ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Effect of Difference in the Dietary Galactose on the Rate of Onset and Progression of Cataract in Rats

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ABSTRACT

Galactose-induced rat model is commonly used for experimental studies on cataract. This study compared the effects of dietary galactose in a close range of 20-30%, on the rate of onset and progression of cataract by clinical and histopathological evaluation. *Sprague-Dawley* rats (80-100g) were divided into 4 groups. Group 1 (ND) received normal diet while groups 2(G-30), 3 (G-25) and 4(G-20) received 30, 25 and 20% galactose diet respectively. Cataract progression was observed by biweekly slit lamp examination. After euthanization, lenses were dissected for histopathological examination. The study showed that the dose-dependent effect of galactose was most evident during the first week with significantly faster progression in G-30 compared to G-25 and G-20. In subsequent weeks, rate of progression of cataract was comparable in 3 galactose-fed groups. Since, G-25 and G-20 showed a slower onset of cataract, 25 and 20% galactose diet may be considered a better representation of human cataract.

Keywords: experimental, galactose diet, cataract, histopathology

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ISSN: 0975-8585

INTRODUCTION

Cataract, a leading cause of blindness, is one of the common complications of diabetes mellitus [1, 2]. Currently no medical therapies are available either for prevention or treatment of cataract. Therefore, it remains an area of intensive research both in terms of investigating anticataract properties of new substances and recognition of potential underlying pathophysiological mechanisms.

Experimental studies have utilized several animal models, of which galactose-induced model of cataract in rats is popularly used. Although, the rats in this model are not diabetic and the development of cataract is not exact representation of human diabetic cataract, the galactose-induced lenticular changes closely mirror the pathophysiological changes seen in diabetic rats [3, 4]. Although, lactose and high carbohydrate diets were also shown to induce sugar cataract as early as in 1930's [5], galactose as a cataract inducing agent is often preferred in cataract research for a few reasons. Galactose-fed rats have very low mortality and importantly, all rats fed with galactose develop lenticular changes [5].

The relationship of the proportion of galactose with the development of cataractous changes has been studied using it in diet over a wide range of 10-40% and over prolonged period of up to 226 days [6, 7]. However, in experimental studies galactose has been used in proportions ranging from 24-50% [8, 9]. The rate of development of cataract with change in the proportion of dietary galactose may closely influence the outcomes and the results of several such studies, therefore, are incomparable. In the current study we examined the effects of dietary galactose in a close range of 20-30%, which is most commonly used experimentally. For the first time, we closely analyzed quantitatively the differences not only in the rate of onset but also the week-to-week progression of cataract both clinically and histopathologically. The results for this study will provide a guide to the selection of most appropriate dietary proportion of galactose for induction of experimental cataract in rats.

Materials and Methods

All experiments and animal handling were performed in compliance with the ARVO statement for the use of animals in ophthalmic and vision research. Thirty-five *Sprague-Dawley* rats of either sex weighing about 80-100g were procured from Laboratory Animal Care Unit of Universiti Teknologi MARA. Animals were maintained under standard laboratory conditions and were given food and water ad libitum. All animals were subjected to systemic and ophthalmic examination and those found normal were included in the study.

Study design

Animals were divided into 4 groups. Group 1 received normal diet (ND) while groups 2 (G-30), 3 (G-25) and 4 (G-20) received D (+)-galactose (Acros Organic, Fisher Scientific) diet. G-30 consisted of 5 animals that were fed with 30% galactose diet for a period of 21 days and were then sacrificed. G-25 consisted of 10 animals that received 25% galactose diet. In this

ISSN: 0975-8585

group, 5 animals were sacrificed at day 21 while the remaining 5 continued on same diet until they were sacrificed on day 28. Among the 15 rats in G-20 that received 20% galactose diet, 5 were sacrificed at day 21, another 5 at day 28 while the remaining 5 continued on same diet until they were sacrificed on day 36. The experimental period was 36 days for group 1, at the end of which all 5 animals were sacrificed (Figure 1).

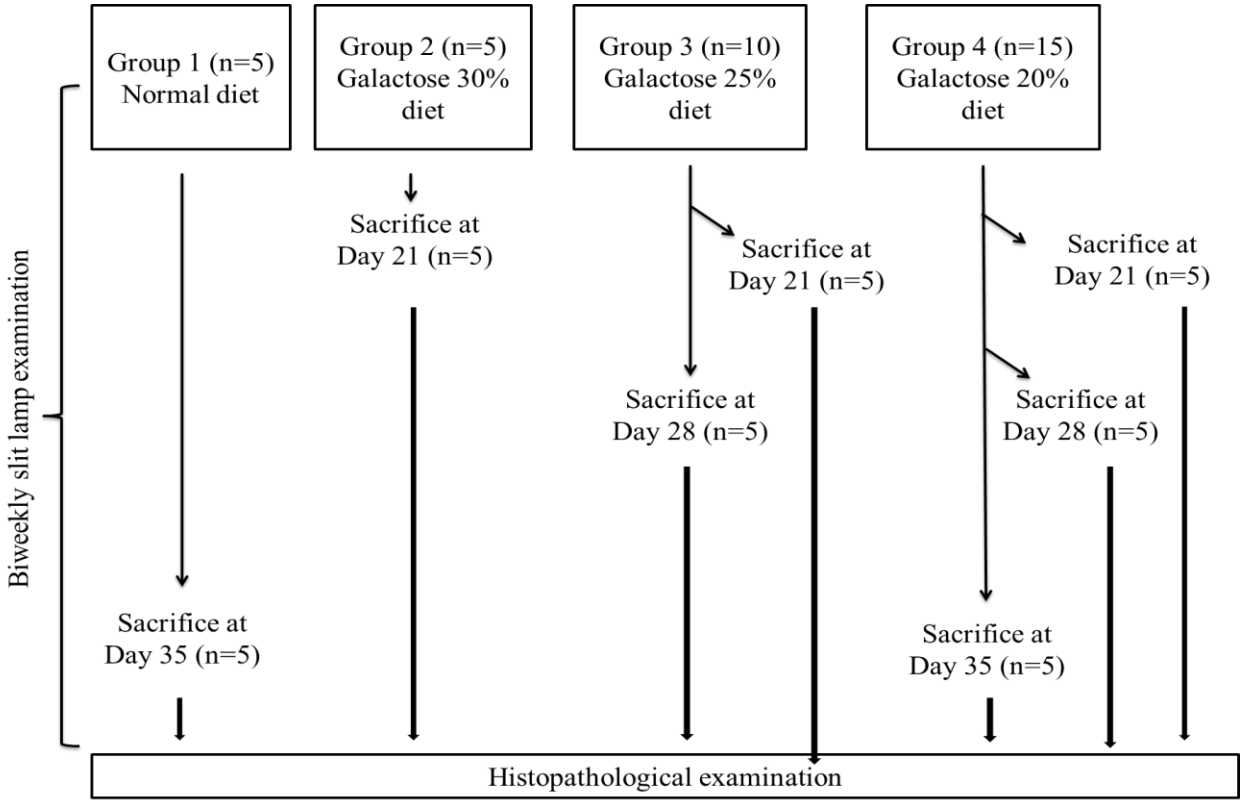


Figure 1: Study design

During the experimental period, body weight was recorded once weekly and anterior segment imaging was done twice a week. At the end of experimental period, lenses from all eyes were removed atraumatically, fixed in 10% formalin and were paraffin embedded. Seven µm thick sections were stained with hematoxylin and eosin and were subjected to histopathological examination under light microscopy.

Assessment of the onset and progression of cataract

Anterior segment imaging by slit lamp

Progression of cataract was assessed biweekly during experimental period using a slit lamp biomicroscope (Portable Digital Slit Lamp-Hawk Eye, Dioptrix). Prior to slit lamp examination, pupillary dilation was achieved with topical application of tropicamide 1% (1% Mydriacyl, Alcon Industries). The cataractous changes were graded according to the classification proposed by Agarwal et al. [10] as described below:

Stage 0- Normal lenses,
Stage1a-Vacoules appearance as an equatorial ring,
Stage1b-Areas of vacuolization covering one-third of anterior cortex, Stage 1c- Areas of vacuolization covering≥ two-third of anterior cortex,
Stage 2a- Coalescence and liquefaction of vacuoles,
Stage 2b- Coalescence and liquefaction of vacuoles and appearance of haziness,
Stage 3- Uniform opalescence,
Stage 4- Nuclear cataract.

The stages of cataract described above were graded on a scale of 1-8. The opacity index was calculated as described previously [11]. The cataract grading was done by two independent and blinded observers.

$$\text{Opacity index} = \frac{\text{no. of eye in each stage x stage of eye}}{\text{total no. of eye}}$$

Histopathological evaluation

Grading

The lenticular histopathological changes were graded according to the method described previously with modification [10, 12, 13, 14]. The grading was carried out in a masked fashion by two independent investigators. The scheme of grading was as outlined below. Stages 0-4 were given grades 1-5 respectively.

Stage 0 – Presence of normal anterior epithelium and lens fibres
Stage 1 – Presence of anterior epithelium, mixture of normal and swollen lens fibres and vacuoles
Stage 2 – Presence of anterior epithelium, mixture of normal and swollen lens fibres, vacuoles and pinkish homogenized area
Stage 3 – Absence of anterior epithelium with presence of mixture of normal and swollen lens fibres, vacuoles and pinkish homogenized area
Stage 4 – Presence of mixture of normal and swollen lens fibre cells and pinkish homogenized area only

Statistical analysis

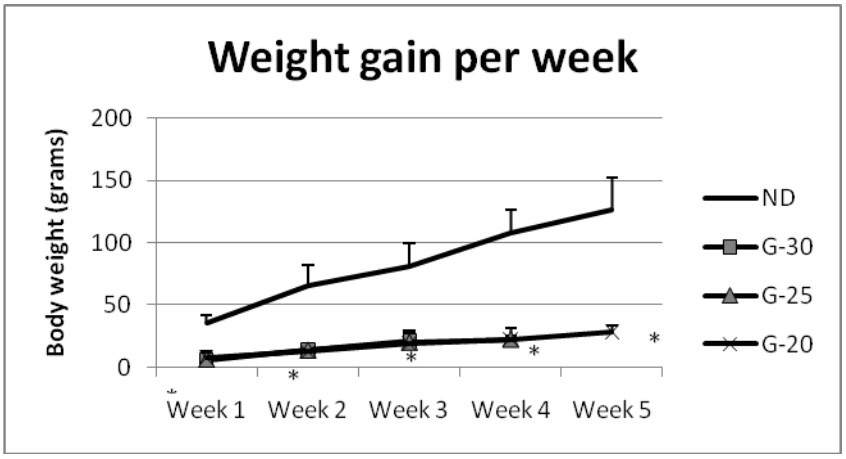
Results obtained were expressed as mean ± standard deviation. All results were analyzed by SPSS software version 20. Intergroup comparison was done using two-way ANOVA and $p<0.05$ was considered significant.

ISSN: 0975-8585

RESULTS

Body weight

Throughout the experimental period, galactose-fed groups showed significantly lower body weight gain compared to normal rats ($p<0.001$). Among the three galactose-fed groups, there were no significant difference in the weight gain throughout the experimental period (Figure 2).



Each data point represents mean ± standard deviation. *p<0.001versus ND. ND: Normal diet group, G-20: Galactose 20% diet group, G-25: Galactose 25% diet group, G-30: Galactose 30% diet group.

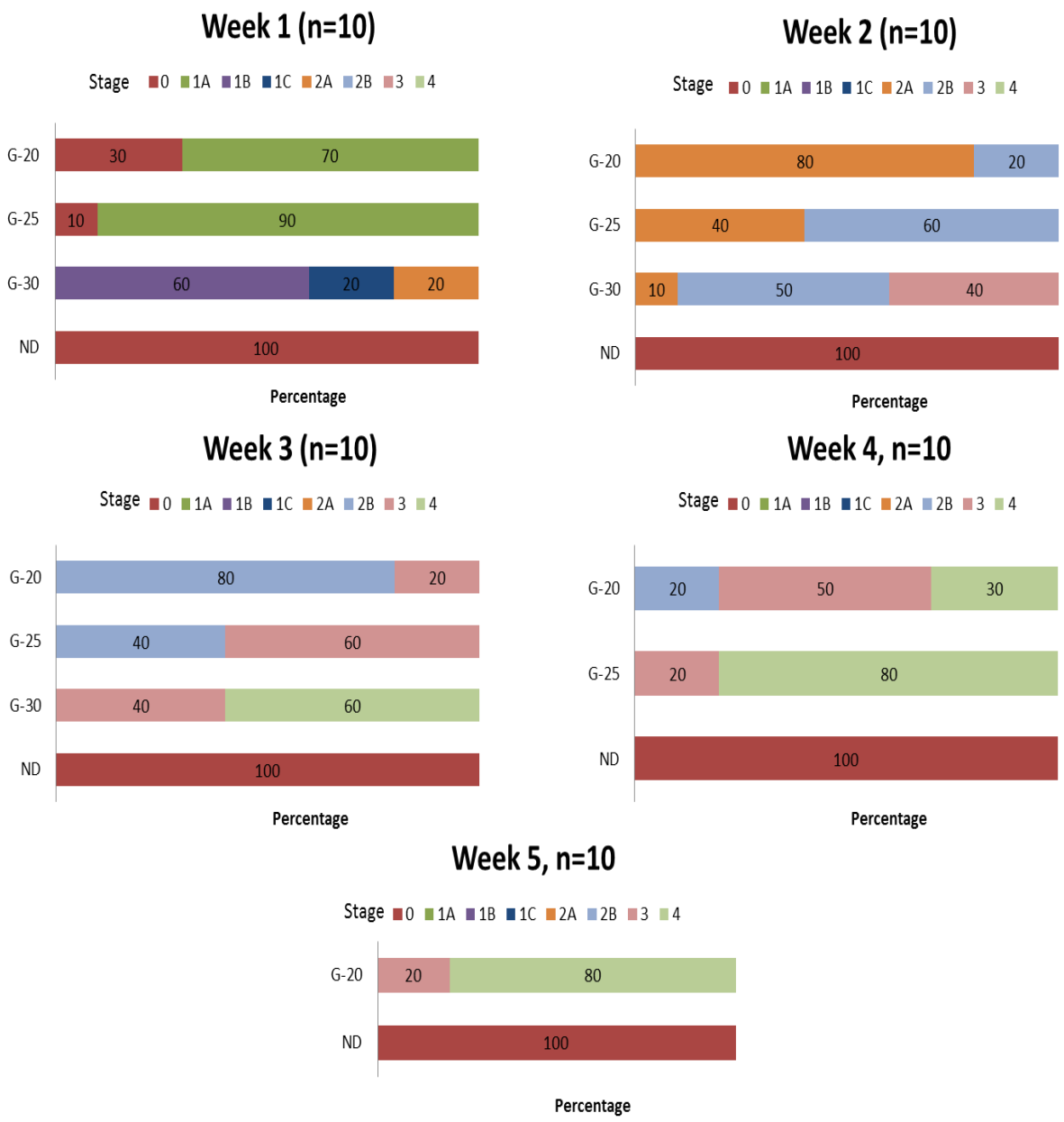
Figure 2: Body weight gain among 4 groups of rats

Progression of cataract in galactose-fed rats

Cataract grading

At the end of week 1, 20% of G-30 eyes progressed to stage 2a. At the same time points 10% eyes in G-25 and 30% eyes in G-20 eyes were still in stage 0. However, at this time point majority of eyes, 90 and 70% respectively, in G-25 and G-20 progressed to stage 1a. At the end of week 2, 40% of G-30 eyes were in stage 3, 60% of G-25 eyes were in stage 2b but in G-20, 80% eyes were in stage 2a. At the end of week 3, 60% of G-30 eyes were in stage 4 but none progressed to stage 4 in G-25 and G-20. At this time point, in G-25, 60% eyes were in stage 3 and 80% eyes in G-20 eyes were in stage 2b. At the end of week 4, 80% of G-25 eyes were in stage 4, while in G-20, 20% of the eyes were still in stage 2b while 50% progressed to stage 3. At week 5, 80% of G-20 eyes were in stage 4. All eyes in ND remained at stage 0 throughout the study (Figure 3).

ISSN: 0975-8585



ND: Normal diet group, G-20: Galactose 20% diet group, G-25: Galactose 25% diet group, G-30: Galactose 30% diet group.

Figure 3: Differences in the onset and progression of cataract among groups of animals fed with 30, 25 and 20% galactose diet over 3-5 weeks.

Opacity index

All galactose-fed groups showed significantly higher opacity index at all-time points. At the end of weeks 1, 2 and 3, the opacity index in both the G-25 and G-20 was significantly lower

than G-30. At the end of week 4, although the mean opacity index in G-20 showed a lower mean value than in G-25, the difference was not significant (Table 1, Figure 4).

Table 1: Differences in the opacity index among groups of animals fed with 30, 25 and 20% galactose diet over 3-5 weeks.

	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5
ND	0.1	0.1	0.1	0.1	0.1	0.1
G-30	0.1	0.36 ± 0.08*	0.63 ± 0.07*	0.76 ± 0.05*	-	-
G-25	0.1	0.19 ± 0.03*#	0.54 ± 0.05*#	0.66 ± 0.05*#	0.78 ± 0.04*	-
G-20	0.1	0.17 ± 0.05*#	0.5 ± 0.08*#	0.62 ± 0.04*#	0.71 ± 0.07*	0.78 ± 0.04*

Data presented as Mean ± standard deviation. *- p<0.05 versus ND, #-p<0.05 versus G-30. ND: Normal diet group, G-20: Galactose 20% diet group, G-25: Galactose 25% diet group, G-30: Galactose 30% diet group

Rate of development of cataract

The rate of week-to-week progression of cataract was estimated by calculating the slope of each week-to-week segment of straight line for all rats in three galactose fed groups. Table 2 presents the mean values of slope for three galactose-fed groups. The slope of the week 0 to week 1 segment of straight line showed a significantly smaller value in groups G-25 and G-20 compared to G-30.
No differences were observed among groups at any other time points (Table 2).

Table 2: Week-to-week progression of cataract among the rats fed with 20, 25 and 30% galactose diet

	Week 0-1	Week 1-2	Week 2-3	Week 3-4	Week 4-5
G-30	0.26±0.08	0.27±0.13	0.13±0.08	-	-
G-25	0.09±0.03*	0.35±0.05	0.12±0.04	0.12±0.04	-
G-20	0.07±0.05*	0.33±0.07	0.12±0.06	0.09±0.06	0.07±0.05

Data presented as Mean ± standard deviation. *p<0.01 versus G-30. ND: Normal diet group, G-20: Galactose 20% diet group, G-25: Galactose 25% diet group, G-30: Galactose 30% diet group.

Histopathological staging

In G-30, at the end of week 3, 33.33% lenses were at stage 4, while 50% were in stage 3 and remaining 16.67% were in stage 2. At the same time point, in G-25, 50% were at stage 2 and 50% were at stage 3. In G-20 at this time point, 66.67% were at stage 2 and remaining 33.33% were at stage 3.

At the end of week 4, in G-25, 50% of lenses were at stage 4, while 33.33% were at stage 3 and remaining 16.67% were at stage 2. At this time point in G-20, 50% were still in stage 2, 33.33% were at stage 3 and 16.67% were at stage 4.

At the end of week 5, 66.67% lenses in G-20 showed progression to stage 3 while remaining 33.33% were at stage 4 (Figure 5).

Table 3: Histopathological scoring at the end of week 3, 4 and 5

	ND	G-30	G-25	G-20
Day 21(Week 3)	-	3.22 ± 0.74	2.45 ± 0.62	2.39 ± 0.62
Day 28 (Week 4)	-	-	3.33 ± 0.82	2.72 ± 0.85
Day 36 (Week 5)	0.00			3.28 ± 0.44

Data presented as Mean ± standard deviation. ND: Normal diet group, G-20: Galactose 20% diet group, G-25: Galactose 25% diet group, G-30: Galactose 30% diet group.

Histopathological scoring among the lenses from 4 groups is shown in table 3. All galactose-fed groups showed significantly higher mean value compared to normal diet group. Although there was no significant difference observed among the three galactose fed groups, there was a trend towards higher mean grade in G-30 followed by G-25 and G-20.

DISCUSSION

Galactose-induced experimental model of cataract is a commonly used tool to investigate potentially new anticataract drugs as well as underlying pathophysiological mechanisms. Lenticular oxidative stress and osmotic stress due to lenticular accumulation of polyols resulting from galactose metabolism through polyol pathways are the major pathophysiological mechanisms in this animal model [4]. Therefore, the galactose-induced lenticular changes closely mirror the changes in diabetic cataract and therefore, this model is frequently used as a representation of diabetic cataract [3, 4].

The current study evaluated effects of differences in the dietary proportion of galactose on the rate of onset and progression of cataract in rats. Rats fed with 30% galactose showed faster development of cataract as indicated by staging and higher opacity index in this group compared to G-25 and G-20, at the end of weeks 1, 2 and 3. These observations were in accordance with the previously done studies that have reported a dose-response relationship between the proportions of galactose in diet with the rate of progression of cataract [6, 7]. In this study, we did not observe significant difference in the rate of cataract development between rats fed with 25 and 20% galactose.

Importantly, our study showed that the slope of straight line in the segment of week 0-1, indicating the rate of onset of cataract, was significantly higher for G-30 compared to G-25 and G-20. No significant difference was observed at any other time point. These results suggest that within the range of dietary proportion of galactose studied, 30% galactose diet causes faster onset of cataract but subsequently the progression of cataract takes place at comparable rates. Continued observations at week 2 and 3 showing higher opacity index in G-30 compared

to G-25 and G-20 appears to result from initial faster onset rather than any difference in the rate of progression subsequently.

In contrast to clinical grading, we did not observe significant differences in histopathological grading among galactose-fed groups. However, the trend towards higher mean grade in G-30 followed by G-25 and G-20 may still be considered suggestive of dose-dependent effect and consistent with opacity index. This is because the method of histopathological grading did not take into account the progressive changes within stage 1 and 2 as was the case for clinical staging of cataract. The lower body weight gain among galactose-fed rats can be attributed to malabsorption resulting from galactose feeding.

The cataract is often a slowly progressively disease even in diabetics. Therefore, using animal models with rapidly progressive cataract appears to be an inappropriate representation of pathophysiological changes in human cataract. Accordingly, 25% or 20% galactose fed animals appear to be better representative models as compared to those fed with 30% or higher proportions of galactose in diet.

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