

# COMPARISON OF TWO FORMS OF VITAMIN C ON GALACTOSE CATARACTS

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## ABSTRACT

**A preliminary *in vitro* study demonstrated the significantly greater effectiveness of ascorbic acid in a citrus extract compared to ascorbic acid alone in diminishing the production of galacticol in rat lenses incubated in a high galactose media. Weanling rats were then given 10% galactose in their drinking water to induce cataracts. Ascorbic acid with or without citrus extract was also added to the drinking water of the experimental groups at 2 different concentrations. Ascorbic acid alone or in a citrus extract slowed the development of cataracts in a dose-dependent fashion. The presence of citrus extract improved the efficacy of ascorbic acid especially at the lower dose. The greatest lens concentration of ascorbate was found in the groups given ascorbate and citrus extract. The combination of ascorbic acid and citrus extract is more effective than ascorbic acid alone as a supplement to increase lens ascorbate and slow down the progression of galactose cataracts.**

KEY WORDS: Cataract, galactose, ascorbic acid, bioflavonoids, galacticol.

## INTRODUCTION

Cataracts, defined as any opacity of the lens (1), are a major cause of vision impairment and blindness. Worldwide, at least 50 million people are estimated to have cataracts (2). In the U.S.A. alone, over 200,000 people suffer from cataracts (3). New medical advances such as surgery and lens replacement have a high degree of efficacy, but are not available or too expensive for most of the world's population. Recently, synthetic aldose reductase inhibitors have been studied for the

treatment of diabetic cataracts. However, these drugs are expensive and sorbinil, the most studied drug in particular, has considerable side effects (4).

Animal studies using various cataract models have demonstrated the effectiveness of nutrients in the prevention of cataracts. An early study showed that a full team of vitamins prevented and regressed galactose cataracts in rats (5). Vitamin C was found to be effective in preventing galactose cataracts in guinea pigs (6). In our

laboratory, we have found ascorbic acid to be capable of both slowing down the progression and speeding up the regression of galactose-induced cataracts in rats (7).

The role of bioflavonoids in cataracts has been considerably less studied. These compounds, which are well-known antioxidants, have been shown to inhibit aldose reductase prepared from rat lenses (8) and to delay cataractogenesis in the diabetic degus animal model (9). Quercetin, applied topically, diminished galactose cataracts in neonate rats (10). Topical quercetin and myricetin had an anti-cataract effect in galactosemic rats (11).

In our laboratory, we have demonstrated that ascorbic acid in the presence of a natural citrus extract containing bioflavonoids was more absorbed than ascorbate alone in two studies; a long-term guinea pig supplementation study (12) and a single 500 mg ascorbate dose in human subjects (13). Therefore, we investigated the effectiveness of ascorbic acid and a citrus extract using the galactose-induced rat cataract model.

## **MATERIALS AND METHODS**

### **Citrus Extract**

Citrus Extract (CE) was a commercial tan powder (Foodform<sup>®</sup> Vitamin C) which was made from an alcohol/water extract of orangette. This contained 10.0% bioflavonoids (naringin, naringenin, and hesperidin), 25.1% ascorbic acid and a minimum of 15% proteins and 30% carbohydrates. High resolution NMR and second derivative UV spectroscopy demonstrated that the ascorbic acid was bound or associated with the matrix (unpublished results).

### **In Vitro Study**

Lenses were enucleated from adult, male Sprague-Dawley rats. They were immediately added to 4.5 ml of an isotonic M199 medium (Sigma Chemical Co., St. Louis, MO) containing 55.5 mM galactose and 0.8 ml of fetal calf serum (Sigma Chemical Co.) Each group had 3 lenses. The control group had no added ascorbate and

the experimental groups had either 0.1 mg ascorbate/100 ml of medium or 0.4 mg citrus extract which contained 0.1 mg ascorbate plus 0.04 mg of bioflavonoids/100 ml of medium. Lenses were incubated for 48 hours at 37° in a 95% air/5% CO<sub>2</sub> atmosphere. They were then removed and homogenized in 3 ml of a 10% trichloroacetic acid solution and an aliquot taken for colorimetric analysis of galacticol (14).

## **SUPPLEMENTATION STUDY**

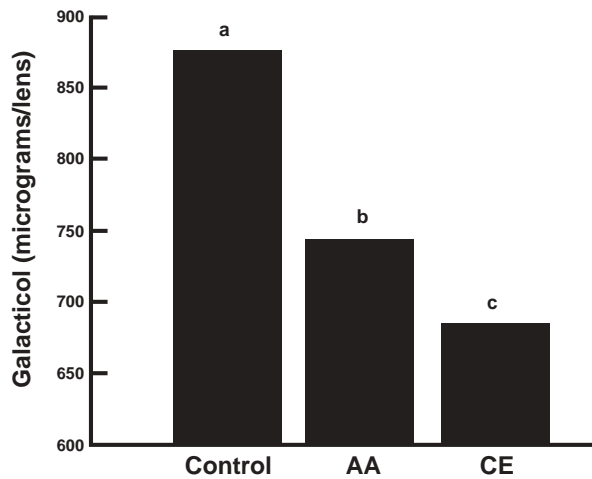
Male weanling Sprague-Dawley rats were divided into 6 groups of 5 animals. All rats received Purina Laboratory Chow *ad libitum*. The blank group received distilled water for drinking. The control and experimental groups received 10% galactose in distilled water alternating with distilled water every other day. The Low AA group received 0.1 g ascorbate/liter of water containing galactose. The High AA group received 1.0 g ascorbate/liter. The Low CE group received 0.4 g of citrus extract which contained 0.1 g of ascorbic acid and 0.04 g of bioflavonoids/liter. The High CE group received 4.0 g of citrus extract, which contained 1.0 g of ascorbate and 0.4 g of bioflavonoids/liter. All ascorbate and citrus extract solutions were freshly prepared each day of use. Each plastic cage contained 5 animals which were allowed to drink as a group a maximum of 200 ml of water/day which was a full container. Thus, assuming 40 ml of water/animal, the animals received an average of 4 g of galactose every other day. The Low AA and CE animals also received an average of 4 mg of ascorbate every other day. The High AA and CE rats consumed an average of 40 mg of ascorbate every other day.

The animals were weighed at the beginning and end of the study. The eyes were examined for opacities using a slit lamp ophthalmoscope for a period of 33 days. The rats were sacrificed at the end of the study using ether anesthesia. The lenses were examined by 2 independent observers and scored for cataracts using the 0-5 system (15) with a score of 5 being a complete opacity. They were then dried on filter paper, weighed, homogenized

in 20% metaphosphoric acid and frozen at -20° C until analysis. The lenses were assayed for reduced ascorbic acid by a fluorometric method (16). Statistical comparison was done by ANOVA with  $p < 0.05$  considered significant for weights and ascorbate and  $X^2$  for cataract ratings. Results are given as the mean  $\pm$  standard deviation.

## RESULTS

The results of the *in vitro* lens incubation are shown below in Figure 1.



**FIG. 1.** Lens galacticol contents following incubation with galactose.

Control = galactose only, AA = 0.1 mg/100 ml ascorbic acid plus galactose, CE = 0.1 mg/100 ml ascorbic acid in a citrus extract plus galactose. Groups with a different letter are significantly different,  $p < 0.001$ .

The presence of galactose in the medium produced over a 10-fold increase in galacticol content, from  $84 \pm 31$  micrograms/lens in the native

lenses to  $876 \pm 18$  micrograms/lens ( $p < 0.001$ ) in the incubated lenses. All incubations which contained ascorbate significantly diminished galacticol formation relative to the control containing no ascorbate ( $p < 0.001$ ). The citrus extract containing bioflavonoids was 41% more effective than ascorbate alone in decreasing galacticol ( $p < 0.001$ ).

The results of the supplementation study are shown in Table 1 and Figure 2. The blank group consistently ate more food and drank less water than the experimental groups which received galactose. This is reflected in the significantly greater weight gain for the blank group compared with the other groups ( $p < 0.05$ ) as shown in Table 1. There was no difference in food or water consumption in the experimental groups given galactose. The control group, given galactose only, did not have a significantly different weight gain than the other experimental groups nor was there any significantly different weight gain for the ascorbate groups compared to the citrus extract groups.

The weight of the lenses at sacrifice had considerable variation within the groups and thus there was no statistical difference among the groups. The galactose-treated groups tended to have lower lens weights than the blank group given no galactose. Other workers have shown a decreased lens weight following the first visible opacities in the galactose cataract model. (17) There also seemed to be a trend toward decreasing lens weight with increasing ascorbate dose in the AA and CE groups.

Table 1.

**Comparison of galactose feeding on rats fed ascorbic acid at two different doses alone (AA) or in a citrus extract (CE).**

Group	Weight Gain (grams)	Lens Weight (mg)	Lens AA	Cataract Rating
			Lens Weight ( $\mu\text{g} / \text{mg}$ )	
Blank	$354 \pm 38$	$35.1 \pm 5.0$	$1.2 \pm 0.58$	0
Control	$284 \pm 8$	$30.5 \pm 13.0$	$1.0 \pm 0.57$	$2.3 \pm 1.2$
Low AA	$303 \pm 39$	$32.0 \pm 10.0$	$0.95 \pm 0.68$	$2.8 \pm 1.2$
High AA	$263 \pm 34$	$28.6 \pm 9.0$	$0.77 \pm 0.32$	$1.3 \pm 1.9$
Low CE	$286 \pm 22$	$26.7 \pm 3.0$	$1.36 \pm 0.57$	$1.6 \pm 1.7$
High CE	$294 \pm 28$	$23.2 \pm 7.0$	$2.32 \pm 1.16$	$1.3 \pm 0.9$

The blank group was given no galactose. The control group was given galactose alone. The results are the mean  $\pm$  standard deviation.

The ascorbate concentration in the lens was quite variable within a group. There was not a clear dose-response of ascorbate supplementation on lens ascorbate perhaps as a result of the effect of 33 days of galactose feeding on lens permeability. Although not significant, there appears to be a greater lens ascorbate concentration in the CE groups compared to the corresponding AA groups. In a previous long-term ascorbate supplementation study with guinea pigs, CE produced a significantly greater liver and plasma ascorbate concentration than did AA (12).

The cataract ratings at the end of the supplementation study are shown in Table 1. Although there was no significant difference between the galactose treated groups, there is a trend toward lower cataract ratings with a higher dose of ascorbate. The low CE dose was almost as effective as the high AA dose.

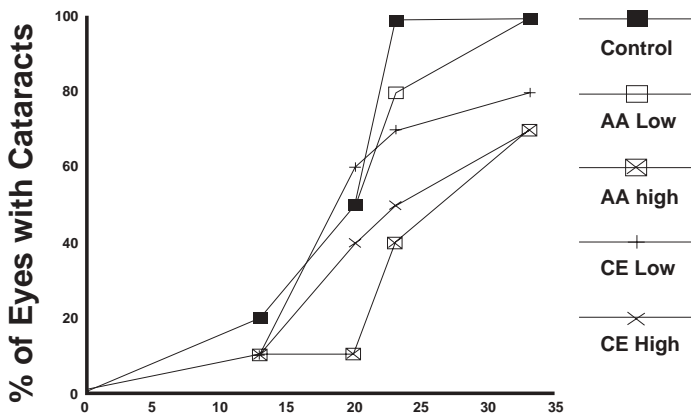


Fig. 2

Percent eyes with visible cataracts as a result of galactose

The progression of cataract formation is seen in Figure 2 above. The blank group (not shown) had no opacities. The control group had 100% visible opacities after 23 days. This result is similar to that obtained after feeding rats 20% galactose in the diet for 30 days (18). Although there was no significant difference in % opacities between low and high AA or low and high CE in Figure 2, there was a dose-response effect of supplemental ascorbate in both the AA and CE treated groups. The Low AA group had essentially no effect on preventing cataracts after 33 days. The Low CE was beneficial and had a lower

cataract rating at the end of the study than the Low AA. The High AA and CE groups were equivalent after 33 days and quite effective at slowing down cataract formation, especially in the early stages of galactose feeding when the biochemical changes in the lens are maximal(19).

## DISCUSSION

The *in vitro* rat lens in a high galactose media produced a large increase in lens galacticol indicative of the activation of the enzyme aldose reductase. Galactose was chosen because it produces a greater increase in its reduced form, galacticol, than does glucose and the fact that galacticol does not further metabolize as does sorbitol, the reduced form of glucose (20).

Ascorbic acid has been hypothesized to be present in the lens as means of protecting the lens against photochemical and oxidative insult. The oxidative stress of 20 mM galactose decreased the concentration of ascorbate in the *in vitro* rat lens over 70% in 24 hours (21). In the present *in vitro* study, ascorbate was added to the medium at a concentration of 0.1 mg/100 ml which is equivalent to the physiological level present in the lens and aqueous humor of nocturnal animals such as rats (22). Even at this very low concentration, ascorbate significantly decreased galacticol in the rat lens and the citrus extract containing bioflavonoids was significantly more effective.

The mechanism for the effect of citrus extract in the lens is unknown. However, from previous work, it is hypothesized that bioflavonoids stabilize the ascorbate preventing its oxidation to dehydroascorbate (23). In addition, we have shown that bioflavonoids increase absorption of ascorbate in both guinea pigs and man (12, 13). Quercetin, a bioflavonoid, has been shown to inhibit the formation of sorbitol from glucose in intact rabbit lenses (24). We have also found that the citrus extract was more effective than ascorbate alone in inhibiting production of sorbitol from human red blood cells incubated in a high glucose medium (25).

As we have shown previously, and is confirmed in this study, ascorbate appears to slow down but not prevent cataract formation in the galactose fed rats (7). Although not a significant difference, it appears that citrus extract is more effective than ascorbic acid at low doses in slowing down the formation of galactose cataracts. It is remarkable that ascorbate can influence cataract formation since the rat synthesizes ascorbate and maintains its concentration in the body, including the lens, by homeostasis.

There now appears to be a large body of evidence supporting the efficacy of ascorbate in cataracts. This includes the galactose animal model presented here and elsewhere and the diabetic rat model (26). A human epidemiological study on 112 human subjects, 77 with at least one lens with a cataract, also indicated the benefit of ascorbate (27). A case controlled study showed that subjects without cataracts took significantly more Vitamin C supplements than did those with cataracts (28). In an intervention trial in 100 patients, a combination supplement of ascorbic acid, amino acids and Vitamin B<sub>6</sub> reduced or even halted the progression of cataract development in comparison with placebo (29). We have recently shown that ascorbic acid alone or in a citrus extract were effective supplements in normalizing the elevated red blood cell sorbitol levels of human diabetic subjects (30). The results indicate a human trial should be undertaken to test the efficacy of ascorbic acid and citrus extract in preventing and treating diabetic cataracts.

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