

ORIGINAL RESEARCH

Evaluating the Cariogenic Potential of Flavored Milk: An Experimental Study using Rat Model

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ABSTRACT

Objective: The purpose of this study was to evaluate and to compare the cariogenic potential of different commonly consumed flavored milk on caries development in rat's molar teeth.

Materials and methods: Sixty Sprague-Dawley rats infected with an active growing culture of *Streptococcus mutans* were offered chocolate flavored milk, strawberry flavored milk, banana flavored milk, plain milk, sweetened plain milk with 5% sucrose, and 10% sucrose *ad libitum*.

Results: Rats consuming flavored milk developed significantly lower sulcal and smooth surface caries scores than rats consuming 10% sucrose. However, rats consuming plain milk developed significantly lower sulcal and smooth surface caries scores than animals consuming flavored or sweetened plain milk except for chocolate flavored milk.

Conclusion: It was concluded that flavored milk containing 5% sucrose showed a moderate cariogenic potential.

Clinical significance: Although the overall results suggest that the cariogenic potential of flavored milk is moderate and despite their nutritional value, occasional consumption of flavored milk as a beverage might be accepted with caution. Parents, dental practitioners and other healthcare professionals should be aware of the cariogenicity of flavored milk. They should keenly encourage the use of plain milk and discourage the use of any cariogenic drink which has a deleterious effect on teeth.

Keywords: Flavored milk, Dental caries, Rats, *Streptococcus mutans*.

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INTRODUCTION

Milk is of a major nutritional value, especially for children and adolescents. Flavors that appeal to children, such as vanilla, strawberry, chocolate, and banana have been introduced and became more popular in recent years.¹ Flavored milk is a nutrient rich beverage that provides essential nutrients similar to unflavored milk except for the added sugar, which adds about 60 to 70 calories per 8-ounce serving.² It provides an option for meeting the recommended intakes of dairy products. According to the data from the US Department of Agriculture and the US Department of Health and Human Services,³ 70% of the milk chosen by children in schools is flavored. Kranz et al⁴ showed that 18% of 4 to 18-year-old children's dairy consumption is flavored milk suggesting its popularity among school children. In Saudi Arabia, more than 50% of adolescents consume chocolate flavored milk compared to 25% who consume plain milk.⁵ Pelsmaecker et al⁶ showed that children prefer flavored milk to plain milk, although it is perceived to be less healthy. Children at this stage are in a calcium crisis as most of them are not meeting current calcium recommendations.⁷ Johnson et al⁸ showed that children who drink flavored milk consume more milk and hence have higher calcium intake. The American Academy of Pediatric⁹ has recognized the value of flavored milk with modest amount of sugar for school children. They recommended the consumption of low fat or fat-free plain or flavored milk as a healthful alternative to soft drinks. When flavored milk was taken out of schools, the overall milk consumption dropped by approximately 38%.⁸

Food flavors can be classified into natural flavors which come from animal and vegetable sources, nature identical flavors which are chemically identical to natural flavorings but are prepared or extracted using chemical methods, and artificial flavors, compounds which are not chemically identical to natural flavoring substances and have not been identified in nature.¹⁰ According to the Code of Federal Regulations, flavors other than natural flavors, are considered artificial.¹¹ Most of the flavors used in food industry are artificial. The impact of contemporary changes in beverage consumption particularly flavored milk, and the presence of these flavors, artificial colors, and added sugar raises some

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questions about their potential effects on children's general health.^{12,13} In addition, the issue about their cariogenicity has also been raised.¹⁴ Miller et al¹⁵ showed that the top sources of added sugars among Americans aged 2 to 11 years were soda, fruit drinks, and flavored milk. Flavored milk typically contains 5% or more of added sugar in addition to 4 to 5% of lactose.¹⁶

Dental caries is one of the most prevalent infectious diseases to afflict mankind.¹⁷ *Streptococcus mutans* is most commonly associated with carious lesions in humans^{18,19} as well as in experimental animals on sucrose diets.²⁰ *S. mutans* metabolizes carbohydrates, such as glucose, lactose and sucrose, to produce acid and induce dental caries.^{21,22} Many types of food promote the development of dental caries and are therefore known as cariogenic. However, some types of food, like milk and milk products contain factors believed to protect against dental caries and are therefore supposed to be anticariogenic or cariostatic.²³ Adding sugar to milk could risk its cariostatic effect. A study on rats showed an increase in cariogenicity with only 2% sucrose added to milk.²⁴ Furthermore, Bowen et al²⁵ reported that 5% sucrose is highly cariogenic.

Although the cariogenic effects of sweetened milk, human milk and infant formula have been studied,^{24,26-28} the interaction between different kinds of flavored milk and caries development should be better understood and investigated. Therefore, the present investigation was carried out to evaluate and to compare the cariogenic potential of commonly consumed flavored milk on caries development in rat's molar teeth.

MATERIALS AND METHODS

Sprague-Dawley pregnant rat dams were obtained from the Centre of Laboratory Animals and Experimental Surgery (CLAES), at King Khalid University Hospital, King Saud University (KSU) in Riyadh and transferred to the animal house of the College of Dentistry, (KSU). The animals were fed with chow diet (F-648; Grain Silos & Flour Mills Organization, Riyadh Branch, Saudi Arabia) and distilled water *ad libitum*. They were maintained under standard conditions of humidity (50-55%), temperature (23-25°C), and light (12 hours light/12 dark cycles). All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals, after the approval of the Ethical Committee at the College of Dentistry Research Centre (CDRC), (KSU). Only pups born within a 24-hour period were used and the dams were circulated among the litters.²⁹ Pups were weaned when aged 21 days.²⁶

Freeze dried pure culture of *S. mutans* (ATCC 25175) available in the microbiology laboratory of the College

of Dentistry, KSU and stored at -20°C was cultured in Todd-Hewitt broth media (BBL Microbiology Systems, Cockeysville, MD, USA) and was incubated at 37°C for 24 hour.³⁰ Each pup was then infected for three consecutive days (21st-23th days of age) by oral inoculation of 3 drops of the bacterial suspension using a pipette.³⁰ The pups were restrained from any fluid or food for at least 1 hour following the infection. During this period, the pups were given laboratory chow containing 25% sucrose.³¹ In addition, a freshly cultured bacterial suspension was diluted 1/100 and was added to their drinking water,³⁰ with 10% sucrose to enhance establishment of infection.²⁶

Forty-eight hours after inoculation was completed, several oral swabs were taken from selected animals and were cultured.³⁰ Oral swabs were placed in 10 ml tube of Todd-Hewitt broth media and were incubated for 24 hours. Then 10 µl was taken and spread on blood agar plate (Oxoid LTD, Basingstoke, Hampshire, UK) and was incubated for 24 hours. After that, the plate was checked for alpha hemolytic organisms then was checked by MicroScan machine (Siemens Healthcare Diagnostic Inc., Ca, USA) to determine the presence or absence of the organism. It revealed that all selected animals were successfully infected by *S. mutans*.

Rats were then randomly divided into 6 groups comprising 10 animals each. All animals were weighed to monitor their growth rates. The animals were maintained under standard laboratory conditions and subjected to the same 12 hours light/dark cycle and the same times of feeding, handling and noise level during the experimental period.

The animals were housed in pairs, in cages with raised wire stainless steel floors with all-time access to distilled water and to a commercial non cariogenic laboratory powdered pellet chow diet *ad libitum*; containing 20% protein, 4% fat, 3.5% fibre, 6% ash, 0.5% salt, 1% calcium, 0.6% phosphorus as well as vitamin A, D and E. Trace minerals, such as cobalt, copper, iodine, iron, manganese, selenium and zinc were also included (F-648; Grain Silos & Flour Mills Organization, Riyadh Branch, Saudi Arabia).

Each group was accordingly offered one of the following fluids to drink *ad libitum*; 1) banana flavored milk; 2) chocolate flavored milk; 3) strawberry flavored milk; 4) sweetened plain milk with 5% sucrose; 5) plain milk (negative control); and 6) 10% sucrose (positive control). Long life plain and flavored milk (2-3% fat) were purchased in a local supermarket and replenished daily in sterile bottles to reduce risk of spoilage. The rats were regularly examined and weekly weighed and the amount of consumed fluids under investigation was monitored and weekly recorded. By the end of the 5th week, the rats were sacrificed using ether; the mandible and maxilla were removed and defleshed.

All molars on each side of the jaws were scored for number and severity of smooth surface (buccal/lingual) and sulcal surface caries by a trained examiner who was unaware of the groups, by means of Nikon stereomicroscope (SMZ 1000) (Nikon Instruments Inc, NY, USA) with magnification of 40× using modified Keyes method.³² Considering unsliced and unstained tooth surfaces: ‘enamel lesions’ were scored when the caries progression was restricted to the enamel, whereas ‘dentinal lesions’ were scored when signs of caries penetration were seen at least in the dentino-enamel junction or further into the dentin. Enamel and dentinal caries were assessed for each molar surface in each rat separately then they were added together giving the total caries score for the smooth surfaces (buccal/lingual) and sulcal surfaces independently.

Total sugar analysis of all tested drinks was performed using high performance liquid chromatographs (HPLC) (Shimadzu, Japan) and the concentration of various sugars was expressed as a percentage. The pH of the consumed fluids was also measured with a pH meter at room temperature.

The data were analyzed using the statistical package for social sciences (SPSS version 16) program. The caries data were calculated as mean ± standard deviation. Statistically significant differences among the groups were determined by analyses of variance (ANOVA) and Tukey’s test with a statistical significance level of 0.05.

RESULTS

The experiment continued for five weeks and all animals survived the experimental procedures and remained in apparent good health.

Table 1: Total sugar and pH level of consumed fluids

Consumed fluids	Sucrose (%)	Lactose (%)	Total sugar (%)	pH
Plain milk	0.00	4.67	4.67	6.5
Chocolate milk	5.90	4.76	10.66	6.3
Strawberry milk	5.15	4.42	9.57	6.3
Banana milk	5.50	5.01	10.51	6.2
Plain milk + 5% sucrose	5.00	4.73	9.73	6.0
Sucrose	10.00	—	10.00	5.7

Table 2: Approximate amount of fluid consumed by rats in each group at the end of the experiment and the effect of drinking fluids on caries scores in rats (n = 10/group)

	10% Sucrose mean (SD)	Plain milk + 5% sucrose mean (SD)	Banana milk mean (SD)	Strawberry milk mean (SD)	Chocolate milk mean (SD)	Plain milk mean (SD)
Amount (ml)	155 (11.2) ^a	157 (7.9) ^a	192 (3.7) ^b	208 (14.1) ^b	186 (6.8) ^b	155 (17.7) ^a
Total smooth surface lesions	30.3 (12.2) ^a	22.8 (5.2) ^b	20.3 (7.3) ^b	19.7 (6.7) ^b	18.9 (4.6) ^b	6.3 (3.5) ^c
Total sulcal lesions	35.7 (12.1) ^a	28.7 (6.1) ^b	23.4 (7.7) ^b	22.5 (10.0) ^b	21.7 (5.4) ^{b,c}	12.7 (4.2) ^c

Different letters in the horizontal rows indicate significant differences (p < 0.05); SD: standard deviation

The relative determinations of total sugar levels as well as the pH of the consumed fluids are indicated in Table 1. The total sugar concentration of the chocolate flavored milk was the highest (10.66%) and the plain milk was the lowest (4.67%) which demonstrated the highest pH value (6.5). The highest consumption was among strawberry flavored milk group (Table 2). Flavored milk drinks were consumed more than other milk.

Rats consuming flavored milk developed significantly lower sulcal and smooth surface caries scores than rats consuming 10% sucrose (p < 0.05) (Table 2). On the contrary, rats consuming flavored and sweetened plain milk had significantly higher caries scores when compared to the caries scores of rats consuming plain milk (p < 0.05) (Table 2). However, for the total sulcal surface caries scores, no significant difference was detected between chocolate flavored milk and plain milk. Although sweetened plain milk group had higher smooth and sulcal caries scores than other flavored milk groups, significant differences were not detected among them (Table 2).

DISCUSSION

Murphy et al³³ emphasized that the effect of added sugar on body mass index (BMI) did not differ between flavored milk drinkers and milk non-drinkers, however, the effects of these sugars on dental health have not been taken into consideration.

The caries process is multifactorial and involves integration of several factors, such as oral bacteria, saliva, tooth enamel, and dietary substrates. It is possible to learn a great deal about each of these factors by studying them individually or in limited combinations.¹⁶ The rat caries model incorporates many aspects of the caries process *in vivo* and is, therefore, considered to be a valuable and suitable model to shed some light on the cariogenic potential of frequently consumed different flavored milk in rat’s molar teeth.

In this experiment, rats consumed flavored milk more than plain milk or sweetened plain milk. Presumably, the smell, the color or the taste of flavored milk made it more attractive than plain or sweetened plain milk.³⁴



In experimental animals, Martínez et al³⁵ showed that rats who were exposed to water sweetened with glucose increased the sweetened water intake gradually; however, no evidence has been provided of whether flavor or sweeteners determined this preference. In humans, Pelsmaeker et al⁶ found that children find plain milk healthier than flavored milk. However, they clearly preferred flavored milk when judging the taste.

In the present study, although the rats were intact (not desalivated),^{29,36} caries have been developed in all groups which might be due to the cariogenic challenge that had been introduced to all animals at the beginning of the experiment.²⁶ The laboratory chow fed to the rats was non-cariogenic and fluoride free, thus any caries developed might be ascribed to the drinks the rats were consuming. All tested fluids produced caries, to varying degrees regardless of the amount being consumed which indicates that the amount of fluids consumed was not directly related to caries development.

Rats consuming 10% sucrose had higher caries scores than all the other groups, while plain milk group had the lowest. Similar observations were reported by many investigators in which sucrose was by far the most cariogenic and plain milk was the least cariogenic of all their examined products.^{25,26,29} In addition to the sugar concentration, fluid acidity is a very important factor in caries initiation as 10% sucrose was the most acidic fluid, while plain milk was the least among the tested fluids.

Significant differences in caries scores were found among the groups receiving flavored milk, sweetened plain milk and plain milk. This result contradicts the result of Bowen and Pearson²⁴ who found that 2% milk containing as much as 10% added sugar was no more cariogenic than 2% milk without sugar. On the other hand, significant differences in caries scores were observed among the groups receiving flavored milk, sweetened plain milk and 10% sucrose, although they had the same total sugar concentration (10%). This observation suggests that milk is exerting some protection against the cariogenic challenge of sucrose included in the milk as reported by Bowen and Pearson.²⁴ Nevertheless, milk containing sucrose is more cariogenic than milk alone. However, when the concentration of sucrose in milk is increased, the pH level is decreased, which suggest that milk might lose its cariostatic properties.

Milk's apparent low level of cariogenicity could be due to its high buffering capacity and to the presence of other components, including high calcium and phosphate contents. In addition, plaque pH studies have shown that milk causes only a negligible decrease in plaque pH.³⁷ Milk, including flavored milk also contains components that may be protective against dental caries.^{38,39} Bowen et al²⁵

reported that 2% milk with 10% added sugar was less cariogenic than water with 10% sucrose which indicates that some components in milk may be protective. The plain milk and the flavored milk which have been tested in the present experiment contain approximately 2 to 3% fat, which aids oral clearance, and adsorbs to the enamel surface and may have a protective role.¹⁴ Casein, the milk protein has been shown to adsorb to the enamel surface, protecting against demineralization.⁴⁰

In the present study, plain milk sweetened with 5% sugar group had more caries score than flavored milk groups without significant difference among them although had the same total sugar concentration. This could be related to the nature of the flavors which might have some effects against caries development. Food flavors are mainly esters which might react with sugar or milk components and elevate the pH and hence reduce the caries scores. This explanation needs further investigation as all flavored milk tested in this experiment contain natural-identical flavors.

Among the flavored milk groups being tested in this study, chocolate flavored milk group had the least caries score, although it had the highest sugar level. This is in agreement with the result of Navia and Lopez²⁹ who found that rat fed with chocolate milk gave the lowest caries scores of the other products evaluated. Chocolate is suspected to contain some caries free inhibitory substances. Some investigators reported that the addition of cocoa extracted powder to cariogenic food could be useful in controlling dental caries.⁴¹⁻⁴³ Chocolate flavored milk tested in this experiment contains cocoa power in addition to chocolate flavor. In the only relevant clinical trial, institutionalized young people received a daily supplement of 0.45 L of chocolate milk (5% sucrose) for 2 years, producing a small but non-significant increase in caries compared with a plain milk supplement.⁴⁴ Numerous nutrition and health organizations agreed that chocolate milk is a healthier alternative to soft drinks and a vital part for meeting the dietary needs of youngsters.⁴⁵⁻⁴⁷

However, milk containing 5% of added sugars is likely to be much less than that of carbonated soft and fruit flavored drinks. Flavored milk is packed with nutrients that make it nutritionally superior to soft drinks and fruit drinks. Children who drink flavored milk have higher total milk intake and consume fewer soft drinks than children who do not drink flavored milk.⁸ Such a result may indirectly and positively affect the teeth through increased level of calcium and milk casein from flavored milk and decreased sugar intake through decreased consumption of soft drinks.

The results of this study should be carefully compared to others findings due to the differences in protocols, diet, handling procedures, strain of bacteria, caging, weaning age, desalivation, and scoring system. However, within the limitations of this animal study, it can be anticipated that the increase of sucrose concentration in milk affects its cariostatic properties. Further animal studies to investigate the effect of flavors on caries development should be studied separately.

CONCLUSION

Lounging between 10% sucrose and plain milk, it can be concluded that the cariogenic potential of flavored milk containing 5% sucrose is moderate. However, an attempt should be made by the dairy manufacturers to decrease the sugar content in flavored milk as the milk naturally contains 5% lactose. Flavored milk should not replace the plain milk and if it is consumed as a beverage, their occasional consumption might be accepted.

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