

Comparative Changes in Biomarkers of Oxidative Stress in Ocular Humour of Honey and Fructose Fed Wistar Rats

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ABSTRACT

Fructose has been shown to spare glucose and induce oxidative stress; honey contains high amount of fructose. Whether honey is capable of causing oxidative stress and lipid peroxidation is not fully documented. In this study therefore, ocular biomarkers of oxidative stress were compared between fructose and honey fed wistar rats. 49 male and female wistar rats were purchased and divided into Honey (20%, 30% and 40%) and Fructose (amounts equivalent to comparable honey group) groups with the control rats given 100% chow. The 7 subgroups (n=70) were fed for 28 days and thereafter, the ocular humour from the anaesthetized rats were obtained and the contents of biomarkers of oxidative stress (glucose, reduced glutathione GSH, and malondialdehyde MDA) were assessed using standard procedures. Results show that honey feeding at 20%, 30% and 40% concentrations for 28 days caused insignificant ($p>0.05$) changes in the values of biomarkers assessed when compared with control. However, fructose induced significant ($p<0.05$) changes in ocular glucose, GSH and MDA levels. Observations with fructose feeding are indications of oxidative stress and lipid peroxidation in the ocular humour. Albeit, the ocular benefits of honey feeding need verification.

Key words: Oxidative stress, Glucose, Ocular humour, Fructose, Honey.

INTRODUCTION

Fructose ($C_6H_{12}O_6$) is a 6 carbon ketose. It is the sweetest simple sugar mainly consumed as sweetener in foods. Food sources of fructose include confectioneries, sucrose and honey.

Honey is a mixture of sugars and other compounds. Honey contains mainly fructose (about 38.5%) and glucose (about 31.0%), and minute amounts of maltose, sucrose, and other complex carbohydrates¹. It also contains tiny amounts of several compounds thought to function as antioxidants, including chrysin, pinobanksin, vitamin C, catalase, and pinoembrin² with trace levels of vitamins and minerals.

Fructose feeding has been reported to induce insulin resistance and increase blood glucose³. These metabolic derangements were observed to increase intraocular pressure (IOP) and augment the level of oxidative stress in the organ³.

The severity of diabetic retinopathy has been observed to be related to length and magnitude of exposure to hyperglycaemia⁴⁻⁵. Patients with evidence of retinopathy have about 15% decrease in aqueous flow and this could impact intraocular pressure (IOP), though mechanism is yet to be clarified.

Although honey contains high amounts of fructose, its consumption lowers blood glucose, lipids, C-reactive proteins and homocysteine when compared with dextrose or sucrose feeding³. Therefore, honey may not significantly affect IOP and ocular glucose, but scientific evidence is yet to be sufficient. In this study, the levels of oxidative stress biomarkers (Glucose, reduced glutathione [GSH] and malondialdehyde, [MDA]) in ocular humour of fructose and honey fed Wistar rats were determined in order to compare the impact of honey and fructose feeding on ocular glucose and associated stress.

MATERIAL AND METHODS

Animal Care and Handling

Forty-nine male and female rats weighing 74-102 g were used in the study. The rats were divided into seven (7) groups. Group C, the control rats were given rat chow. Groups H₁, H₂ and H₃ were fed with 20%, 30% and 40% honey, respectively. The other experimental groups F₁, F₂ and F₃ received fructose quantities equivalent to amounts in 20%, 30% and 40% honey. The rats were fed for 28 days and given water *ad libidum*. Cages were cleaned regularly and animals were kept in 12 h light / 12 h dark cycle at room temperature (28°C-31°C). Animal care and handling complied with standard recommendations⁶.

Collection of Humour from the Rats

The two whole eyes of chloroform anaesthetized rat were plucked and punctured in order to collect the humour. The humour obtained was centrifuged at 1000rpm for 15min at 37°C. The

supernatant was carefully removed with Pasteur pipette and stored frozen until needed for analysis.

Analysis

The amounts of glucose⁷, glutathione⁸ and malondialdehyde⁹ in the eye humour were determined using standard procedures as previously described.

Statistical Analysis

Values were expressed as Mean±SEM and significant differences between means were evaluated by analysis of variance (ANOVA). Post test analysis was carried out using the Turkey multiple comparison test and values of $p < 0.05$ were considered as statistically significant¹⁰.

RESULTS

The results obtained from this investigation into the changes in biomarkers of oxidative stress in ocular humour of fructose and honey fed rats are presented in Table 1.

Table 1: Changes in the biomarkers of oxidative stress in the ocular humour of honey and fructose fed rats

| Group | Biomarkers of oxidative stress in ocular humour | | |
|----------------|---|------------------|---------------------------------|
| | Glucose(mmol/L) | GSH(mg/g tissue) | MDA (X10 ⁻⁵ mmol/mL) |
| C | 2.16±0.06 | 48.2±4.3 | 0.47±0.07 |
| H ₁ | 2.30±0.07 | 46.7±4.2 | 0.53±0.07 |
| H ₂ | 2.36±0.11 | 45.9±5.2 | 0.57±0.03 |
| H ₃ | 2.44±0.12 | 47.9±6.6 | 0.61±0.07 |
| F ₁ | 2.39±0.20 | 44.9±4.8 | 0.58±0.07 |
| F ₂ | 2.56±0.23* | 40.1±4.1 | 0.69±0.07 |
| F ₃ | 2.65±0.32* | 35.6±5.6** | 0.76±0.05** |

Values are expressed as Mean ±SD for n=7 rats/group.

GSH =Glutathione (reduced).

MDA = Malondialdehyde.

C =100% grower's mash (Control).

H₁ =80% grower's mash+20% honey.

H₂=70% grower's mash +30% honey.

H₃ =60% grower's mash +40% honey.

F₁=84.4% grower's mash+7.2g (Glucose) and 8.4g (Fructose).

F₂=76.6% grower's mash +10.8g (Glucose) and 12.6g (Fructose).

F₃ =68.8% grower's mash +14.4g (Glucose) and 16.8g (Fructose).

The quantities of glucose/fructose for Group E, F, and G were equivalent to amounts in 20%,30%,40% honey, respectively.

* Significantly different ($p < 0.05$) from control value

** Significantly different ($p < 0.05$) from both control and honey fed groups.

The administration of honey ($p>0.05$) or fructose ($p<0.05$) increased the concentration of glucose in ocular humour in a dose-dependent manner when compared with control. There was a significant ($p<0.05$) indication of oxidative stress, as judged by the levels of GSH and MDA induced by the highest amount (40%) of fructose. The level of stress induced by honey was minimal and insignificant ($p>0.05$). Honey reduced GSH levels ($p>0.05$) and the associated lipid peroxidation as indicated by the MDA amounts (Table I) compare well with control value.

DISCUSSION

Recall that in this study, the concentrations of glucose, GSH and MDA in ocular humour were measured in rats fed with either fructose or honey. Overall, fructose feeding when compared with control and honey fed groups significantly ($p<0.05$) increased the amounts of glucose, levels of oxidative stress and lipid peroxidation in ocular humour of exposed Wistar rats. But values obtained with honey feeding compare well with control values.

Concentrations of oxidative stress biomarkers (GSH and MDA) in the ocular humour of fructose fed rats indicate significant cellular and molecular levels of oxidative stress.

This stress which can increase IOP has been shown by the over expression of intracellular nitric oxide synthase, iNOS, an enzyme primarily involved in mitochondrial lipid peroxidation and damage of cell membrane¹¹. Such over expression has been validated by the accumulation of intracellular MDA. Fructose feeding also increased ocular glucose and this observation agrees with previous reports³. It has been reported that fructose feeding affects IOP via increased blood glucose. Compromise in GLUT 5 (fructose transporter) and receptors has been shown¹² to elicit metabolic disturbances associated with increase in blood glucose and lipids. Honey consumption has been observed to lower plasma glucose, C-reactive proteins, homocysteine and blood lipids in healthy, diabetic and hyperlipidaemic subjects³. Honey, unlike fructose appears not to seriously induce ocular stress and this may not affect IOP significantly. Nevertheless, further studies are required to fully document the ocular benefits arising from honey consumption.

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