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Comparison of the Cariogenicity of Carbonated Soft Drinks and Fruit-flavored Drinks Using Experimental Rats

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Research Article

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ABSTRACT

Objective: To test and compare the cariogenicity of carbonated soft drinks and fruit-flavored drinks using experimental rats.

Materials and Methods: Seventy Sprague–Dawley rats were divided into six experimental groups comprising 10 rats each. Each group was offered one of the following drinks: carbonated cola, carbonated orange, carbonated citrus, apple-flavored, orange-flavored, and mango-flavored. In addition, 10 rats were offered distilled water as a control. The experiment continued for 5 weeks. The rats' molar teeth were scored for smooth surface and sulcal caries using the modified Keyes method. Caries scores were compared between different groups using one-way analysis of variance and Tukey's test.

Results: Rats in the carbonated and fruit-flavored drink groups had higher caries scores with significant differences when compared with the control group. Rats consuming carbonated cola developed higher smooth surface and sulcal caries than rats consuming the other drinks. However, rats consuming other carbonated and fruit-flavored drinks developed similar smooth surface and sulcal caries.

Conclusion: Carbonated soft drinks, especially cola, and fruit-flavored drinks are cariogenic; however, cariogenicity was comparable between the various drinks evaluated in this study.

INTRODUCTION

The consumption of "sugar-sweetened beverages" (SSBs), including carbonated soft drinks, fruit-flavored drinks, fruit juices, and energy beverages, has increased significantly over the past decade, particularly among children and adolescents [1,2]. An earlier study evaluating beverage consumption and nutrient intake among children <5 years of age found that 30% of children consumed carbonated drinks, and approximately 37% of children consumed fruit-flavored drinks [3]. Fruit drinks provided children with less than 5% of their daily recommended nutrient intake; however, carbonated drinks provided very little nutritional value [3]. Fruit-flavored drinks typically consist of water with a large amount of added sugar, citric acid, and artificial flavors and colors. Carbonated and fruit-flavored drink consumption habitually starts during the preschool years and usually increases with age [1,4,5]. Patterns of beverage consumption developed during the first years of life can have long-term implications throughout childhood, adolescence, and into adulthood [6].

Frequent consumption of high sugar, high calorie, and low nutrient beverages has raised the concern of health professionals that the consumption of these drinks might displace nutrient-rich beverages such as milk [7]. In addition, frequent consumption of SSBs is a commonly identified risk factor for the increased incidence of many systemic health problems in children, such as obesity, diabetes, and dental caries [8–10]. These problems have become prominent public health concerns, and in response to these concerns, many countries, including Saudi Arabia, have persistently targeted adolescents' and children's carbonated soft drink consumption, often by banning their sale in schools [11]. However, evidence suggests that adolescents and children may be replacing carbonated soft drinks with other SSBs. Since 2005, carbonated soft drink consumption dropped in the US in favor of sports and energy drinks [2]. In Saudi Arabia, carbonated soft drinks are still the most frequently consumed drinks by Saudi adolescents [12]. In response to Saudi authority banning of carbonated soft drinks, fruit drinks and fruit juices have become the most highly consumed SSBs in schools [12]. Parents often perceive fruit-flavored drinks as healthy alternatives to carbonated soft drinks and as less harmful to the teeth and general health, despite their high sugar and acid content.

Dental caries is a common disease among children, and sugars play a major role in the caries process [13]. A number of epidemiological studies have found that SSBs are commonly consumed by children with a high incidence of caries [14–16]; however, direct experimental evidence that demonstrates the high cariogenicity of SSBs is lacking. The purpose of this study was to test and compare the cariogenicity of different carbonated soft drinks and fruit-flavored drinks that are frequently consumed by children, using experimental rats.

MATERIALS AND METHODS

After approval from the Ethical Committee at the College of Dentistry Research Centre (CDRC), King Saud University (KSU), 20 Sprague–Dawley pregnant rat dams were obtained from the Centre of Laboratory Animals and Experimental Surgery at King Khalid University Hospital in Riyadh and transferred to the College of Dentistry animal house at KSU. The procedures were performed according to the Guide for the Care and Use of Laboratory Animals.

In this experiment, all experimental procedures were followed according to the methods prescribed in a previous paper investigating the cariogenic potential of flavored milk [17], and in accordance to the previous studies [18,19]. Seventy pups born within a 24-hour period were used in the current experiment. The pups were weaned when aged 21 days and were infected with an active culture of *S. mutans* (ATCC 25175) for three consecutive days by oral inoculation. During this period, the animals were fed laboratory chow and 10% sucrose to enhance establishment of infection. At the age of 25 days, oral swabs were taken from selected animals to confirm the bacterial implantation. The rats were then randomly divided into two experimental groups, which received either carbonated soft drinks or fruit-flavored drinks, and a control group. The carbonated soft drink group was divided into three subgroups of 10 rats each and each subgroup was offered either cola, orange, or citrus carbonated drinks ad libitum. The fruit-flavored drink group was divided into three subgroups of 10 rats each and each subgroup was offered either apple, orange, or mango flavored drinks ad libitum. In addition, 10 rats were offered distilled water to act as a control group.

Drink consumption was monitored and recorded weekly. The rats were regularly examined and weighed weekly. By the end of the 5th week, the rats were sacrificed using ether; both jaws were then removed and defleshed. The maxillae and mandibles were fixed in 10% neutral buffered formalin and coded.

In each rat, all 12 molars were scored for both the severity and number of sulcal and smooth surface (buccal and lingual) caries by a trained examiner, who was uninformed about the different groups, using the modified Keyes method [20] and a Nikon stereomicroscope (SMZ 1000; Nikon Instruments Inc, NY, USA) with 40× magnification. Enamel and dentinal caries were evaluated discretely in each rat for each molar surface. The scores were then added to give the total caries score for the sulcal and smooth surfaces independently. To assess the reproducibility of the caries diagnosis, seven rats were re-examined and the intra-examiner kappa statistics were 0.89 for sulcal caries and 0.86 for smooth surface caries. SPSS version 20 software was used for the statistical analyses. The caries scores were calculated as means ± standard deviation. One-way analysis of variance and Tukey's test were used to compare caries scores among different groups at a significance level of 0.05.

High-performance liquid chromatographs (HPLC, Shimadzu, Japan) were performed to analyze the total sugar content of all tested drinks and the different sugar concentrations were expressed as percentages. A pH meter was used to measure the pH of the consumed drinks at room temperature.

RESULTS

The selected rats were successfully inoculated with *S. mutans*. The study was continued for 5 weeks, and all rats survived the duration of the investigation. The relative determinations of total sugar levels, acid concentration, and pH values of the consumed drinks are shown in **(Table 1).** Among the carbonated soft drinks, cola scored the highest sugar level and the lowest pH value, and it was the only drink that contained phosphoric acid. However, the mango-flavored drink scored the highest sugar level, the highest citric acid concentration, and the lowest pH among the fruit-flavored drinks.

A summary of the sulcal and smooth surface mean caries scores is presented in **(Table 2).** The highest sulcal and smooth surface caries was noted in the rats that consumed carbonated soft drinks, followed by fruit-flavored drinks. The caries scores in these groups did not differ significantly ($P>0.05$); however, they all differed significantly from the distilled water group ($P<0.05$). The highest consumption occurred in the carbonated citrus group, while the lowest occurred in the apple-flavored drink group

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(Table 3). The detailed caries scores for each consumed drink are presented in **Table 3.** Among the experimental drinks, the highest sulcal caries score was noted in the cola group, while the lowest occurred in the apple-flavored group. Furthermore, the highest smooth surface caries score was also noted in the cola group, while the lowest score occurred in the carbonated citrus group. Rats that consumed the carbonated cola drink developed significantly more sulcal caries than rats that consumed the apple-flavored drink (P<0.05). Additionally, they developed significantly more smooth surface caries than rats in the other groups (P<0.05). Rats that consumed carbonated orange, carbonated citrus, mango-flavored, orange-flavored, and apple-flavored drinks developed similar sulcal and smooth surface caries, with no significant differences between the groups (P>0.05).

Table 1. Total sugar content, acid concentration, and pH level of tested drinks.

		Fructose (%)	Glucose (%)	Sucrose (%)	Total sugar (%)	Citric acid (ppm)	Phosphoric acid (%)	pH
Carbonated soft drinks	Carbonated cola	4.75	4.16	4.80	13.71	-	1.07	2.3
	Carbonated orange	1.48	1.54	10.5	13.52	1250	-	2.6
	Carbonated citrus	2.26	2.51	6.69	11.73	928	-	3.0
Fruit-flavored drinks	Orange-flavored	1.33	1.27	11.7	14.3	1401	-	2.8
	Apple-flavored	3.36	3.03	6.60	12.9	2318	-	2.6
	Mango-flavored	3.90	3.76	8.77	16.4	2615	-	2.4
Control	Distilled water	-	-	-	-	-	-	7.1

Table 2. Effect of tested drinks on caries scores in the experimental and control groups.

Consumed drink	Carbonated soft drinks Mean ± SD N=30	Fruit-flavored drinks Mean ± SD N=30	Distilled water Mean ± SD N=10
Total sulcal caries	44.9 ± 13.42[a]	37.9 ± 12.76[a]	16.90 ± 3.9[b]
Total smooth surface caries	33 ± 11.58[a]	26.23 ± 9.44[a]	10.782 ± 4.80[b]
Different letters indicate significant differences (P<0.05); SD: standard deviation.			

Table 3. Approximate amount of drinks consumed by rats in each group at the end of the experiment and the effect of each tested drink on caries scores in rats.

	Carbonated soft drinks Mean±SD			Fruit-flavored drinks Mean±SD			Control Mean ± SD
Drink	Cola N=10	Orange N=10	Citrus N=10	Mango N=10	Orange N=10	Apple N=10	Distilled water N=10
Amount (ml)	228 ± 6.2[a]	233 ± 3.1[a]	243 ± 12.4[a]	187 ± 18.5[b]	198 ± 14.1[b]	184 ± 13.5[b]	169 ± 15.7[c]
Total sulcal caries	53.3 ± 12.6[a]	43.0 ± 13.7[a,b]	38.4 ± 10.1[a,b]	40.1 ± 14.0[a,b]	38.6 ± 11.0[a,b]	35.0 ± 13.8[b]	16.9 ± 3.9[c]
Total smooth surface caries	44.2 ± 9.3[a]	32.7 ± 7.1[b]	22.2 ± 4.9[b]	30.0 ± 9.5[b]	25.7 ± 7.2[b]	23.0 ± 10.7[b]	10.7 ± 4.8[c]
Different letters indicate significant differences (P<0.05); SD: standard deviation.							

DISCUSSION

The aim of the present investigation was to evaluate various popular SSBs from a cariological perspective. For this purpose, the total sugar concentration, acid concentration, pH value, and the effect of the consumption of these drinks on the tooth surface were assessed.

Dental caries is primarily a multi-factorial microbial disease, but diet plays a very important role in the caries process. Diet affects the dental structure, salivary pH, salivary quantity and quality, and plaque pH [21]. SSBs are thought to cause damage to the teeth because of two processes. First, the low pH and titratable acidity of acidic drinks may cause enamel erosion, and second, the fermentable sugar in such drinks is metabolized by plaque micro-organisms to produce organic acids in the dental plaque and saliva, resulting in demineralization and leading to dental caries [22,23]. Although erosion and caries differ histologically, concurrent occurrence of these conditions often has detrimental effects on the teeth [22,23].

In the current study, rats in the carbonated drink groups developed more caries than rats in the fruit-flavored drink groups, but the difference was not significant. Both carbonated and fruit-flavored drinks have a high sugar content, to enhance their taste, and a high acid concentration, which causes enamel demineralization. Birkhed [24] studied the sugar content and acidity of fruit drinks, fruit juices, carbonated soft drinks and sport drinks, and found that all investigated drinks had a similar cariogenic potential.

All tested experimental drinks, particularly fruit-flavored drinks, contained high sugar content. This included glucose, fructose, and sucrose, which was the main sugar found in these drinks. It was previously believed that drinks containing natural sugars (glucose and fructose) were harmless for the teeth [25,26]. However, several studies have shown that all sugars have a similar potential for acid production in plaque; hence, their cariogenicity may be similar to that of sucrose [25,26]. The sugars found in SSBs may be the main sugars linked with infant caries [27,28]. However, Cury et al. [29] found that dental plaque produced in the presence of sucrose was more cariogenic than that produced in the presence of glucose and fructose. Al-Jobair and Khounganian [17] found that flavored milk was more cariogenic than plain milk due to the added 5% sucrose. Sucrose is considered to be the most important sugar in dental caries development [29]. Bacterial adherence is dependent on the generation of plaque dextrans produced by sucrose; thus, cariogenic bacteria are easily implemented in the oral cavity [27,28]. However, the cariogenicity of a drink or food is associated with the frequency and duration of sugar intake rather than the type of sugar ingested [30]. In this study, all experimental drinks produced caries, despite differences in the amount consumed, which indicates that the quantity of drink consumption was not directly related to caries development. As such, frequent exposure to sugary and acidic drinks appears to play a role in caries development. Therefore, few sugar exposures are of low significance, but if frequent exposure occurs, the ability for saliva to resist the acid produced by bacteria decreases. If the challenge is repeated sufficiently and there are no protective factors, as in the case of high caries risk children, the effect is likely to be destructive.

Besides sugar and cariogenicity, fruit-flavored drinks generally have a higher citric acid concentration than carbonated orange and citrus, which have relatively higher pH values. Although not investigated in this experiment, fruit-flavored drinks have a significantly greater buffering capacity as compared with carbonated drinks, which is related to their titratable acidity [31,32]. Titratable acidity, which is a measure of the total acid content, is a more significant indicator than the pH value in determining the destructive potential of drinks, as the initial pH value does not indicate the underlying buffering capacity. Conversely, carbonated soft drinks have inherent acidity because of other acids that are added to stimulate taste and neutralize sweetness. Carbonated soft drinks often contain acids other than citric or phosphoric acid, such as carbonic acid, which is formed from carbon dioxide, resulting in lower pH values. Even if the carbon dioxide has been blown off, the pH remains low [25].

The highest caries score in this experiment was registered in the carbonated cola group, which is consistent with the results of Bowen and Lawrence [18], who found that cola drinks were the most cariogenic among different fluids commonly consumed by infants and toddlers, when tested on experimental rats. Cola had the lowest pH when compared with the other tested drinks, which is in accordance with other studies [33,34]. The carbonated cola drink tested in this experiment had high total sugar content, the lowest pH value, and it was the only drink that contained phosphoric acid. This suggests that it is not only the sugar concentration that is responsible for caries initiation; the type of acid also plays a role. In addition, Ireland et al. [35] showed that cola drinks were more adherent to tooth enamel than human saliva, which suggests that cola drinks are less likely to be removed from tooth enamel by saliva, making cola drinks more cariogenic than other drinks. Concerning the other tested drinks, rats in the carbonated citrus group scored less caries than rats in the carbonated orange group. This could be related to the lower total sugar concentration (especially sucrose), lower citric acid concentration, and the higher pH value of the carbonated citrus drink when compared with the carbonated orange drink. Similarly, these factors may have resulted in lower caries scores in rats consuming the apple-flavored drink than rats in the mango- or orange-flavored drink groups.

The results presented here support previous epidemiological and experimental reports on the cariogenic potential of carbonated and fruit-flavored drinks [15,16,18]. Caution should be exercised in applying these results to humans. The enamel layer of rat teeth is approximately 20 times thinner than that of human teeth. Rat teeth are also highly hypomineralized after eruption and posteruptive maturation of the teeth may continue for at least 2 months [36]. In humans, primary tooth enamel is more susceptible to the effects of carbonated and fruit-flavored drinks because primary enamel has a higher degree of porosity and a lower degree of mineralization than permanent enamel [37]. In addition, immature permanent enamel is porous and easily dissolved by acids until the enamel surface is fully matured and becomes less penetrable, progressively harder, and relatively resistant to acid attack [38, 39]. However, enamel maturation occurs over time; therefore, children and adolescents are at greater risk of dental caries if the acidogenic challenge is high.

CONCLUSION

In conclusion, this study revealed the high cariogenicity of carbonated and fruit-flavored drinks. All of the investigated experimental drinks showed a similar and high cariogenic potential when compared with distilled water. The findings from the present study indicate that the reported high sugar content, high acid concentration, and low pH values of carbonated and fruit-flavored drinks are strongly related to their high cariogenic load. Although the findings from the present rat model cannot be definitively applied to humans, it would be prudent for dental personnel, including students, dentists, and dental educators, to alert children and adolescent patients and their parents about the potential consequences of frequent consumption of these drinks, and to highlight their detrimental role in establishing caries and dental erosion.

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CONFLICT OF INTEREST

Authors Al-Jobair and Khounganian declare that they have no conflict of interest.

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