. ** p<0.01, *** p<0.001 Vs treated groups, diabetic group

Figure 2: Blood glucose of the control, diabetic, Chrysin treated and Chrysin with pioglitazone combination treated groups



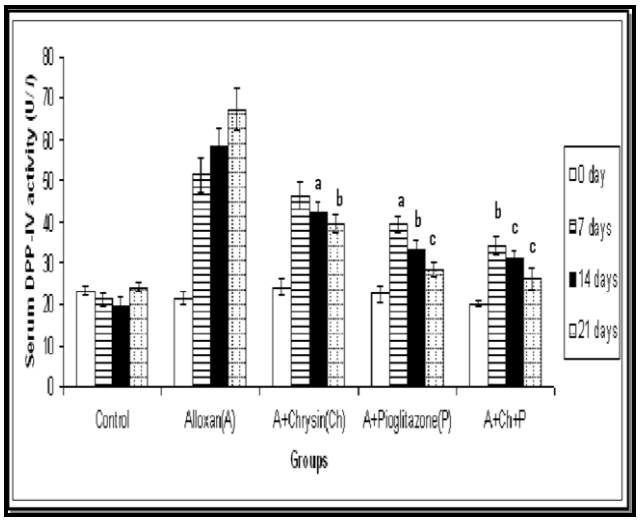
Values were Mean ± SD; n=6. *** p<0.001 Vs treated groups, diabetic group.

Figure 3: Blood glucose of the control, diabetic, Hesperidin treated and Hesperidin with pioglitazone combination treated groups



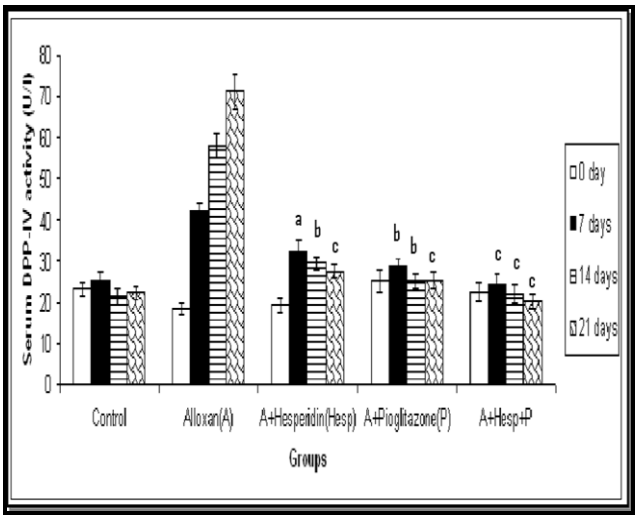
(Data were Mean ± SD; n=6 a p<0.05, **b** p<0.01, **c** p<0.001 vs. diabetic and control)

Figure 4: Serum Dipeptidyl peptidase –IV activity of control, diabetic, Quercetin treated and Quercetin with pioglitazone combination treated groups



(Data were Mean ± SD; n=6 a p<0.05, **b** p<0.01, **c** p<0.001 vs. diabetic and control

Figure 5: serum Dipeptidyl peptidase –IV activity of the control, diabetic, Chrysin treated and Chrysin with pioglitazone combination treated groups



(Data were Mean ± SD; n=6 a p<0.05, **b** p<0.01, **c** p<0.001 vs. diabetic and control)

Figure 6: serum Dipeptidyl peptidase –IV activity of the control, diabetic, Hesperidin treated and Hesperidin with pioglitazone combination treated groups

Table 1: Effect of flavonoids (Quercetin, Chrysin and Hesperidin) alone and pioglitazone combination on serum lipid profile (mg/dL) levels in diabetic rats

Parameters/ Groups	Total cholesterol (mg%)	Triglycerides (mg%)	High density lipoprotein (mg%)	Very low density lipoprotein (mg%)	Low density lipoprotein (mg%)
Control	56.42±2.3	48.61±3.3	36.45±2.6	9.72±0.5	10.24±0.6
Alloxan(A)	125.31±10.3	165.23±11.2	15.26±0.8	33.04±1.2	77.04±6.1
A + Quercetin (Q)	76.51±5.6*	68.34±5.1*	23.51±1.3*	13.66±0.6	39.33±1.5
A + Pioglitazone(P)	61.34±5.1*	58.24±4.3*	26.43±1.2**a**	11.64±0.7**a**	23.26±1.4
A+Q+P	57.62±4.1**b**	52.34±3.2**b**	31.23±1.2**b**	10.46±0.6**b**	15.92±1.6**b**
A + Chrysin(Ch)	74.56±3.2	66.32±5.2*	25.31±1.5*	13.26±1.2*****	35.98±2.4*
A+Ch+P	49.62±3.3**a**	48.67±2.5*	31.46±2.4**a**	9.73±0.7**a**	8.42±0.4**a**
A + Hesperidin(Hesp)	98.61±8.2*	66.53±4.6*	22.31±1.1*	13.30±0.9*	62.99±3.7*
A+Hesp+P	59.46±4.2**b**	51.23±3.6**b**	31.26±2.2**b**	10.24±0.7**b**	17.95±1.5**b**

(Data values were mean ± SD; n=6 ***** p<0.05, **a** p<0.01, **b** p<0.001 vs. diabetic and control)

DISCUSSION

The present study Dipeptidyl peptidase activity, total cholesterol, triglycerides, and low density lipoprotein (LDL), very low density lipoprotein (VLDL) levels were increased in alloxan induced group but above all were decrease after treatment with flavonoids and combination with pioglitaone and increased the High density lipoprotein (HDL) levels and protected the necrosis of pancreasis.

Positive association of plasma DPP-IV activity to blood glucose and serum lipid profiles. Previous study reported that the Streptootacin-induced increase in plasma DPP-IV activity is attributed to enhanced biosynthesis of DPP IV enzyme and its secretion in endothelial cells by high blood glucose. [16] In addition, the increased activity of plasma DPP IV could still worsen hyperglycemia since DPP IV activation may lead to decreases in the anti-diabetic effects of GLP-1 and GIP.[17] and reputed that the β-cell necrosis, Oxidative stresses and inflammatory and cholestasis conditions also increases the DPP-IV activity. Lipid lowering activity of Metformin and pioglitazone controlled the DPP-IV levels which associable elevated cholesterol and triglycerides.[18, 19, 20]

The Present study result shows that in alloxan induced diabetic rats' increased DPP-IV activity due to destruction of β-cells and release of ROS, which were prevented by flavonolds treatment and also decreased elevated DPP-IV activity. The previous study reveals that flavonoids protects against oxidative stress-induced cellular damage as well as chelatory property.[21, 22, 23]

Anti-diabetic potency of flavonoids, particularly hesperidin and quercetin, has been highlighted in many reports and attributed in part to their antioxidant and hypoglycemic effects.[24, 25, 26]

Flavonoids inhibited the dyslipidemia in our study reported that Hesperidin can inhibit lipogenesis and lower plasmatic triglycerides levels by enhancing LDL receptors expression and increasing fat bile rejection.[26] From the results of clinical studies Insulin resistance was compensated by the enhanced insulin secretion, whereas persistently elevated free fatty acids may contribute to progressive β-cell failure (β-cell lipotoxicity) in individuals genetically predisposed to DM2.[27, 28] The other report specifies that a potential mechanism by which obesity and inflammation could affect GLP-1 degradation is by enhancing the expression or activity of DPP-IV.[29] The previous reports on the flavonoids possess anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral and anticarcinogenic activities.[30,31, 32]

The present study results reveal that pioglitazone reduce the DPP IV activity reported that treatment with metformin and pioglitazone significantly increase in circulating GLP-1 amide after an oral glucose load and inhibition of DPP-IV activity.[20, 33] Thiozolidine dione derivatives has been to prevent TNF-Alpha indced inflammatory activity and insline resistsance.[34, 35] In the same animal model, thiozolidin diones treatment was preventing the loss of β-cell mass indicated previously and in the same animal model, thiozolidin diones treatment was preventing the loss of β-cell mass indicated previously.[36]

The flavonoids combination with pioglitazone is increase the bioavailability of pioglitazone reduce the DPP IV activity, It has an earlier reports that administration of pioglitaone along with quercetin to diabetic rats increases its bioavailability.[37] Pioglitazone also prevented the loss of β-cell mass by reduction of oxidative stress.[38]

CONCLUSION

Our results show that oral administration of quercetin, hesperidin and chrysin has a beneficial effect on the alloxan-induced diabetics by reducing hyperglycemia, dyslipidemia and elevated DPP-IV expressions in diabetic rats. This study suggests that the induction of diabetes mellitus by alloxan in rats may be protected by quercetin, hesperidin and chrysin administration. It hypothesized that this effect may be result of antiradical/chelatory/anti-inflammatory properties of flavonoids used. However,

inhibition of DPP-IV expression which elevated along with diabetes mellitus.

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