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Correlation of liver and blood reduced glutathione levels and neutrophil cell count in honey fed wistar rats

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ABSTRACT

Liver and blood reduced glutathione (GSH) levels and neutrophil cell counts were estimated in a group of wistar rats fed with honey. The rats were divided into four groups. Group1 was the control and the animals in this group were fed with rat chow without honey substitute, the group2 was fed with 20% honey, group3 was fed with 30% honey and the group4 was fed with 40% honey. The results of the liver and blood GSH and neutrophil cell count are liver GSH: Gp 1(14.66%) Gp2 (16.05%) Gp3 (19.00%) Gp4(19.30%); blood GSH: Gp1 (28.94%) Gp2 (31.21%) Gp3(30.28%) and Gp 4(29.99%), neutrophilcellcount$\times 10^9$/l:Gp1(1.43±0.19)Gp2(1.21±0.32)Gp3(2.55±0.89) Gp4(1.56±1.13). When these results were compared with the results obtained from white blood cell differential count(WBC $\times 10^9$/l: (Gp1=2.82±0.53),Gp2(2.78±0.45),Gp3,(7.29±2.21),Gp4(4.61±3.54). Observations show that there was reduced neutrophil cell count. This reduction widens with increasing honey in feed. Honey may reduce blood GSH level. This observation requires further investigation in order to ascertain the overall effect of honey on the oxidative homeostasis.

Key word: Liver, glutathione, neutrophil count, white blood cell count, honey.

INTRODUCTION

Oxidation stress could be defined as an imbalance between the generation of reactive oxygen species and antioxidant defense capacity of the body. It is closely associated with a number of diseases like cancer, rheumatoid arthritis, HIV, cardiovascular diseases, diabetes and aging. The understanding of the reactive oxygen species generation and biology is necessary for designing an optimal nutritional countermeasure against nutritional based disorders like diabetes mellitus. Free redicals (e.g. superoxide, nitric oxide and hydroxyl radicals and other reactive species (e.g. hydrogen peroxide, peroxynitrate and hydrochlorous acid) are produced in the body, primarily as a results of aerobic metabolism. Antioxidants (e.g. glutathione, organic, citrilline, taurine, creatine, solenuim, zinc vitamins E, C and A, and tea polyphenols) and antioxidants enzymes (e.g. superoxide dismutase, catalase, glutathione reductase, and glutathione peroxides) exert synergistic action in scavenging free radicals.

Free radicals are defined as molecules having an unpaired electron in the outer orbit (Gilbert, 2000). They are generally unstable and very reactive. Examples of Oxygen free radicals are superoxide, hydroxyl, peroxyl (RD_2), alkoxyl (RD^-) and hydroperoxyl (HO_2^-) radicals. Nitric oxide and nitrogen dioxides (NO_2) are two nitrogen free radicals. Oxygen and nitrogen free radicals can be

converted to other non-radicals reaction species as hydrogen peroxides, hydrochlorous acid (HOCL) hypobromonous acid [HOBr] and peroxynitrite ($ONOO^-$). Both ROS, reactive Nitrogen species (RNS), and reative chlorine species are produced in animals and humans under physiologic and pathologic condition (Evans and Halliwill 2001). Free radicals may play important role in the origin of life and biological evolution, implicating their beneficial effect on the organism. For example, oxygen radicals exert critical actions such as signal transduction, gene transcription and regulation of soluble guanylate cyclase activity in cells (Zheng and Storz 2000; Lander, 1997). Also, NO is one of the most widely spread signaling molecules and participates in virtually every cellular and organ function in the body(Ignarro, *et al.,* 1999). Physiological levels of NO produced by endothelial cells are essential for regulating the relaxation and proliferation of vascular smooth muscles cell, leukocytes, adhesion, plalelet aggregation, angiogenesis, thrombosis, vascular tone and hemodynamics(Iqnarro *et al.,* 1999). NO produced by neurons serves as neurotransmitters and those generated by activated macrophages is an important mediators of the immune response (Freidovich 1999).

As oxidants and inhibitors of enzymes containing an iron-sulfur centre, free radicals and other reactive species cause oxidation of biomolecules (e.g. protein, amino acids, lipids and DNA), which leads to cell injuring and death (mcCord, 2000., Freidovich, 1999). Radiation induced ROS will severely alter the physical, chemical and immunologic properties of SOD, which would further generate oxidative damage in cells (Fang, 1991). The cytotoxic effects of free radical is damaging to mammalian cell and mediates the pathogesis of many chronic diseases, but is responsible for the killing of pathogens by activated macrophages and other phagocytes in the immune system. (McCord, 2000). Thus, there are two faces of free radicals in biology in that they serve as signaling and regulatory molecules at physiologic levels but as highly deleterious and cytotoxic oxidants at pathologic levels (Freidovich, 1999).

Honey is a biological product with a very complex chemical composition. It is composed primarily of fructose and glucose and also contain about 3 to 4% fructooligosacharrides (FOS) (Chow, 2002). Honey has been used in many pathologic cases where it is believed it could have medicinal benefit. It has been used in dressing burnt wounds; also used to soothe the throat in coughing condition; and more especially, it has gain preference to refined carbohydrate product with the believe that it will prevent such diseases like diabetes. Many diabetic patients are also found to be using it in place of refined carbohydrate products. Although, there is little evidences that modest amount of fructose have detrimental effects on carbohydrates and lipid metabolism, larger doses have been associated with numerous metabolic abnomalities in laboratory animals and humans, suggesting that high fructose consumption adversity affects health (Henry, *et al,* 1991.,Hallfrisch, 1990). It has been evidently observed that honey fed rats exhibited lipid peroxidation which is one of the cardinal evidence of oxidative stress. It is there fore necessary to assess oxidative stress in honey fed rats to enable us defermine the physiologic benefit of substituting honey for refined carbonhydrate product which seen to be gaining acceptability among people with such carbohydrate metabolic disorders. It has also been reported (Busserolles, et al 2002) that substituting honey for refined carbohydrate protects rats from hyppertriglyceridemic and pro-oxidative effects of fructose.

The aim of this study is to access the effects of feeding wistar rats with honey on the levels of liver and blood reduced glutathione and neutrophil cell count.

MATERIAL AND METHODS.

Twenty four Wistar rats weighing approximately 240g, were placed in the animal house cage for 14 days in the first instant to acclimatize, and then were fed with animal feed a product of *Topfeeds and flour Mills Ltd;* Sapele, Delta State, Nigeria. The animals were divided into four groups.

The first group was fed without honey, that is, the control group. The second group was fed with feed and 20% honey by weight (i.e. eighty grams of feed and twenty grams of honey). The

third group was fed with feed and 30% of honey by weight (i.e seventy grams of feed and thirty grams of honey), while the fourth group was fed with sixty grams of feed and forty grams of honey. The honey used was purchased from A and Shine International Limited, Abuja, Nigeria (undiluted, no artificial flavors or colour, no preservatives added) with NAFDAC No. 01-6025.

The four groups were given water without any other additives. This feeding regime was maintained throughout the 56day period of the experiment.

Collecton of blood samples

The rats were sacrificed on the morning of the 57th day after an overnight fast and blood samples were collected into EDTA Bottles, and liver tissue was also collected for liver glutathione analysis.

Estimation of liver and blood reduced glutathione

The blood reduced glutathione level was estimated using the method of Beutler *et al.* (1963). The liver reduced glutathione was estimated using the method of Ellman, 1959.

Hematological analysis

Abacus Junior Hematology Analyzer (an automatic analyzer) was used to perform the white blood cell count Red blood cell count, Platelets cell count and haematcrits analysis.

Statistical analysis

Data were expressed as mean + standard error of mean (SEM). Multiple comparisons of means were carried out using SPSS 10.0 Statistical Package

RESULTS

The results of the various hematological parameters in honey fed rats are shown in Table 1. Data revealed that the control group and the group that received 20% honey had significantly ($p<0.05$) lower WBC count when compared with the groups fed 30% honey(group 3) and 40% honey (group 4). However, there was an observed significant difference between group3 and group4, but there was no significant difference between the count of WBC in group1 and group2. Lymphocyte (Lym) count was observed to be increasing, Lymphocyte count in group 1 was much lower than the count in groups 2, 3 and 4. These increases have been found

Table 1: Various Hematological parameters in honey fed wistar

Parameters	Group1(n=4)	Group2(n=4)	Group3(n=2)	Group4(n=4)	Pvalue
WBC $\times10^9$/l	2.82±0.53	2.78±0.45	7.29±2.21	4.61±3.54	0.69
Lym. $\times10^9$/l	1.00±0.19	1.28±0.17	3.25±0.82	2.34±2.54	-
MID. $\times10^9$/l	0.73±0.42	0.43±0.23	1.49±0.50	0.74±0.55	-
GRA. $\times10^9$/l	1.43±0.19	1.21±0.32	2.55±0.89	1.56±1.13	-
RBC$\times10^{12}$/l	5.95±1.71	6.16±1.55	7.84±0.76	6.95±1.82	0.172
HGB g/l	92.33±31.48	99.25±29.78	114.50±8.50	97.00±22.05	0.172
HCT %	36.62±9.95	38.26±9.07	47.00±2.47	43.95±9.34	0.427
MCV fl	61.67±3.10	62.25±2.59	62.50±2.50	64.00±4.90	0.111
MCH pg	15.33±1.23	15.93±1.43	14.80±2.50	14.47±3.43	0.123
MCHC g/l	246.33±29.8	255.00±29.55	244.00±30.50	223.00±35.95	-
RDWc %	18.1±2.10	18.60±1.78	18.25±0.45	19.20±1.11	0.324
PLT$\times10^9$/l	440.33±200.40	532.25±234.28	566.00±70.00	806.00±263.72	-
PCT %	0.34±0.16	0.43±0.18	0.52±0.06	0.76±0.26	-
MPV fl	7.93±0.29	8.05±0.17	9.15±0.25	9.37±0.12	-
PDWc %	34.33±0.25	34.50±0.26	35.95±0.35	36.20±1.07	-

to be statistically significant($p>0.05$). Granulocyte (Gra) count was also remarkable, there was decrease granulocyte Count with increased honey and this decrease is also significant ($p>0.05$) (Table 1).

The results of reduced glutathione in the liver and blood is also shown in Table 2. The result show an increase in the liver reduced glutathione level in all the experimental groups (i e groups 2, 3 and 4). The increase was significant ($p>0.05$). The result for the blood reduced glutathione also shows an increased reduced glutathione level in all the experimental groups but the increases was not statistically significant (Table 4). The values of reduced glutathione in the blood is relatively higher than the levels in the liver. In the blood there was a noticeable drop in the reduced glutathione level in the groups fed with 30% and further drop in 40% when compared with values in group 2, and these reduced values were not statistically significant.

Table 2: Showing the valeus for liver and bloob reduced glutathione

Parameters	Goup1(n=4)	Group2(n=4)	Group3(n=2)	Group4(n=4)
Liv. GSH	14.66 %	16.05 %	19.00 %	19.30 %
Blood GSH	28.94%	31.21%	30.28%	29.99%

Table 3: ANOVA table

			Sum of sq	Df	Mean Square	F	Sig.
Liver GSH Status	Between Groups	(Combined)	54.587	3	18.196	3.866	*0.045
		Linearity	51.788	1	51.788	11.004	*0.008
		Deviation from Linearity	2.800	2	1.400	0.297	0.749
	Within roups		47.062	10	4.706		
	Total		101.649	13			

*Indicates significant test at 95% degree confident limit

Table 4: ANOVA table

			Sum of sq	Df	Mean Square	F	Sig.
Blood GSH* Status	Between Groups	(combined)	10.420	3	3.473	.888	.480
		Linearity	.927	1	.927	.237	.637
		Deviation From linearity	9.494	2	4.747	1.214	.337
	Within groups		39.101	10	3.910		
	Total		49.521	13			

*Indicates significant test at 95% degree confident limit

DISCUSSION

Oxidative stress occurs when the balance between free radical generation and antioxidant defense is upset. In such cases, active oxygen species and free radicals are so reactive and short lived that their levels are difficult to measure directly. For this reason, most methods measure only the product of oxidative stress. Glutathione (GSH) is a tripeptide and a major intracellular antioxidant which accounts for over 90% of the intracellular non-protein thiols. One mechanism of action of GSH is through removal of intracellular hydrogen peroxide by providing substrate for GSH peroxidase, the major hydrogen peroxide removing enzyme.

Indirect evidence indicates that HIV infection is associated with an increased consumption of antioxidants. The concentration of intracellular GSH in the peripheral blood mononuclear cells and lymphocytes of asymptomatic HIV-seropositive patients was found to be lower than in the healthy control group (Roederer, *et al.,*1990; Smith *et al.,*1990; Staal *et al.,*1992). In addition, GSH levels were profoundly depressed in patients with AIDS and AIDS-related complex.

A high plasma glutamate is associated with a low level of thiol, leading to a reduction of intracellular GSH and impairment in T cell function (Eck, *et al.,* 1989). The consumption of intracellular non-protein thiol in these situations may play a role in increased oxidative stress. HIV-infected individuals show a 30-40% decrease in GSH in both CD4+ and CD8+ T cells. This decrease is due primarily to the specific removal from the circulation of a class of T cells with high GSH content (Roederer, *et al.,* 1990).

Neutrophils serve as the major defense of the body against acute bacterial and certain fungal infections. Neutrophils usually constitute about 45 to 75% of all white blood cells in the bloodstream (Kuby,1997). When the neutrophil count falls below 1,000 cells per microliter of blood, the risk of infection increases and when it falls below 500 cells per microliter, the risk of infection increases greatly.Without the key defense provided by neutrophils, people have problems controlling infections and are at risk of dying from an infection. From the results (Table1), it can be observed that in Group 1(i. e. the control group), neutrophil count was about 75% of the WBC. But from Group 2 to 4 neutrophil count was greatly reduced to about 35% of the WBC. The reason for the reduced neutrphil count in honey fed rats have not been documented. Neutropenia is defined as abnormally low number of neutrophils in the blood and as noted above this will signicantly increase the risk of life threatening infections. The physiopathology of neutropenia in honey feeding has not been clearly linked.

Tables 1 and 2 will a relationship between reduced glutathione levels and neutrophil cell count in honey fed rats. It can be observed that between Groups 1 and 2, there was increase in the reduced glutathione level in the Group 2 compared with the level in Group1. There was a significant reduced level of glutathione in Groups 3 and 4 when compared with Group 1. From this observation, it could be suggested that 20% honey intake may have anti-inflammatory and antioxidant benefits, but higher doses (30% or 40%) could upset oxidative homeostasis.

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