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Evaluation of the Effect of Dairy Products on *Streptococci* and *Lactobacilli* in Saliva on Group of Egyptian Children

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ABSTRACT

Objective: to evaluate the effect of dairy products on *streptococcus mutans (SM)* and *lactobacilli (LB)* in saliva on group of Egyptian children. **Material and Methods:** One hundred and twenty Egyptian children from both sexes were included in this study, their age ranged from 4-12 years old. These children were equally divided into 3 groups, each group consisted of 40 children. First group **(group A)** was instructed to drink plain cow's milk, second group **(group B)** was instructed to drink milk plus sugar and third group **(group C)** was instructed to drink milk plus honey. Three saliva samples were collected from each child, one before drinking the beverage (S_1) then immediately after drinking (S_2) and finally one hour after drinking the beverage (S_3) by spitting in a sterile plastic container, then submitted to the Culture and Sensitivity Unit at The Regional center for Mycology and Biotechnology at Al-Azhar University to evaluate the salivary concentration on *SM* and *LB* for each subject. **Results:** The use of plain milk resulted in decrease in the mean value of *SM* immediately after drinking then subsequently showed a slight increase after one hour. And *LB* mean value increased immediately after beverage, then subsequently showed a significant increase after one hour. The use of milk plus sugar resulted in significant increase in mean value of *SM* immediately and one hour after beverage. And the mean value of *LB* significantly increased immediately and one hour after beverage. The use of milk plus honey resulted in significant decrease in the mean value of *SM* one hour after beverage .And the mean value of *LB* significantly decreased immediately and one hour after beverage. **Conclusion:** the results revealed that, Milk and dairy products have a low cariogenic potential. Adding sugar makes whole milk as cariogenic as a sucrose solution. Natural honey had an antibacterial activity on *SM* and *LB* bacteria.

KEYWORDS

Milk, SM, LB, Saliva, Honey, Sugar

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INTRODUCTION

Children's eating habits have dramatically changed in the last years. Milk consumption has decreased whereas the consumption of soft drinks, juices, non- citric beverages and carbohydrates has increased. Unfortunately, these habits have been correlated with a rise in the prevalence of dental caries [(1)].

Milk, in its various forms, is well known to be beneficial for the development of teeth and bone. More importantly, milk is the major nutritional source in the first years of life [(2)]. Around the world, people drink milk from many animals including cow, buffalo, sheep, goat and camel. But today, cow's milk is still one of the most popular animal milks consumed by humans as nine out of every ten glasses of milk consumed by people come from cows [(3)].

The nutritional value of milk is particularly high due to the balance of the nutrients that compose it. The composition varies among animal species and breeds within the same species, and also from one dairy to the other, depending on the period of lactation and diet. Bovine (cow) milk contains 87.2% water, 3.5% protein, 3.7% fat, 4.9% lactose, calcium (118mg/100g) and phosphorus (96mg/100g), with some small variation[(4)]. In milk, approximately 80% of the protein is *casein* and 20% is *whey*. Both *casein* and *whey* proteins are rich sources of peptides that significantly lower blood pressure in those with hypertension and may contribute to satiety and regulate food intake [(5)].

About 98% of milk fat is a mixture of triacylglycerides. Milk fat acts as a solvent for the fat-soluble vitamins A, D, E and K and also supplies essential fatty acids [(6)]. Non-cariogenic and protective properties of bovine milk may be due to the lactose content is low and of limited cariogenic potential, the high calcium and phosphate content resists demineralization and aids remineralization of enamel and dentine, *casein* reduces demineralization of tooth tissue. Finally, other components of milk as (Lactoferrin, Lactoperoxidase and Lysozyme) may reduce the ability of plaque microflora to adhere to enamel and produce acids[(7)].

Many children like to drink sweetened milk either with sugar or honey. The effect of adding sucrose to cow's milk has been investigated in a variety of studies. In an uncontrolled, observational epidemiological survey it was recorded that children who had drink milk with added sugar had higher caries experience than children who had drink milk with no added sugar[(8)]. Honey in combination with milk provides excellent nutritional value[(9)], and reduces the survival of bacteria faster than either honey or milk alone [(10)].

MATERIALS AND METHODS

One hundred and twenty Egyptian children from both sexes were included in this study, their age ranged from 4-12 years old. Ethical approval was obtained from the Research and Ethics committee of the Faculty of Dental Medicine of Al- Azhar University (Girls Branch), Cairo –Egypt.

Case Selection

These children were chosen from the outpatient clinic of Faculty of Dentistry (Girls'branch) Al-Azhar University, or their relatives attending with them.

They were selected according to the following criteria:

- They were in a good physical condition with no systemic diseases.
- No history of recent antibiotic administration (last 2 weeks) or antimicrobial mouth-rinse (last 12 hours).
- They were free of oral inflammation or any oral septic foci.
- There were no orthodontic appliances.
- They were with low caries index (DMF ≤ 4, def ≤ 4 and dmf ≤ 4).

Methods:

Preparation of the Beverages:

- Cow's milk was purchased from the milk shop (Sonista shop, Cairo, Egypt) and boiled then packed into a bottle after cooling, after that a 150ml of the milk [(11)] was measured by a graduated cup and then given to each child.
- Milk plus sugar was prepared by adding 3 small spoons (12 grams) of sucrose sugar [(12)] to 150ml of cow's milk.
- Milk plus honey was prepared by adding 2 small spoons of honey (11.5 grams of sugar) [(13)] (IMTENAN Co.) to 150 ml of cow's milk.

Collection of saliva samples

Children were equally divided into 3 groups, each group consisted of 40 children:

Group A: the first sample was collected first thing in the morning (before breakfast or at least 2 hours after meal) to establish baseline levels (S_1), then 40 children were instructed to drink 150 ml of cow's milk, then the second sample (S_2) was obtained by spitting immediately after drinking the beverage in a sterile plastic container, then the children were instructed to wait in the waiting area and avoid eating anything for 1 hour then the third sample was taken (S_3).

Group B: After collection of the first sample (S_1), 40 children were instructed to drink 150 ml of cow's milk with 3 small spoons of sucrose sugar added to sterile plastic container, then the children were instructed to wait in the waiting area and avoid eating anything for 1 hour then the third sample was taken (S_3).

Group C: After collection of the first sample (S_1), 40 children were instructed to drink 150ml of cow's milk with 2 small spoons of honey added to milk, then the second sample (S_2) was obtained by spitting immediately in a sterile plastic container, then the children were instructed to wait in the waiting area and avoid eating anything for 1 hour then the third sample was taken (S_3). Saliva samples (S_1) and (S_2) were kept in ice until obtaining third sample (S_3), then submitted to the Culture and Sensitivity Unit at The Regional center for Mycology and Biotechnology at Al-Azhar University to evaluate the salivary concentration on *SM* and *LB* for each subject.

Laboratory Diagnosis:

For SM counts and LB counts

The saliva samples were diluted at least four dilutions 1:10, 1:100, 1:1000, 1:10000 in sterile diluent (peptone water), then 1 ml of each dilution of saliva specimen was homogeneously spread on the surface of the selective media {Mitis Salivarius agar (Diffco Co. USA) and Rogosa agar (Unipath Co. UK) }.The plates were incubated anaerobically in CO_2 anaerobic incubator at 37°C for 48-96 hours[(14)], then the colonies of *SM* and *LB* were counted and calculated.

Statistical Analysis

Analysis of data was performed using SPSS 17 (Statistical Package for Scientific Studies) for Windows. Description of quantitative variables was in the form of mean, standard deviation (SD). Data were explored for normality using Kolmogorov-Smirnov test of normality. The results of Kolmogorov-Smirnov test indicated that most of data were normally distributed (parametric data) so parametric tests were used for the comparisons. Different milk types (plain, plus sugar, plus honey) were compared using analysis of variance (ANOVA) test, followed by Tukey's post hoc test when a significant difference was detected. The significance level was set at $P \leq 0.05$.

RESULTS

I- Streptococcus mutans

A) Difference between types of beverages

At baseline, there was no statistically significant difference between the three different groups regarding the values of colony forming unit of streptococcus mutans. The greatest mean count was found in the milk plus sugar group immediately ($21351.11X10^4$) and one hour after beverage ($57408.89x10^4$), with a statistically significant difference revealed by One way analysis of variance (ANOVA) test ($p<0.0001$). Tukey's post hoc test revealed no significant difference between milk plus honey and plain milk groups (table 1).

B) Difference between baseline and post beverages

In the milk plus honey group, the greatest mean value was recorded at baseline, however, there was no statistically significant difference between baseline and immediately after beverage regarding the values of colony forming unit of streptococcus mutans. On the other hand, the mean value ($16.1642x10^4$) significantly decreased one hour after beverage ($p<0.0001$).

In the milk plus sugar group, the lowest mean value was recorded at baseline ($3368.889x10^4$), subsequently, there was a statistically significant increase immediately after beverage ($21351.11 x10^4$) and one hour after beverage ($57408.89x10^4$), with a p value <0.0001.

In the plain milk group, the greatest mean count was found at baseline ($5390.222 X10^4$). This value decreased immediately after beverage ($4344.444x10^4$), then subsequently showed a slight increase after one hour ($4470.889x10^4$), with a non- statistically significant difference revealed by one way analysis of variance (ANOVA) test ($p=0.638603$), (table 2).

C- Percent change in colony forming unit after beverage

Immediately after beverage, the milk plus honey group showed a percent decrease in mean number of colony forming units of streptococcus mutans (-18.3968%). Although plain milk showed a percent increase in the same interval (53.04883%), both groups were not statistically significantly different. However, ANOVA test revealed a statistically significant percent increase in the milk plus sugar group ($p<0.0001$).

Regarding the overall percent change, the milk plus honey group showed a percent decrease in mean number of colony forming units of streptococcus mutans (-66.58503%), both groups were not statistically significantly different. However, ANOVA test revealed a statistically significant percent increase in the milk plus sugar group ($p<0.0001$), (table 3).

II- Lactobacilli

A- Difference between types of beverage

At baseline, there was no statistically significant difference between the three different types of beverages regarding the values of colony forming unit of lactobacilli. The greatest mean count was found in the milk plus sugar group immediately ($5208X10^4$) and one hour after beverage ($32344x10^4$), with a statistically significant difference revealed by One way analysis of variance (ANOVA) test ($p<0.0001$). Tukey's post hoc test revealed no significant difference between milk plus sugar and plain milk groups immediately after beverage (table 4).

B- Difference between baseline and post beverages

In the milk plus honey group, the greatest mean value ($5502.222 x10^4$) was recorded at baseline. The mean value significantly decreased immediately ($46.3267x10^4$) and one hour after beverage ($11.138x10^4$), with a P value <0.0001. Tukey's post

hoc test revealed that there was no statistically significant difference between immediately after beverage and one hour after beverage regarding the values of colony forming unit of lactobacilli.

In the milk plus sugar group, there was no statistically significant difference between baseline and immediately after beverage regarding the values of colony forming unit of lactobacilli. On the other hand, the mean value ($32344.44x10^4$) significantly increased one hour after beverage ($p<0.0001$).

In the plain milk group, the lowest mean count was found at baseline ($4345.577X10^4$). This value increased immediately after beverage ($5108.667x10^4$), then subsequently showed a significant increase after one hour ($18148x10^4$), ($p=<0.0001$). Tukey's post hoc test revealed that the difference between the baseline and immediately after beverage value was not significant (table 5).

C) Percent change in colony forming unit after beverage

Immediately after beverage, the milk plus honey group showed a percent decrease in mean number of colony forming units of lactobacilli (-99.01675%). On the other hand, both milk plus sugar and plain milk groups showed a percent increase in the same interval (1421.26% and 1199.704% respectively. ANOVA test and Tukey's post hoc test revealed a statistically significant difference between the milk plus honey group and the other 2 beverage types ($p<0.0001$).

Regarding the overall percent change, the milk plus honey group showed a percent decrease in mean number of colony forming units of lactobacilli (-99.6311%). Although plain milk showed a percent increase in the same interval (4571.353%), both groups were not statistically significantly different. However, ANOVA test revealed a statistically significant percent increase in the milk plus sugar group ($p<0.0001$), (table 6).

Table (1) *Mean ±SD of colony forming unit of streptococcus mutans ($x10^4$) in different beverages at baseline and after beverage and significance of the difference between beverages using ANOVA test*

	Baseline	**Immediately after beverage**	**after 1hour of beverage**
Milk plus honey	5502.222 ±1975.703	4668.444 ±2829.498	16.16422 ±5.3023
Milk plus sugar	3368.889 ±2714.387	21351.11 ±15293.72	57408.89 ±16116.72
Plain milk	5390.222 ±977.54	4344.444 ±2496.006	4470.889 ±2521.029
F value	2.04	51.47	104.99

**significant, ns= non-significant at p<0.05*

Table (2) *Mean ±SD of colony forming unit of streptococcus mutans ($x10^4$) in different beverages at baseline and after beverage and significance of the difference between baseline and post beverages values using ANOVA test*

	Baseline	**Immediately after beverage**	**After 1hour of beverage**	**F value**	**P value**
Milk plus honey	5502.222 ±1975.703	4668.444 ±2829.498	16.16422 ±5.3023	99.06	<0.0001*
Milk plus sugar	3368.889 ±2714.387	21351.11 ±15293.72	57408.89 ±16116.72	66.14	<0.0001*
Plain milk	5390.222 ±977.54	4344.444 ±2496.006	4470.889 ±2521.029	0.45	0.63860^{ns}

**significant, ns=non-significant at p<0.05*

Table (3) *Mean ±SD of percent change of colony forming unit of streptococcus mutans (%) in different beverages and significance of the difference between beverages using ANOVA test*

	Percent changes immediately after beverage	**Over all percent change**
Milk plus honey	-18.3968±5.94006	-99.6311±0.617492
Milk plus sugar	1026.913±601.568	2701.471±993.355
Plain milk	53.04883±20.8157	66.58503±29.4748
F value	13.97	27.17
P value	<0.0001*	<0.0001*

**significant at p<0.05*

Table (4) *Mean ±SD of colony forming unit of lactobacilli (x10⁴) in different beverages at baseline and after beverage and significance of the difference between beverages using ANOVA test*

	Baseline	immediately after beverage	after 1hour of beverage
Milk plus honey	5204.667 ±2680.544	46.3267 ±19.645	11.138 ±5.3023
Milk plus sugar	5055.5778 ±307.9736	5208.667 ±3312.432	32344.44 ±26479.46
Plain milk	4345.5778 ±232.6135	5108.667 ±2652.447	18148 ±13337.83
F value	8.53	65.25	28.45
P value	0.247^{ns}	<0.0001*	<0.0001*

**significant, ns=non significant at p<0.05*

Table (5) *Mean ±SD of colony forming unit of lactobacilli (x10^4) in different beverages at baseline and after beverage and significance of the difference between baseline and post beverages values using ANOVA test*

	Baseline	**immediately after beverage**	**after 1hour of beverage**	**F value**	**P value**
Milk plus honey	5204.667 ±2680.544	46.3267 ±19.645	11.138 ±5.3023	168.38	<0.0001*
Milk plus sugar	5055.5778 ±307.9736	5208.667 ±3312.432	32344.44 ±26479.46	55.87	<0.0001*
Plain milk	4345.5778 ±232.6135	5108.667 ±2652.447	18148 ±13337.83	20.62	<0.0001*

*significant at p<0.05

Table (6) *Mean ±SD of percent change of colony forming unit of lactobacilli (%) in different beverages and significance of the difference between beverages using ANOVA test*

	Percent change immediately after beverage	**Over all percent change**
Milk plus honey	-99.01675 ± 1.118815	-99.6311 ± 0.617492
Milk plus sugar	1421.26 ±337.609	13899.89±2153.76
Plain milk	1199.704 ±79.1254	4571.353±1715.33
F value	12.17	7.77
P value	<0.0001*	0.000645*

**significant at p<0.05*

DISCUSSION

Bovine (cow) milk is one of the most consumed food products by humans. Whole cow's milk has been typically considered as healthy and caries-protective. This protective effect would come from anticariogenic properties of the fluid [15].

Thus, many components in cow's milk have been reported to have anticaries properties, including calcium, phosphates, fats, vitamins, iron, and some enzymes [16]. Caseins have an antibacterial effect. Furthermore, these proteins lead to an increase in calcium and phosphate within the oral biofilm [17]. Besides casein; LFe, lysozymes, and antibodies present in milk have an antibacterial effect against *SM* [18].

In the present study *SM* and *LB* were tested as many studies have confirmed *SM* and *LB* as the sole causative microbiological agents and their presences in the dental structure cariogenic biofilm are indicator of dental caries [19].

In the this study the children's age (4-12 years old) was selected as cow's milk is a rich source of protein and calories which is important for growing kids [20], also children who drink milk tend to have higher intakes of specific nutrients, such vitamin A, folate, vitaminB12, calcium and magnesium, and have better overall nutritional status than non-milk drinkers [21]. Cow's milk was sweetened either with sugar or honey as many children like to drink sweetened milk.

In plain milk group there was a decrease of *SM* count immediately after drinking. This is supported by certain study [22] which proved that daily consumption of milk and dairy products may reverse soft and leathery caries and decrease the salivary level of *SM* in adults.

After one hour of drinking plain milk *SM* count showed slight increase but non- statistically significant. This agreed with certain study [15] which proved that lactose in milk gives rise to the monosaccharides glucose and galactose, thus, it still contains sugars that are fermentable by *SM*. This also agreed with some studies [23]which proved that milk contains 4-5% of the disaccharide lactose which can be fermented by oral biofilm bacteria (*SM*) and unless the bacteria are adapted to lactose, fermentation is significantly less than from sucrose .

In the present study the value of *LB* increased immediately after drinking plain milk then showed significant increase after one hour (P= < 0.0001) . This result agrees with some studies[24]which proved that cow and buffalo milk exhibit a wide diversity of *LB* occurring naturally in the milk and can be used as a potential natural source to isolate a variety of strains of *LB*.

In milk plus sugar group the greatest mean count for both *SM* and *LB* was found immediately

and after one hour of drinking the beverage. This agreed with a study [25] which proved that consequence of sucrose exposure when compared with other carbohydrates (lactose,maltose and glucose) resulted in increased biomass of *SM* . The results also agreed with certain study [26] which proved that the increase in the frequency of sugar consumption increased the number of *LB* in saliva as the sugar was too rapidly eliminated to create an acidic oral environment as favors the aciduric *LB*.

In the present study honey was used as a sweetener for milk as honey is a delicious, natural sweet food and sweeteners are one of the most common causes in developing dental caries. Thus, the use of a low harmful sweetener in the diet is very important, especially, if it was confirmed that honey has antibacterial activity against cariogenic bacteria in vitro and in vivo [27].

Results of this current study showed percent decrease in mean number of colony forming unit (CFU) of both *SM* and *LB* (-99.01675 %) and (-99.6311%) respectively. These results are in agreement with certain studies [27]which studied the antibacterial activity of honey on cariogenic bacteria and reported that natural honey had an antibacterial effect on cariogenic bacteria (*SM* and *LB*) in experimental studies.

It was proved that honey is effective against *SM* and *LB* which are responsible for initiation and progression of dental caries respectively [28] and this agrees with the results of this study. But the results of this study didn't agree with some studies [29] which proved that there is no general agreement about the cariogenic potential of honey and some studies have reported honey to be cariogenic.

CONCLUSION

From this study we concluded that:

1. Milk and dairy products have a low cariogenic potential, cow's milk provides protection against caries even in caries - susceptible conditions.
2. The low cariogenic potential of milk would appear to be due to (a) lactose being the least cariogenic of dietary sugars, (b) the protective role of casein and possibly fats, and (c) the protective role of calcium and phosphorus.
3. Adding sugar makes whole milk as cariogenic as a sucrose solution.
4. Several studies have recorded that children who had drank milk with added sugar had higher caries experience than children who had drank milk with no added sugar.
5. Results demonstrated that natural honey had an antibacterial activity on *SM* and *LB* bacteria.

RECOMMENDATIONS

- Children should drink milk for better overall health.
- The practice of adding sugar to milk should be discouraged, and honey can be used as an alternative sweetener to sucrose.
- It's advisable that children must brush their teeth after drinking milk.
- Cow's milk is not recommended for babies until at least 1 year of age .

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