

Changes in Hepatic Glutathione and Malondialdehyde Levels in Honey Fed Wistar Rats

U.E. UZUEGBU^{1*}, C. AMADI², A. OSASUYI³, A.O. OPAJOBI¹ and O.M. UNUERO¹

¹Department of Medical Biochemistry, Delta State University, Abraka (Nigeria).

²Department of Medicine, Lagos University Teaching, Hospital, Lagos (Nigeria).

³Department of Nursing Science, Delta State University, Abraka (Nigeria).

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ABSTRACT

Researchers have shown that fructose consumption induces oxidative stress and lipid peroxidation. Whether honey, which contains high amount of fructose could also elicit such tendency is not clearly established. In this study, changes in biomarkers of oxidative stress (glutathione [GSH]) and lipid peroxidation (malondialdehyde [MDA]) induced in hepatic tissue of Wistar rats exposed to varying doses (20%, 30% and 40%) of honey and equivalent amounts of fructose were investigated. Results show that both honey and fructose administrations for 4 weeks significantly ($P < 0.05$) increased hepatic GSH in a dose-dependent manner when compared with control. The hepatic MDA for the control, honey and fructose fed groups were not significantly ($P > 0.05$) different. Honey or fructose challenges the liver to improve oxidative defense mechanisms and lower lipid peroxidation activity. These might be initial responses. Overall, long term effects need proper documentation.

Key words: Biomarkers, Hepatic Glutathione, Malondialdehyde Levels, Wistar rats.

INTRODUCTION

Honey is a delicious viscous sweetener made naturally by bees for their own nourishment. Honey comes in a range of colours including white, amber, red, brown and almost black. Honey is a mixture of sugars and other compounds. With respect to carbohydrates, honey is mainly fructose (38.2%) and glucose (31.1%) though others include maltose, sucrose and complex carbohydrates (Anon, 1995).

Honey is known to exhibit a broad spectrum of activities including antiviral, antibacterial and immunostimulant (Molan, 2002; Mato, *et al.*, 2003). It was found to have antioxidant activity due to its high content of flavonoid (Mabrouk, *et al.*, 2004). Honey has been found to have a hepatoprotective activity against methyl nitrosourea-induced oxidative stress and inflammatory response in rats by 100% by keeping normal defense system

(Mabrouk, *et al.*, 2002). Honey also has hepatoprotective activity against CCl_4 -induced liver damage in sheep (Al-Waili, 2003) and in mice (Resende, *et al.*, 2003) by improving liver functions.

A wide variety of oxidising molecules such as ROS and depleting agents can alter the glutathione (GSH) redox state, which is normally maintained by the activity of GSH-depleting and GSH-replenishing enzymes (Halliwell and Gutteridge, 1996). Formation of ROS is a normal consequence of a variety of essential biochemical processes. It is also known that oxygen radicals may be formed in excess in chronic diseases of the gastrointestinal tract. The main source of oxidants in the liver are probably phagocytes and inflammatory state mediators, which are present in the tissue of patients with liver diseases and could generate oxidants upon activation, which might contribute to the increased risk of cancer (Szatrowski and Nathan, 1991). Oxygen radical

production which increases with clinical progression of diseases, involves increased lipid peroxidation. The process of lipid peroxidation consists of the oxidative conversion of polyunsaturated fatty acids to products known as malondialdehyde or lipid peroxides which are the most studied, biologically relevant, free radical reaction product.

GSH homeostasis at cellular level is maintained by the balance between biosynthesis, uptake, oxidation and export. Its decrease in cirrhotic and cancer tissues is due to a reduced synthesis of the tripeptide by the diseased liver. The alteration may influence the capability of the liver to provide protection against oxidative damage (Dalhoff, *et al.*, 1992; Loguercio and Di Pierro, 1999; Fernandez-Checa and Kaplowitz, 2005). A low GSH level is associated with an increased production of reactive oxygen species and free radicals in cirrhotic and liver cancer tissues. These ROS is actively scavenged by GSH, resulting in the formation of the oxidized form of GSH (GSSG). This oxidized form is rapidly converted to GSH by GSH reductase (Kikkawa, *et al.*, 1992).

Studies have shown that decreased GSH content, increased MDA level and changes in activities of GSH related enzymes (Casaril, *et al.*, 1985; Skrzydlewska *et al.*, 2003). A number of clinical trials have been done on the benefits of honey and its effect on different biochemical parameters in the body. The aim of this study was to evaluate the level of glutathione and malonaldehyde, the respective indicators of antioxidants and lipid peroxidation status in hepatic tissue of Wistar rats fed with honey.

MATERIAL AND METHODS

Animals

Animal care complied with the guideline of the National Institute of Health and the recommendation of the INRA Ethics committee in accordance with Degree N0.87-848. Forty nine adult Wistar rats of both sexes obtained from the Animal House, Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria, were randomly assigned into seven groups. They were put in a wire-bottom cages (7rats/cage) at room temperature (25-28°C) with a 12hour light/12hour dark cycle. All rats

were first adapted to a starch-based semi purified diet (growers' mash), and animal hold environment for ten days. Thereafter, diets were composed (Table 1) and fed to the groups (A-G) accordingly. Rats were given unrestricted access to food and clean potable water. Feed consumption was estimated daily and body weight was determined weekly. The animals were 10 weeks old and weighed between 78g and 95g.

Sample Collection and Tissue Preparation

At the end of the four weeks of dietary treatment, the rats were deprived of food overnight. All the rats were sacrificed under mild anaesthesia (chloroform) and the liver was rapidly excised and washed in ice-cold normal saline (9g NaCl/L). The liver tissue was sliced, homogenised and centrifuged at 1200 x g for 15min at room temperature. The supernatant obtained was stored frozen and used for the assay of GSH and MDA.

Sample assay/biochemical analysis

Determination of Liver GSH

Reduced glutathione (GSH) was determined by the Trichloroacetic acid (TCA) method (Ellman, 1959). To the homogenate was added 10% TCA and the resulting content was centrifuged. The supernatant obtained was then treated with 0.5ml of Ellman's reagent (19.8mg of 5,5'-dithobisnitro benzoic acid (DTNB) in 100ml of 0.1% sodium nitrate) and 3.0ml of phosphate buffer (0.2M, pH 8.0). The absorbance was read at 412nm and GSH concentration was expressed as mg/g tissue.

Determination of Liver MDA

Lipid peroxidation was estimated using the thiobarbituric acid reacting species (TBARS) and malondialdehyde (MDA) as standard (Buege and Aust, 1978). One millilitre (1.0ml) of the tissue homogenate was added to two millilitre (2.0ml) of the TCA-TBA-HCl reagent (15% w/v TCA, 0.375M TBA and 0.25N HCl). The content were boiled for 15min, cooled and centrifuged at 10,000g to remove the precipitate. The absorbance was read at 535nm and the malondialdehyde concentration of the sample was calculated using extinction coefficient of $1.56 \times 10^{-5} \text{ M}^{-1} \text{ cm}^{-3}$

Statistical Analysis

The data obtained were expressed as

Mean \pm Standard Deviation. Statistical analysis was evaluated using ANOVA and $p < 0.05$ were regarded as statistically significant (Armitage, 1971).

RESULTS

Glutathione (GSH) and malondialdehyde (MDA) levels were determined in hepatic tissues of forty-nine Wistar rats fed honey-based diets for four weeks and the results obtained are summarized (Table 2).

Results (Table 2) show the changes in hepatic GSH and MDA induced by honey based diets in Wistar rats. Honey administration (20%, 30% and 40%) significantly ($p < 0.05$) increased hepatic GSH in a dose dependent manner when compared with the level for the control animals. The groups given the equivalents of fructose and glucose in 20%, 30% and 40% honey (i.e. Groups E, F and G) also had significant ($p < 0.05$) increase in liver GSH in a trend similar to that observed for the honey-treated groups (Group B; 20%, Group C; 30% and Group D; 40%)

Table 1: Composition of diets for the different groups of animals

Composition	A	B	C	D	E	F	G
Growers' mash (g)	100	80	70	60	84.4	76.5	58.8
Honey (g)	-	20	30	40	-	-	-
Fructose (g)	-	-	-	-	8.4	12.6	16.8
Glucose (g)	-	-	-	-	7.2	10.9	14.4
Total (g)	100	100	100	100	100	100	100

Groups E, F and G were given fructose and glucose equivalent to the amounts in 20g, 30g, and 40g honey.

Table 2: Changes in rats' hepatic GSH and MDA levels induced by honey-based diets

Group	GSH(mg/g tissue)	MDA (nmol/mL)
A(n=7) 100	15.02 \pm 4.01	5.9 \pm 0.06
B(n=7)	40.63 \pm 7.92*	5.9 \pm 0.05
C(n=7)	41.05 \pm 6.21*	5.6 \pm 0.02
D(n=7)	44.05 \pm 5.80*	5.7 \pm 0.03
E(n=7)	23.3 \pm 3.44*	5.3 \pm 0.08
F(n=7)	43.3 \pm 4.42*	5.5 \pm 0.07
G(n=7)	45.62 \pm 7.73*	5.7 \pm 0.04

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

GSH = Glutathione (reduced).

MDA = Malondialdehyde.

A = Control (100% growers' mash).

B = 20% honey (80% growers' mash).

C = 30% honey (70% growers' mash).

D = 40% honey (60% growers' mash).

E = 7.2g glucose and 8.4g fructose, (84.4g growers' mash)

F = 10.8g glucose and 12.6g fructose (76.6g growers' mash)

G = 14.4g glucose and 16.8g fructose, (68.8g growers' mash)

* $P < 0.05$

Glucose and fructose quantities were equivalent amount in 20%, 30% and 40% honey.

The hepatic MDA levels for both honey and fructose/glucose-treated animals were not significantly ($p>0.05$) different from the control value.

DISCUSSION

In this present study the levels of glutathione were determined as the protective mechanism against oxidative damage and the level of malondialdehyde as a final product of lipid peroxidation.

The data obtained from this research show an increased GSH level in the hepatic tissue of honey administered rats. There was a statistical significant increase in the level of GSH ($p<0.05$) in the liver tissue of honey fed rats when compared with the control group. The increased GSH may be due to challenged hepatic synthesis related to increase in production of reactive oxygen species leading to enhanced oxidative reaction in hepatic tissue (Maborouk, *et al.*, 2004). The inference is drawn from the evidence (Table 2) that fructose/glucose fed animals also had increased ($p<0.05$)

hepatic GSH levels. Metabolites of fructose has been observed to induce free radical production (Kelley *et al.*, 2004). Therefore the metabolism of fructose content of honey may have caused free radical generation and the challenge by the liver to synthesize more GSH. This tendency may have overwhelmed the antioxidant capacity of honey.

The results of the present study revealed that administration of honey caused a decrease in MDA level in the liver and a significant increase ($p<0.05$) in antioxidant GSH level. These observations agrees with other studies which have shown increased GSH content, decreased MDA level and changes in the activities of GSH related enzymes (Casaril, *et al.*, 1985; Skrzydlewska, *et al.*, 2003). GSH provides powerful antioxidant protection to hepatic tissue exposed to reactive oxygen species (Mabrouk, *et al.*, 2004). Nevertheless, the long term sustenance of GSH system and the associated protection of liver tissue from peroxidative injuries arising from honey feeding should be further investigated.

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