Alpha amylase inhibitory activity of flavonoids in diabetic induced rats

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ABSTRACT

Purpose: The study aim is to investigate the amylase inhibitory activity and antioxidant effects of the flavonoids (Quercetin, Chrysin & Hesperdin) in alloxan-induced diabetic rats. Methods: The effects of orally administered flavonoids (Quercetin, Chrysin & Hesperdin) on serum glucose and antioxidant activity were examined in diabetic control and flavonoids treated diabetic rats. While the activity of the a-amylase levels, in the serum were assessed. The drugs were administered over a period of 21 days. Results: The quercetin, chrysin & Hesperdins were significantly (P<0.05) reduced serum glucose, α-amylase activity but increased serum antioxidant status in all the flavonoids and pioglitazone treated groups. Conclusion: The present investigation suggests that flavonoids and pioglitazone combination with flavonoids was inhibits alpha-amylase activity and hypoglycemic effect in diabetes rats.

Keywords: α-amylase, flavonoids, blood glucose, antioxidant activity.

INTRODUCTION

Diabetes mellitus (DM) is a multifactorial disease which is characterized by hyperglycemia (Ugochukwu et al., 2003), lipoprotein abnormalities (Scoppola et al., 2001), raised basal metabolic rate (Owu et al., 2006), defect in reactive oxygen species (ROS) scavenging enzymes (Kesavulu et al., 2000) and high oxidative stress induced damage to pancreatic beta cells (Nayeemunnisa, 2009). Diabetes mellitus is the leading causes of death and its fatal complications were taken into account (Trivedi et al., 2004). Non-insulin dependent diabetes mellitus (NIDDM), a common disorder of glucose and fat metabolism is strongly associated with diets (Garg et al., 1994). Postprandial hyperglycemia has been linked to the onset of the diabetic complications, generation of free radicals and oxidation-related damage in the retina, renal glomerulus and peripheral nerves (Kwon et al., 2005).

Studies have shown that the glucose-induced increased levels of mitochondrial reactive oxygen species (ROS) responsible for hyperglycemia induced vascular complications (Kaiser et al., 1993). Various approaches are used to improve diabetes via different modes of action such as stimulation of insulin release, increase, the number of glucose transporters, and inhibition of gluconeogenesis and reduction of absorption of glucose from the intestine (Youn et al., 2004). One of the most beneficial therapies for type II diabetes is said to be the control of postprandial hyperglycemia(Kim et al., 2005; Mai and Chuyen, 2007). The best therapeutic approach to decrease postprandial hyperglycemia is to retard absorption of glucose through inhibition of carbohydrate hydrolyzing enzymes in the digestive organs (Kim et al 2005). The enzymes are responsible for the breakdown of Oligo and disaccharides, a-amylase is one of the enzymes that catalyses the breakdown of starch to maltose and finally to glucose, which is the only sugar that can be utilized by the body (Kotowaro et al., 2006). The inhibition of these enzymes leads to a decrease in blood glucose level, since monosaccharides are a form of carbohydrates which are absorbed through the small intestine (Funke and Melzig, 2006).
Hesperidin (Hesp) 300 mg/kg (p.o.) and Pioglitazone 15 mg/kg (p.o) for 21 consecutive days (after alloxan administration).

**Experimental design**

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

- Group I: normal control animals (sod. carboxymethyl cellulose-1%, orally).
- Group II: diabetic control animals
- Group III: diabetic animals + Quercetin (Q).
- Group IV: diabetic animals + Chrysin (Ch)
- Group V: diabetic animals + Hesperidin (Hesp)
- 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 (Mahesh and Menon 2004; De Boer et al., 2005).

**Biochemical evaluation**

Blood samples were collected from all the groups from the retro orbital of rats on 0, 7, 14 and 21 days of treatment, centrifuged at 1000 rpm for 15 min and the blood glucose levels (Trinder, 1969) and Serum antioxidant (Blios, M.S.1958), Superoxide dismutase (SOD) (Misra et al., 2002) and a-amylase (Street and Close, 1956) levels were estimated.

**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 effects of quercetin, chrysin and hesperidin on blood glucose levels (0 day, 7days, 14 days and 21 days) of control, diabetic and flavonoids treated diabetic rats were summarized in Figs. 1, 2 and 3. The data of SOD, TAS levels (21days after flavonoids treatment) were presented in Table 1. a-amylase activity (0 day, 7days, 14 days and 21 days) is represented in figs 4, 5 and 6. Flavonoids quercetin, chrysin, hesperidin and pioglitazone combination had no effect on normoglycaemic animals. On the other hand, the alloxan-induced animals consistently exhibited hyperglycaemia. The simultaneous treatment with quercetin and combination with pioglitazone, significantly reduced in the blood glucose concentration in diabetics (p < 0.001). Completely controlled, elevation of serum glucose by Chrysin, hesperidin and combination with pioglitazone (p < 0.01). All the flavonoids significantly increased SOD, TAS levels, and reduced the activity of a-amylase levels as per dose and schedules.

<table>
<thead>
<tr>
<th>Groups/Parameters</th>
<th>SOD (IU)</th>
<th>Total antioxidant status (TAS (nm of ascorbic acid))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>38.61±10.26</td>
<td>21.67 ± 9.41</td>
</tr>
<tr>
<td>Alloxan(A)</td>
<td>13.8±9.82</td>
<td>6.2±1.11</td>
</tr>
<tr>
<td>A+Quercetin (Q)</td>
<td>29.3±11.1*</td>
<td>13.3±1.68*</td>
</tr>
<tr>
<td>A+Pioglitazone (P)</td>
<td>28.7±8.25*</td>
<td>12.7±3.25*</td>
</tr>
<tr>
<td>A+Q+P</td>
<td>34.2±3.46**</td>
<td>14.26±3.56**</td>
</tr>
<tr>
<td>A+Chrysin (Ch)</td>
<td>28.3±9.1*</td>
<td>13.4±1.41*</td>
</tr>
<tr>
<td>A+Ch+P</td>
<td>31.2±12.5**</td>
<td>15.1±3.5**</td>
</tr>
<tr>
<td>A+Hesperidin (Hesp)</td>
<td>32.1±6.36**</td>
<td>16.3±1.61**</td>
</tr>
<tr>
<td>A+Hesp+P</td>
<td>35.1±10.93**</td>
<td>19.5±4.9**</td>
</tr>
</tbody>
</table>

(Da were significant values *p < 0.05, **p < 0.01, vs. control, diabetic and treated rats)
DISCUSSION
Many natural products have been investigated with respect to suppression of glucose production from carbohydrates in the gut or glucose absorption from the intestine. Alpha amylase catalyses the hydrolysis of 1, 4-glucosidic linkages of starch, glycogen and various oligosaccharides into simpler sugars which can be readily available for the intestinal absorption. Inhibition of alpha amylase enzyme in the digestive tract of human is being considered to be effective in controlling diabetes by this enzyme can delay the carbohydrate digestion and reduce the rate of glucose absorption.

In our study results inhibition of alpha amylase levels in treatment with flavonoids these findings were support of Tadera et al. (2006), reported that Inhibition of alpha-glucosidase and alpha amylase by flavonoids. Another
Our results show that oral administration of quercetin, hesperidin and chrysin has a beneficial effect on the alloxan-induced diabetes mellitus which were controlled to normal by treatment with flavonoids and combination with pioglitazone. The indirect mechanism of flavonoids and pioglitazone inhibited the alpha amylase levels, these finding were support of Mira et al. (2006), reported that the flavonoids in longer duration studies of compounds on chronic models are necessary to develop a potent antidiabetic drug. Anti-diabetic potency of flavonoids, particularly hesperidin and quercetin, has been highlighted in many reports and attributed in part to their antioxidant and hypoglycaemic effects (Frode and Medeiros, 2008; Lean et al., 1999; Jung et al., 2006).

In present study elevated the alpha amylase levels due to cause of reactive oxygen species (ROS)and inflammatory mediators in diabetes mellitus which were controlled to normal by treatment with flavonoids and combination with pioglitazone. The indirect mechanism of flavonoids and pioglitazone inhibited the alpha amylase levels, these finding were support of Mira et al. (2006), Anjaneyulu and Chopra (2004), reported that flavonoids to protect against oxidative stress-induced cellular damage as well as its chelatory properties. The anti-inflammatory activity of quercetin protects pancreatic ß-cells from type 2 diabetes, by inhibiting inflammation-producing enzymes (cyclooxygenase, lipooxygenase) and subsequent inhibition of inflammatory mediators, including leukotrienes and prostaglandins (Della-Logia et al; 1988; Kim et al; 1998). PPAR ? agonists inhibit the oxidative stress (Barter et al., 2004; Jung et al., 2006; 2008; 2011). The present study results reveals that decreased the blood glucose with flavonoids (quercetin, Chrysin, Hesperidin), these study support of Ali et al. (2006), reported that the flavonoids in longer duration studies of compounds on chronic models are necessary to develop a potent antidiabetic drug. Anti-diabetic potency of flavonoids, particularly hesperidin and quercetin, has been highlighted in many reports and attributed in part to their antioxidant and hypoglycaemic effects (Frode and Medeiros, 2008; Lean et al., 1999; Jung et al., 2006).

In present study elevated the alpha amylase levels due to cause of reactive oxygen species (ROS) and inflammatory mediators in diabetes mellitus which were controlled to normal by treatment with flavonoids and combination with pioglitazone. The indirect mechanism of flavonoids and pioglitazone inhibited the alpha amylase levels, these finding were support of Mira et al. (2006), Anjaneyulu and Chopra (2004), reported that flavonoids to protect against oxidative stress-induced cellular damage as well as its chelatory properties. The anti-inflammatory activity of quercetin protects pancreatic ß-cells from type 2 diabetes, by inhibiting inflammation-producing enzymes (cyclooxygenase, lipooxygenase) and subsequent inhibition of inflammatory mediators, including leukotrienes and prostaglandins (Della-Logia et al; 1988; Kim et al; 1998). PPAR ? agonists inhibit the oxidative stress (Barter et al., 2004). All these studies and our present findings stress the importance of pioglitazone in combination therapy.

In the present study clearly indicated that flavonoids exhibited anti-diabetic as well as lowering the alpha amylase activity in type 2 diabetic model rats.

CONCLUSION
Our results show that oral administration of quercetin, hesperidin and chrysin has a beneficial effect on the alloxan-induced diabetes by reducing hyperglycaemia, a-amylase activity and improving the antioxidant status. This study suggests that the induction of diabetes mellitus by alloxan in rats may be protected by quercetin, hesperidin and chrysin administration. We hypothetized that this effect may be result of antiradical/cherlatory/anti-inflammatory properties of flavonoids used. However, inhibition of a-amylase activity which elevated along with diabetes mellitus effect of these flavonoids.

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