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Changes in Biomakers of Ocular Pressure and Oxidative Stress in Aqueous Humour of Honey Fed Rats

A.E. OJIEH[1], I.E. AWIRE[2]*, P.E. AWHIN[2] and H.N. MADOJEMU[2]

[1]Department of Physiology, Delta State University, Abraka (Nigeria).
[2]Department of Medical Biochemistry, Delta State University, Abraka (Nigeria).
E-mail: ighele@yahoo.com

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ABSTRACT

Fructose has been reported to increase the concentrations of solutes in eye humour. Honey has been observed to contain high amount of fructose. Whether honey displays similar effect in the eye is not clearly documented. Therefore, this study attempts to assess the concentrations of the biomarkers for ocular pressure and oxidative stress in the eye humour of honey fed rats. Forty nine (49), ten weeks old, male and female Wistar rats, weighing 78-95g were obtained from Laboratory Animal Centre, Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria. They were divided into seven groups. The first group (Group A) served as the control group and the other six were the experimental groups. The experimental groups were fed with 20% (Group B), 30% (Group C) and 40% (Group D) honey. Fructose quantities equivalent to amounts in 20% (Group E), 30% (Group F) and 40% (Group G) honey were given to the remaining experimental animals. At the end of the 28 days, the animals were sacrified under mild anaesthesia and the two whole eyes were plucked and punctured in order to obtain the humour. Biomarkers of ocular pressure (glucose and albumin) and oxidative stress (glutathione [GSH] and malondiadehyde [MDA]) were assayed using standard procedures. Results show that honey and lower amounts of fructose did not yield any statistically significant ($P>0.05$) difference in the levels of the biomarkers determined, but fructose feeding at quantities equivalent to the amount in 40% honey, significantly ($P<0.05$) reduced GSH, but increased ($P<0.05$) the humours MDA and glucose levels. Unlike fructose, honey may not be associated with the risk of increased ocular pressure arising from enhanced glucose concentration. The honey induced peroxidative activity was also minimal.

Key words: Biomarkers, Ocular pressure, Oxidative stress, Honey Fed Rats.

INTRODUCTION

Honey is a mixture of sugars and other compounds. With respect to carbohydrates, honey is mainly fructose (about 38.5%) and glucose (about 31.0%) (Crane, 1999), making it similar to the synthetically produced inverted sugar syrup which is approximately 48% fructose, 47% glucose, and 5% sucrose. Honey's remaining carbohydrates include maltose, sucrose, and other complex carbohydrates (Crane, 1975). As with all nutritive sweeteners, honey is mostly sugars and contains only trace amounts of vitamins or minerals. Honey also contains tiny amounts of several compounds thought to function as antioxidants, including chrysin, pinobanksin, vitamin C, catalase, and pinocembrin (Gheldof, *et al.,* 2002). The specific composition of any batch of honey depends on the flowers available to the bees that produced the honey.

It has been previously reported that aqueous flow in the retina was decreased by 15% in patients with type 1 diabetes without evidence of microvascular complications, and severity of diabetic retinopathy has been observed to be related to length and magnitude of exposure to hyperglycaemia (Ensminger, *et al*., 1983). Diabetic retinopathy appears to exist concurrently with microvascular abnormalities in the kidney, and

signals diabetic nephropathy (Cook, *et al*., 2005). If a decrease in aqueous flow accompanies diabetic retinopathy, and if these changes are related by common pathophysiologic mechanisms, it is hypothesized that patients with evidence of retinopathy will have decreased aqueous flow that could impact intraocular pressure (IOP). The relationship between rate of aqueous flow and the severity of complications in the eyes of patients with diabetes is not known.

Although fructose does not appear to acutely increase insulin levels, chronic exposure seems to indirectly cause hyperinsulinemia and obesity through other mechanisms. One proposed mechanism involves GLUT5, a fructose transporter that is found to have significantly higher expression levels in young Zucker obese rats compared with lean controls. As the rats age and become diabetic, GLUT5 abundance and activity is compromised, causing an even more marked insulin resistance over lean rats, implying a possible role of GLUT5 receptors in the pathology of metabolic syndrome associated with fructose feeding and insulin resistance (Hadjduch, 2004). In rats fed 66% fructose for 4weeks, insulin receptor mRNA, and subsequent insulin receptor numbers in skeletal muscle and liver were significantly lower compared with rats fed a standard chow diet. Compromise in insulin receptor and signaling may elicit metabolic effects associated with increase in blood glucose and lipids. Fructose feeding affects IOP, but honey consumption lowers plasma glucose, C-reactive protein, homocysteine and blood lipids in healthy, diabetic and hyperlipidemic subjects (Al-Waili, 2004). However, the effect of honey on biomarkers of ocular pressure (glucose, albumin) and oxidative stress (GSH, MDA) are not clearly documented. This study attempts to provide useful evidence regarding the effect of natural honey on ocular pressure and oxidative status.

MATERIAL AND METHODS

Animal care and handling

Forty-nine male and female rats weighing 78-95g were used in the study. The rats were divided into seven (7) groups. Group A, the control rats were given rat chow. Groups B, C and D were fed with 20%, 30% and 40% honey, respectively. The other experimental groups E, F and G received fructose quantities equivalent to amounts in 20%, 30% and 40% honey. The rats were fed for 28 days and allowed free access to water. Cages were cleaned regularly and animals were kept in 12 h light and 12 h dark cycle. Animal care and handling complied with standard recommendations.

Collection of humour from the rats

The two whole eyes of chloroform anaesthetized rats were plucked and punctured in order to collect the humour. The humour obtained was centrifuged at 1000rpm for 15min at 37^{0}C. The supernatant was carefully removed with Pasteur pipette and stored frozen until needed for analysis.

Analysis

The amounts of glucose (Trinder, 1969), albumin (Braford, 1976), glutathione (Kikkawa, *et al*., 1992) and malondialdehyde (Buege and Aust, 1978) in the eye humour were determined using standard procedures previously adopted by other workers.

Statistical analysis

The group Mean ±SEM was calculated for each biomarker and significant differences between means were evaluated by analysis of variance (ANOVA). Post test analysis was carried out using the Turkey multiple comparison test. Values of $P< 0.05$ were considered as statistically significant.

RESULTS

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

Increase in albumin and glucose concentrations in the aqueous humours could contribute to the changes in ocular pressure. The administration of honey or fructose, increased the concentration of glucose in the humour in a dose-dependent manner when compared with control. There was also an indication of oxidative stress, as judged by the levels of GSH and MDA induced by increased amount of fructose. The level of stress induced by honey was minimal. It increased GSH with little associated lipid peroxidation

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

Markers	A	B	C	D	E	F	G
Ocular pressure							
´ Glucose(mmol/L)	2.44±0.08	2.50±0.09	2.52±0.14	2.54±0.14	2.49±0.22	2.52±0.25	2.55±0.31
´ Albumin(g/dL)	3.04±0.09	2.97±0.08	3.08±0.61	3.14±0.72	2.88±0.50	2.90±0.80	2.99±0.06
Oxidative Stress							
´ GSH(mg/g)	44.6±4.1	44.7±4.0	46.9±5.6	47.9±7.3	46.9±4.6	40.1±3.9	34.6±5.5
´ MDA ($\times 10^{-5}$ nmmol/mL)	0.40±0.07	0.43±0.07	0.45±0.03	0.46±0.07	0.44±0.07	0.49±0.07	0.56±0.06

Values are expressed as Mean ±SD for n=7 rats/group.
GSH =Glutathione (reduced).
MDA = Malondiadehyde.
A =100% grower's mash (Control).
B =80% grower's mash+20% honey.
C=70% grower's mash +30% honey.
D =60% grower's mash +40% honey.
E =84.4% grower's mash+7.2g (Glucose) and 8.4g (Fructose).
F=76.6% grower's mash +10.8g (Glucose) and 12.6g (Fructose).
G =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

DISCUSSION

In the present study, the concentrations of glucose, albumin, GSH and MDA in aqueous humour were measured in rats fed with honey. We observed a significant higher concentration of glutathione (GSH) in aqueous humor of rats fed with honey compared with control. These groups of experimental rats have enhanced defense against oxidative stress when compared with the fructose fed groups.

One of the greatest challenges in oxidation research today is the determination of oxidation stress *in vivo*, because proteins are ubiquitous in all cells and tissues, and are susceptible to oxidation modification. Concentrations of the oxidative biomarkers (GSH and MDA) in the aqueous humour activates diverse cellular and molecular signals of stress, such as the upregulation of the ocular pressure which, increases IOP and results in oxidative stress. This stress is shown by the over expression of iNOS, and enzyme primarily involved in mitochondrial lipid peroxidation and damage of cell membrane (Halliwell and Gutterridge, 1984), validated by the accumulation of intracellular MDA. Honey, unlike fructose appears not to seriously affect ocular stress, but the induced increase in humour's glucose may disturb ocular pressure. Further studies are required to fully document the implication of the observed increase in ocular glucose.

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