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Full Length Research Paper

A study on the oxidative properties of dietary galactose on reduced glutathione (GSH) in experimental rats

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Twenty (20) albino rats of the wistar strain were self-bred and divided into two groups, each containing ten (10) rats and acclimatized for two (2) weeks in separate wooden, but well ventilated cages, labeled test and control. The test group was placed on a diet composed of 30% D-galactose and corn starch as the carbohydrate source, while the control group was placed on the same diet composition but with corn starch only, serving as the carbohydrate source. Both groups were fed ad libitum with food and water, and observed closely for a period of four (4) weeks. Weight and weight changes of the rats were measured on a daily basis. At the end of four (4) weeks, about 80% of the rats in the test group had developed cataract (as evidenced by the appearance of white patches in the lenses of the rats), while there was no sign of cataract in the lenses of the rats in the control group. For all the organs analyzed, the concentration of reduced glutathione (GSH) in the test rats was found to be much reduced, when compared with that of the rats in the control group. The result of this study thus indicates that with continued administration of excess amount of dietary D-galactose, the concentration of reduced glutathione (GSH) in the body organs will be significantly depleted.

Keywords: D-galactose, Reduced Glutathione (GSH), Cataract.

INTRODUCTION

A cataract is the clouding or opacity that develops in the human lens (Geddes and Grosset, 2001). Many causes such as ageing (senile cataract), diabetes mellitus, radiation therapy or UV rays, ocular disease uveitis, glaucoma, intraocular tumors (*Retinitis pigmentosa*), skin diseases (atopic dermatitis, scleroderma), drugs (corticosteroids) and trauma had been implicated in cataract formation (Gardiner, 2001).

The development of lenticular opacities becomes more apparent with increased regularity if the animals are fed with adequate diet in which galactose is the sole carbohydrate constituent, provided that galactose comprises 25% to 70% of the whole diet. Galactose, flooding into the retinal tissue in excess, initiates non-enzymatic glycation and enters into the polyol pathway to generate free radicals and induce oxidative damage, as well as activating protein kinase C and other diabetes-like abnormalities (Kern and Engerman, 1996; Kowluru *et al*., 2000). The younger the animal and the higher the dose, the more rapidly the cataract appears. It is delayed by the administration of Insulin and exercise, and hastened by the topical or systematic administration of steroids (Ortonello *et al*., 2000).

The polyol pathway has been implicated as the primary cause of cataractogenesis in diabetics. The formation of hydroxyl radical was detected in sugar cataracts induced by galactose in rats using ESR (Electron spin resonance) spin trapping method with a spin trapping agent (Kubo *et al*., 1999).

In normal animal, galactose can be metabolized to some extent by specific enzymes to glucose and then to glycogen, but when fed in large quantities, as it is in this study, it is undoubtedly toxic. Such animal survives little longer than starved controls, probably owing to the fall in

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the glucose content of the blood, a depletion of the liver's glycogen store (Gardiner, 2001). When fed with galactose, weaning rats show an increase of this substance in the aqueous humor and a smaller increase in the lens, suggesting that it penetrates into the extracellular spaces but not into the fibres (Geddes and Grosset, 2001). The excess sugar is reduced by the enzyme aldose reductase to produce large quantities of sugar alcohols, particularly galactitol and dulcitol. This change results in high osmolarity of the lens, which presumably accounts for the early appearance of vacuolation (Rahi and Ganner, 1976; Kubo *et al*., 1999; Thomas *et al*., 2005).

Due to some free radical production in animal cells, which are inevitable and because they can be very damaging, defenses against the deleterious actions of free radicals have evolved. These are known as antioxidant defenses and the two main categories include; those which prevent the generation of free radicals and those that intercept any that are generated. They exist in both the aqueous and non-enzyme states (Suryanarayana *et al*., 2003). Catalase and Glutathione peroxidase are enzymes whose role is to safely decompose peroxides. The former is mainly located in peroxisomes and acts upon hydrogen peroxides while the latter is found in the cytosol of most cells. In the aqueous phase, other compounds act as free radical scavengers. Ascorbic acid (vitamin C) is an important antioxidant both within cells and in the plasma (Stocker *et al*., 1991). Uric acid in plasma and glutathione in cell cytosol also possess strong free radical scavenging properties (Stocker *et al*., 1991). It is readily apparent that cells have evolved an array of antioxidant defenses designed to severely limit damage caused by free radicals, wherever and whenever it might occur.

Although, the toxicity of galactose on the lens has been studied for over 70 years, and galactose-induced cataract is well accepted as an animal model for an investigation of the cataracts in humans (Suryanarayana *et al*., 2003; Ai *et al*., 2000), very few studies had been reportedly carried out on the investigations into the interactions of excess dietary galactose and oxidative stress in extra-ocular tissues. This study aims at finding out the oxidative effect of excess dietary galactose on the lens and other tissues, using experimental weaned rats as a model.

MATERIALS AND METHODS

Materials

Galactose, reduced glutathione (GSH), Ellman's reagent [5-5'-dithiobis-2- nitrobenzoic acid (DTNB), trisodium trimetaphosphate were purchased from Sigma Chemical Co. , USA. All other chemicals and materials used in the research were obtained from the biochemistry laboratory of Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria.

Experimental subjects

Twenty (20) albino male rats of the wistar strain, and three (3) weeks old with an average weight of 250g, were used for the research. All were self-bred in the animal house of Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria. The rats were randomly assigned into two groups; test and control. The control group was fed with a normal stock chow based on the AIN-93 formula comprising of; corn starch (19.7% for test rats and 49.7% for control rats), α-D-galactose (30% for test rats only), sucrose (10.2%), groundnut (23.3%), groundnut oil (7%), vitamin mix (1%), salt mix/minerals (3.5%) and methionine (0.3%), while the test group was fed with the same chow but with the inclusion of 30% α-D-galactose (Reeves, 1997). All animals were housed in wooden but adequately ventilated cages and maintained at room temperature. Animals were housed in individual cage in a temperature and humidity controlled room having a 12hr light and dark cycle. All of the animals had free access to their respective feed and clean drinking water. Their weights were monitored weekly throughout the research period. The experiment was carried out for four (4) weeks after which food and water were withdrawn from the animals overnight. The rats were then sacrificed by cervical dislocation after anaesthesis with diethylether. Eyes were removed from each rat and lenses were extracted. The kidney and liver were also removed from all the rats sacrificed, cleaned of blood, weighed and then homogenized.

Care of animals

The care of the animals was in accordance with the U. S. Public Health Service Guidelines and approved by the Olabisi Onabanjo University, College Of Health Sciences, Animal Ethics Committee.

Preparation of crude homogenate

[a] Lens: A pair of lenses from rat in each group were pooled together and homogenized in 10% 0.05M ice-cold sodium phosphate buffer, pH 7.4. The homogenate was centrifuged at 15,000xg for 30minutes at 4^{o}C. The biochemical parameter was analyzed with the supernatant of the

Table 1a. Weights and weight changes of the liver, eye lens and kidney of the control and test rats

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	Liver (g)	Lens (g)	Kidney (g)
Control rats	2.1464 ± 0.1359	0.2185 ± 0.0016	0.4908 ± 0.0334
Test rats	2.4045 ± 0.1769	0.2073 ± 0.0012	0.6188 ± 0.0080

Table 1b. Percentage relative weight of organs to body weight of control and test rats.

	Liver (%)	Lens (%)	Kidney (%)
Control rats	4.51	0.46	1.03
Test rats	4.28	0.37	1.13

Values are expressed as Mean ± SEM; n = 10

Table 2. The results of the GSH analysis in the organs of experimental rats.

GSH (mg/g tissue)			
	Lens	Liver	Kidney
Control rats	2.66 ± 0.81^{a}	0.55 ± 0.13^{a}	0.94 ± 0.10^{a}
Test rats	0.50 ± 0.11^{b}	0.26 ± 0.05^{b}	0.50 ± 0.08^{b}
Percentage reduction in GSH level	81.20%	52.73%	46.81%

Values are presented as Mean ± SEM; n = 10. Values in the same column with different superscripts are significantly different from each other.

lens homogenate.

[b] Liver and Kidney: The liver and the kidney of each animal were homogenized separately in 10% 0.1M ice-cold phosphate buffer, pH 7.4. It was then centrifuged at 9,000xg for 20 minutes at 4^{o}C. The supernatant was carefully removed and used for the biochemical analysis.

Biochemical Analysis

Reduced glutathione (GSH) was assayed by the method of Beutler *et al*., (1963).

Statistics

Statistical analysis was carried out with student's t-test, with a $p<0.05$ level considered significant.

RESULTS

Weights of the selected organs of rats

Table 1 (a and b) is the result of the weights of the organs, and the percentage relative weight of organs to body weight, of both the control and test rats. The results indicate that there was a decrease in the percentage relative weight of organs to body weight of rats in the test group when compared to that of the control group for the liver and the lens. The apparent increase observed in kidney weight signifies toxicity of the over-consumed sugar on the organ.

Effect of overconsumption of dietary galactose on reduced glutathione (GSH) in the organs of control and test rats.

Shown in table 2 is the result of the effect of overconsumption of dietary galactose on reduced glutathione (GSH) in the lens, liver and the kidney of experimental rats. The result indicates that there was a significant reduction in the GSH levels of the organs of the test rats when compared to that of the control rats. The percentage reduction is highest in the lens, followed by that of the liver and lastly that of the kidney. This shows that the oxidative effect of galactose is highest in the lens, thereby depleting most of the glutathione stores in the lens.

DISCUSSION

Aldose reductase is the key enzyme for the polyol pathway. This enzyme has been found to play a pivotal role in the development of "sugar" cataracts (Pallavi, 2003). Galactokinase is the first enzyme in the metabolism of galactose; the conversion of galactose to galactose-1-phosphate. Galactokinase deficiency results in galactosemia, galactosuria and cataracts. Cataracts form in galactokinase-deficient individuals because ingested galactose cannot be metabolized and is therefore available for reduction to galactitol through the action of aldose reductase. The study of the enzymatic mechanism involved in the reduction of galactose to galactitol has revealed that the lens is a particularly favourable site for the accumulation and production of galactitol. Galactose concentration must be fairly high before the enzyme aldose reductase can convert significant amounts of the sugar to the alcohol form. In other tissues, even though the organ may be exposed to high levels of galactose, the phosphorylation mechanism is sufficiently active to keep the sugar at low levels. Severe oxidative stress has been reported to cause cell damage and death. In mammalian cells, oxidative stress appears to lead to increase in the levels of free Ca^{2+} and iron within the cells. It has been suggested that excessive rises in intracellular free Ca^{2+} may cause DNA fragmentation by activating endonucleases (Okezie, 1998).

Oxidative stress is caused by an imbalance between the production of reactive oxygen and biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage. GSH is an extremely important cell antioxidant. It is known to directly quench reactive hydroxyl free radicals and other oxygen-centered free radicals (Ajani *et al*., 2008). In healthy cells and tissues, more than 90% of the total glutathione is in the reduced (GSH) state and less than 10% exists in the disulfide form (GSSG). It is known as substrate in both conjugation and reduction reactions catalyzed by glutathione-S- transferase enzymes (Kidd, 1997). It can be reported based on the data generated in this study that the reduced activity of reduced GSH content may be implicated in damage to the liver, lens and kidney during cataract development resulting from overconsumption of dietary galactose.

The results of this study agrees with the work of Ajani *et al* (2008), who also observed a significant reduction in GSH, catalase and superoxide dismutase levels in the tissues of experimental rats after being placed on a high dosage of galactose. But the highest reduction was observed in the lens in contrast to the findings of Strother *et al* (2001), who reported the highest reduction of GSH level in the liver. This thus indicates that the highest oxidative damage occurs in the lens in galactose-fed rats, followed by the liver and then the kidney.

CONCLUSION

From this study, it could be concluded that with the continued administration of excess dose of dietary galactose, there is high risk of oxidative damage on the body organs with the eye lens being the most susceptible, as a result of generation of free radicals and the lowering of the body antioxidant defence mechanisms, of which reduced glutathione is significantly implicated.

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