INHIBITORY ACTIVITY OF FLAVONOIDS ON THE LENS ALDOSE REDUCTASE OF HEALTHY AND DIABETIC RATS

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Abstract- Aldose reductase is a critical enzyme in the polyol pathway that plays an important role in diabetes mellitus. Inhibition of the activity of this enzyme can prevent cataract in diabetic patients' lenses. In this study the inhibitory effect of two flavonoids, quercetin and naringin, in the activity of aldose reductase in streptozotocin-induced diabetic and healthy rats were investigated. Thirty male rats were divided in six groups. The first, second and third group were healthy rats that received water, quercetin and naringin, respectively. The fourth, fifth and sixth groups were streptozocin-induced diabetic rats that received water, quercetin and naringin, respectively. These rats were fed orally in a definite dose from each substance for 12 days. After this period rats were scarified and their lenses were separated and homogenized. The activity of aldose reductase was measured in each homogenized sample separately. The effect of feeding of these substances in blood sugar was also determined. Aldose reductase activity was reduced 73 and 69 percent in diabetic rats fed by quercetin and naringin, respectively, and the difference compared to control group was significant. In healthy rats this reduction was 63 and 59 percent, respectively, and the difference was significant compared to those who did not receive flavonoids. It was concluded that these substances were effective in reduction of aldose reductase activity *in vivo* and consequently could delay the progress of cataract.

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INTRODUCTION

In diabetes mellitus the increased availability of glucose in insulin-insensitive tissues such as lens, nerve, and retina leads to the increased formation of sorbitol through the polyol pathway (1). In this pathway, glucose converts to sorbitol and then fructose through the enzymatic activity of aldose reductase and sorbitol dehydrogenase. Sorbitol is a tissue poison and its accumulation increases osmotic pressure and may damage the tissues by causing them to swell (2). The polyol pathway is involved in diabetic cataract. Since aldose reductase is localized primarily in lens epithelial cells, osmotic insults induced by the accumulation of sugar alcohols occur first in these cells (3).

It is possible to prevent cataract via inhibition of the activity of aldose reductase (2). It is shown that apoptosis in epithelial cells can be prevented by an aldose reductase inhibitor, suggesting that this apoptosis is linked to the accumulation of sugar alcohols (3). The first synthetic inhibitors tested *in vivo* were some flavonoids such as Quercitrin-3-rhamnosid (4). Varma *et al.* investigating different flavonoids showed that oral consumption of quercitin led to decrease sorbitol accumulation in

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lenses of diabetic rats and also cataract development was retarded (5). Lim *et al.* aim to provide a potential inhibitor of aldose reductase synthesized a series of 35 flavonoid derivatives and examined their effect on sorbitol accumulation in different rat tissues including lenses (6). These studies showed the 4-oxo-4h-chromen ring in flavonoid structure is necessary to inhibit the aldose reductase.

Two other isoflavone compounds, tectorigenin and irigenin, were found to show a strong aldose reductase inhibition (7). Oral administration of these compounds also was shown that inhibited sorbitol accumulation in the lenses of streptozotocin induced diabetic rats.

Vincent *et al.* also have shown that high ascorbic acid concentration can directly inhibit erythrocyte aldose reductase (8). Effects of aminoguanidine and aspirin have been examined on the development of retinopathy. This study has shown that administration of aminoguanidine prevented the retinopathy in diabetic rats (9). Recent studies show that beta isoform of protein kinase C is involved in the pathogenesis of diabetic retinopathy and it is a possible therapeutic benefit to inhibit this enzyme (10-11). On the other hand quercetin is known to have inhibitory activity on protein kinase C (12).

This is a very controversial area because differences in potency of the inhibitors, experimental designs and length of trails have resulted in different studies reaching different conclusions (13). As quercetin and naringin (a grapefruit flavanone) have the flavone's structure, it is possible that these compounds have also inhibitory activity on aldose reductase *in vivo*. This study was aimed to show the effect of feeding with two flavonoids, naringin and quercetin, on the activity of aldose reductase in healthy and diabetics rats.

MATERIALS AND METHODS

A total of 30 Wistar male rats were divided into 6 groups and each group was kept in a separate cages. Animals were maintained under controlled temperature (21-23º C) and light (12:12 h light, dark cycle, lights on at 7: am). They had free access to tap water. All experiments on animals were performed in accordance with UK legal requirements.

The first, second and third groups were healthy rats that received distillated water, quercetin and naringin, respectively. The fourth, fifth and sixth groups were streptozocin induced diabetic rats that received distillated water, quercetin and naringin, respectively. Diabetes was induced in rats using intera-peritoan injection of 60 mg/kg streptozotocin (Pharmaci & Upjohn) (14). The flavonoids were administrated in 1 ml solution using tube feeding, containing 10 mg/kg weight from each substance for 12 days.

Quercetin (3,3',4'5,7-pentahydroxy flavone, $C_{15}H_{10}O_7$) was from Sigma and naringin (4',5,7-trihydroxyflavanone 7-rhamnoglucoside, $C_{27}H_{32}O_{14}$) was from Merck; other chemicals (unless stated) in pure grade was purchased from Sigma.

After 7 days, the blood sugar was measured using an enzymatic method of glucose oxidase (Pars–Azmoon Kit, Iran). The blood samples were collected from the lateral caudal vein using heparinized hematocrit tubes. The rats that had blood glucose more than 300 mg/dl were considered as diabetic. The blood sugar of the rats was measured before the experiments and 12 days after the regime started. The lenses of rats were isolated and homogenized using the Heidolph-DIAX900 (Germany) homogenizer. The removed lenses from each rat were homogenized for 30 seconds in a tube containing 10 mM sodium phosphate and 2-mercaptoethanol (pH 7) at 0º C (15). Measurement of aldose reductase activity was assayed according to the method described by Lee *et al.* (14). The incubation mixture contained 0.4 M $(NH_4)_2SO_4$ in 0.1 M Hepes buffer (pH 7), 10 mM DL-glycerol as substrate and 0.12 mM NADPH as coenzyme in a total volume of 1 ml. The homogenate of lenses were centrifuged in 17300g for 10 min and 200 µl from supernatant were added to quartz cuvette contained the incubation mixture and decreases in absorbance at 340 nm were recorded after 15 min in a spectrophotometer (Pye Unicam UV-VIS). The one unit of activity was the activity of the enzyme that can produce 1 µmol NADP+ from NADPH in 1 min (15).

The data were compared according to descriptive statistics, ANOVA and Dunnett tests and using the Minitab software.

RESULTS

The effect of quercetin and naringin on blood sugar of diabetic rats is shown in table 1 and it is compared with those of control diabetic rats that did not receive the flavonoids. As these results show that blood sugar of diabetic rats after 12 days feeding was reduced and the difference compared to healthy rats was statistically significant ($P < 0.01$ for quercetin and $P < 0.001$ for naringin).

In table 2 the effect of quercetin and naringin on the activity of aldose reductase in healthy and diabetic rats are shown. In healthy rats that received quercetin and naringin, the activity of aldose reductase was reduced (63 and 65%, respectively) and compared with the healthy group that received water, the difference was significant ($P < 0.001$). In diabetic rats that received quercetin and naringin the activity of aldose reductase was reduced (73 and 69%, respectively) and the difference in activity in comparison to those rats that did not receive flavonoids was significant ($P < 0.001$).

Table 2. The effect of quercetin and naringin feeding on aldose reductase activity in lenses of healthy and diabetic rats*

Group	Aldose reductase activity		*P* value
	U/mg lens wet weight	U/whole lens weight	
Healthy control	2.39 ± 0.18	56.2 ± 3.31	-
Naringin fed	1.89 ± 0.16	45.9 ± 2.34	< 0.001
Quercetin fed	1.82 ± 0.13	41.40 ± 2.79	< 0.001
Diabetic control	3.17 ± 0.23	75.56 ± 2.23	-
Naringin fed	2.50 ± 0.19	56.94 ± 3.18	< 0.001
Quercetin fed	2.31 ± 0.17	55.66 ± 215	< 0.001

*Flavonoids were administrated in 10 mg/kg weight for 12 days.

DISCUSSION

Since polyol pathway has been known a cause of diabetic microvascular complications, there is an increased interest in inhibition of aldose reductase involved in this pathway. Aldose reductase inhibitors including quercetin are currently the most commonly used oral agents for their good penetrations through cellular membranes and fast metabolism of sorbitol by sorbitol dehydrogenase (15). They are considered more importantly as therapeutic prospects for treatment of diabetic complications such as retinopathy and cataract. However there is some evidence about the inhibitory activity of quercetin on aldose reductase, most of were carried out *in vitro* (16).

Varma and associates performed an *in vitro* experiment to determine the most effective flavonoid on inhibition of aldose reductase (17). They reported that 10^{-4} molar of quercetin had 100% inhibition on this enzyme (17). In a recent study reported by Matasuda *et al.* (18) some flavonoids including quercetin were shown that inhibited aldose reductase. They concluded that flavonol and flavanone having the 7-hydroxy and/or catechol moiety at the B ring exhibit the strong activity in inhibition of aldose reductase (18). As quercetin is flavone and naringin is a flavanone, they might have this activity too. In vitro inhibitory activity of quercitirin (a glycoside of quercetin) was reported and also compared with Cinnamaldehyde from Cinnamomum that showed higher activity (19). Nevertheless there is no evidence about the inhibitory activity of naringin.

Table 1. The effect of quercetin and naringin feeding on blood sugars of diabetic rats*

Groups	Blood sugar (mg/dl)		Difference	*P* value
	t_0	t_{12}	$t_{12} - t_0$	
Diabetic Control	489.2 ± 36.4	528.2 ± 21.9	39.0 ± 42.4	-
Naringin fed	456.0 ± 24.1	273.0 ± 23.6	183.0 ± 29.9	<0.01
Quercetin fed	462.6 ± 21.7	204.8 ± 16.01	257.8 ± 23.6	<0.001

*Blood sugars before flavonoid feeding (t_0) and after 12 days feeding (t_{12}) are expressed as Mean ± SD. Flavonoids were administrated in 10 mg/Kg weight.

Our study showed that oral consumption of two studied flavonoids *i.e.* quercetin and naringin could inhibit aldose reductase activity in lenses of diabetic rats. However vitamin C a different compound has also been shown that is effective in inhibition of aldose reductase activity (8).

As both ascorbic acid and flavonoids have antioxidant activity their inhibitory activity may be due to this property (4). Since flavonoids can chelate the metals, this activity may involve in their inhibitory action. Metal chelating property of these compounds is also involved in their antioxidant activity (20). It is shown that quercetin can chelate Fe and Cu (20). Also the main structural features of the inhibitors are considered as a polar head group and a hydrophobic ring system (21).

Our results also showed that rats fed with quercetin and naringin had reduced blood sugar and this reduction was more in diabetic rats compared to healthy rats. Increase in insulin concentration by narginin has been reported (22). A large number of synthetically prepared inhibitors, as well as plant derivatives have been examined, and some of them are used therapeutically, however still none of them is satisfactory (21). In spite of this fact, still it is believed that some will be developed promising in the treatment of diabetic complications. The plants that contain the aldose reductase inhibitors may prevent diabetic complications.

Conflicts of Interests

We have no conflicts of interest.

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