GARLIC EXTRACT INHIBITS ACCUMULATION OF POLYOLS AND HYDRATION IN DIABETIC RAT LENS

V. K. SRIVASTAVA and Z. AFAQ
Department of Chemistry, University of Gorakhpur, Gorakhpur 273 009, India.

EXCESS glucose, galactose or xylose leads to sugar cataract formation in experimental animals and in man[1-3]. The importance of polyol pathway in sugar cataract formation has been very well established[4]. The polyol pathway, which consists of two reactions, constitutes one of the most thoroughly studied series of reactions in the lens.

$$\left.\begin{matrix}\text{Glucose} + \text{NADPH} \\ + \text{H}^+\end{matrix}\right\} \xrightarrow{\text{aldose reductase}} \begin{matrix}\text{sorbitol} \\ + \text{NADP}\end{matrix}$$

$$\left.\begin{matrix}\text{Sorbitol} \\ + \text{NAD}\end{matrix}\right\} \xrightarrow[\text{(polyol pathway)}]{\text{Sorbitol dehydrogenase}} \begin{matrix}\text{fructose} \\ + \text{NADH} + \text{H}^+.\end{matrix}$$

It is the first reaction of this polyol pathway which plays a very significant role in the pathogenesis of sugar cataracts. A common factor in these cases is the availability of high concentration of glucose, galactose or xylose in the aqueous humour from where aldose reductase catalyses their reduction to respective polyols[5]. Membranes are impermeable to these polyols which are non-toxic to the cell and its accumulation within the cell renders the lens hypertonic. In order to neutralize this hypertonicity extracellular water moves into the cell and as a result of which the lens swells. This swelling of the lens results in a series of physicochemical changes which eventually leads to cataract formation[6]. The formation of sugar cataracts has been shown to be inhibited by aldose reductase inhibitors[7,8]. These inhibitors block the conversion of the sugars to their respective polyols and also inhibit the hydration of diabetic lenses. Such inhibitors are known as flavonoids[9] which are ubiquitously distributed within the plant kingdom. We have earlier reported that garlic extract inhibited non-enzymatic glycosylation of diabetic rat lens protein[10]. The purpose of the present study was to investigate the role of garlic extract in terms of its effect on the level of polyols, in rats fed with galactose, glucose and xylose-rich diet (50% each) and also on the extent of hydration in lens incubated in medium containing glucose, galactose and xylose (50 mM each).

Rats weighing 50–90 g were divided into seven groups each of ten animals. Group I rats received regular rat diet, groups II, III and IV rats received a diet of 50% glucose, galactose and xylose, whereas groups V, VI and VII rats were orally given 0.2 ml aqueous extract of garlic (3.5 g/10 ml) per day in addition to diet containing galactose, glucose and xylose (50%). After 30 days the sugar levels in rats of groups II, III and IV reached 350 mg dl^{-1}. The rats of all the groups were then sacrificed, the lenses were homogenized in 5% solution of $ZnSO_4.7H_2O$ and deproteinized by addition of an equivalent volume of 0.3N $Ba(OH)_2$ solution. The protein-free filtrate was lyophilized and the polyols in the residue were determined by gas liquid chromatography[4]. Goat lenses were incubated in glucose, galactose and xylose (50 mM each) in the presence and absence of garlic extract in the culture medium. The weight of these lenses was determined before and after incubation. The difference in the weight of the lenses was taken as the extent of hydration.

The results reported are the mean ± standard error of the mean. Because of the variability in animals, lenses in all cases were paired for statistical analysis using Students' *t* test for paired data. A confidence level of 95% or more was considered to be significant.

The results of the levels of polyols in rats maintained on a diet containing 50% glucose, galactose and xylose (table 1) show that the levels of sorbitol, dulcitol and xylitol were 14.7, 35.6 and 2.9 (mg/g wet weight of the lens) respectively. Feeding garlic extract inhibited the formation of these polyols, the levels being 10.9, 16.8 and 1.8 (mg/g wet weight of the lens) respectively.

Water content in lenses incubated in 50 mM glucose, galactose and xylose (table 2) increased by 12.7, 10.7 and 9.6% respectively. The presence of garlic extract in the incubation medium, however, resulted in a significant inhibition in increase in

Table 1 *Levels of polyols in the rat lens*

Diet of rats	Concentration of polyols (mg/g wet weight of the lens)
Regular diet	0.4 ± 0.1
50% glucose	14.7 ± 1.2
50% glucose + garlic extract	10.9 ± 0.9
50% galactose	35.6 ± 2.6
50% galactose + garlic extract	16.8 ± 1.5
50% xylose	2.9 ± 0.2
50% xylose and garlic extract	1.8 ± 0.1

Table 2 *Increase in water content in lens*

System	% water content	Change in water content (%)
Rat lens incubated in medium	68.66	—
Rat lens incubated in medium +50 mM glucose	79.3	10.7
Rat lens incubated in medium +50 mM glucose+garlic extract	71.4	2.8
Rat lens incubated in medium +50 mM galactose	81.3	12.7
Rat lens incubated in medium +50 mM galactose+garlic extract	73.6	7.0
Rat lens incubated in medium +50 mM xylose	78.2	9.6
Rat lens incubated in medium +50 mM xylose+garlic extract	74.8	6.2

water content, the value being 2.8, 6.2 and 7.0% with glucose, xylose and galactose respectively.

The inhibition in the levels of accumulated polyols in the lens in the presence of garlic suggests its new role like flavonoids. This property of garlic is further strengthened by its ability to inhibit the hydration of incubated lenses in the presence of glucose, galactose and xylose. The inhibitory effect of garlic extract is probably due to its sulphur compounds, which are good acceptors of hydrogen and the biological activity may be due to their reaction with thiol group substances and NADPH[11,12]. The consumption of NADPH will thus retard the conversion of glucose and galactose to their corresponding polyols which is the key factor of polyol pathway[13].

It can thus be suggested that by proper regulation of dose of garlic extract, the formation of diabetic cataract, due to the excessive accumulation of polyols and hydration of the lenses can be effectively inhibited.

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1. Chylack, L. T. Jr. and Kinoshita, J. H., *Invest Ophthalmol.*, 1964, **8**, 401.
2. Ramakrishnan, S., Ranganathan, R. and Lamba, P. A., *Arch. Ophthalmol.*, 1971, **9**, 33.
3. Van Heyningen, R., *Biochem. J.*, 1959, **73**, 197.
4. Varma, S. D. and Kinoshita, J. H., *Biochem. Biophys. Acta*, 1974, **338**, 632.
5. Varma, S. D., Schocket, S. S. and Richards, R. D., *Invest Ophthalmol. Vis. Sci.*, 1979, **18**, 237.
6. Chylack, L. T. Jr. and Cheng, H. M., *Surv. Ophthalmol.*, 1978, **23**, 26.
7. Varma, S. D., *Proc. of the Int. Congr. of Eye Res. Jpn.*, 1978, **3**, 113.
8. Juliani, H. R., Pohl, M. G., Richards, R. D. and Varma, S. D., *Invest Ophthal. Vis. Sci.*, 1979, **15**, 212.
9. Varma, S. D. and Kinoshita, J. H., *Biochem. Pharmacol.*, 1976, **25**, 2505.
10. Srivastava, V. K., Afaq, Z., Chaturvedi, N. and Garg, M., *Nat. Acad. Sci. Lett.*, 1987, **10**, 191.
11. Srivastava, K. C., *Ind. Eur. Med.*, 1987, **2**, 40.
12. Adamu, I., Joseph, P. K. and Augusti, K. T., *Experientia*, 1981, **38**, 889.
13. Goodnight, S. H. Jr., Haris, W. S., Connier, W. E. and Illingworth, D. R., *Arteriosclerosis*, 1982, **2**, 87.

C-BANDING TECHNIQUE FOR KINETOCHORE AND HETEROCHROMATIN DIFFERENTIATION IN *NITELLA MIRABILIS* (DIV. CHAROPHYTA)

S. K. BHATNAGAR, ABHA VERMA and V. K. SINGH
Department of Botany, Bareilly College, Bareilly 243 005, India.

KARYOTYPES, which may be defined as the phenotypic appearance of chromosomes in contrast to their genic content[1,2], have been used extensively for species differentiation in the Charophyta[3-5]. However, the karyotypes studied so far are anaphasic configurations based on assessment of centromere position. The most distinguishing characteristics of a karyotype are the number, position, size and distribution of differentially staining heterochromatic segments[6]. These are being used largely in species differentiation of higher plants. The heterochromatin in Charophyta is probably of the constitutive type and highly localized in the centromeric regions[7]. The C-banding procedure has been found to differentiate the repetitive DNA in centromeric heterochromatin[7]. However, the method also gives bands on the chromosome arms besides the