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Original Article

Effect of Different Concentrations of Molybdenum on Dental Enamel Microhardness, an *In Vitro Study*

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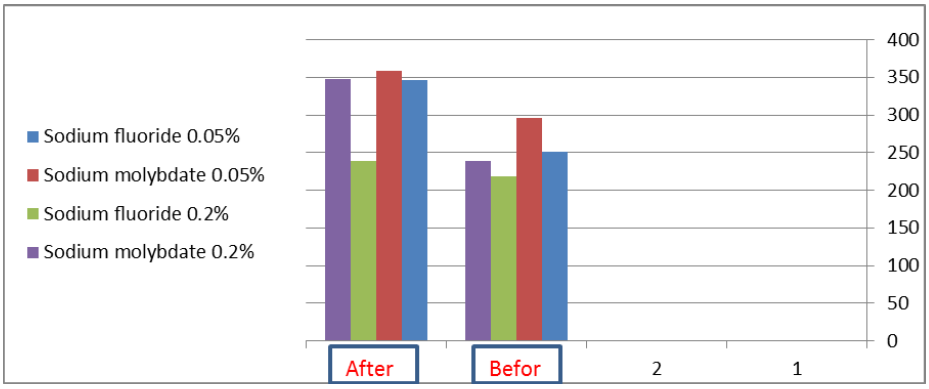
ABSTRACT

Introduction: Molybdenum is an essential trace element with several biological functions and therapeutic uses, and reported to have a cariostatic effect and is suggested as one of the agents that could be used as an alternative to fluoride as one the effective ways of preventing dental caries.
Aim: This study aimed to evaluate the effect of molybdenum compounds on the microhardness of dental enamel.
Materials and Methods: This in vitro experimental study was performed on 50 extracted sound premolar teeth. These teeth were extracted for an orthodontic cause. Enamel blocks were divided randomly into five groups. Micro-hardness of tooth enamel was measured by the Vickers microhardness test before and after the use of different concentrations of molybdenum solutions. ANOVA and a Dunnett t-test (2-sided) were used under $P < 0.05$.
Results: The comparison of means change in microhardness before and after the use of molybdenum compound showed an increase in microhardness number for all concentrations of molybdenum solutions with a significant difference when compared to the control group ($p<0.000$), and that between sodium fluoride and sodium molybdate with highly significant difference between the initial and final measurements ($P<0.000$). The difference in sodium molybdate was higher in the concentration 0.2% than in 0.05% concentration.
Conclusion: Results indicated that the molybdenum compound significantly increased the microhardness of dental enamel rendering it to be more resistant to acid attack, and its effect will approximate that of fluoride that could use in the prevention of dental caries.

GRAPHICAL ABSTRACT



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Introduction

Molybdenum is an essential dietary trace element [1], and it may act synergistically with fluoride in reducing enamel dissolution [2]. Molybdenum is an essential trace element that has several biological functions and therapeutic uses [3]. Dental enamel showed several changes due to the action of the demineralization process [4], and it can resist this process by the role of its minerals contents such as calcium and phosphate [5]. Fluoride was proved to inhibit and prevent the demineralization process and increase the resistance of dental enamel against acid attack [6]. Rinsing with 0.05 and 0.2% of sodium fluoride mouth rinse can cause an increase in surface microhardness [7]. Enamel microhardness is a measurement used for the evaluation of the effects of different types of minerals and other agents on the enamel surfaces used in dental experiments to measure the microhardness and hardness of teeth [8]. It resembles the microhardness properties of enamel surfaces determined by indentation performed by applying a diamond indenter such as a Vickers or Knoop indenter into the surface of the tested agents under the average of 1 to 1000 gm load measured by a microscope because the indentations are very small [9]. Despite the success of fluoride in the prevention of dental caries, its prevalence remains high [10], and because of dental fluorosis, an alternative element that gives the maximum benefit in prevention without showing any side effects was studied [11]. Enamel microhardness demonstrated the resistance of dental enamel against several conditions and agents [12]. The Microhardness test is used to determine the effect of different agents on tooth microhardness. The Microhardness test is a widely used method in *in vitro* experimental studies to evaluate tooth hardness which demonstrates resistance against demineralization and determines the indentation of the hardness tester [13]. Some are concerned with dental fluorosis especially in a highly fluoridated area, due to the incorporation of high concentrations, so they decided to seek other alternative safe cariostatic agents [14]. One of these methods is the Vickers Hardness test used for the measurement of the microhardness of surfaces and is considered a good method because of its high accuracy by applying force with the use of different sizes and allowing re-measurement at any time [15]. This method is also very useful for measuring the surface microhardness of small parts or areas, and very thin materials as well as to determine the depth of hardness by making several indentations to determine the change in hardness [16]. Concerning dental enamel microhardness, the experimental studies use the prepared test samples to determine the effects of different minerals and chemicals applied on dental enamel surfaces [8, 9]. There are two types of indenters; a narrow rhombus-shaped indenter used for a Knoop tester and a square base pyramid-shaped diamond used for testing in a Vickers microhardness tester [17]. In experimental studies, a microhardness test was used to evaluate the enamel mineralization after the development of subsurface caries lesions with increased porosity due to subsurface demineralization, so the indenter face less resistance and penetrates down until reaching the sound enamel [8]. One of these methods is Vickers Hardness test which used for measurement of microhardness of surfaces and is considered as a good method because of its high accuracy by applying force with the use of different sizes and allow re-measurement at any time [18]. This method is also very useful for measuring surface microhardness of a small parts or areas, and very thin materials as well as to determine the depth of hardness by making several indentations in order to determine the change in hardness [19]. There are two types of indenters; a narrow rhombus shaped indenter used for a Knoop tester and a square base pyramid shaped diamond used for testing in a Vickers microhardness tester [20]. The aim of this *in vitro* study was to determine the effect of molybdenum on the microhardness of dental enamel.

Materials and Methods

Groups of study

Enamel samples were derived from sound, un-stained, caries-free upper first permanent premolar teeth of a healthy 50 female patients, their age range from 14-16 years old recorded according to their birthday. Teeth were extracted for orthodontic treatment cause, collected in the dental clinic under the supervision of the author. This study was conducted in the period between November 2020 and April 2021, in Dentistry Department/Al-Rafidain University College, Baghdad, Iraq.

Enamel block preparation

Enamel samples were obtained from the buccal surfaces of these teeth sliced using a high-speed turbine handpiece (Cavo, 636C) with a flow of deionized water to prepare rectangular enamel slabs. These enamel slabs were placed in prepared acrylic blocks and the enamel surface of the slab was exposed to different concentrations of molybdenum as sodium molybdate and the inner surface was fixed on the acrylic block by a resin. Enamel slabs were polished with sandpaper to become smooth. The prepared samples of dental enamel blocks were mounted in acrylic blocks to facilitate the preparation measurement and testing, to provide a specimen that can fit suitably into the Vickers teste that allows a sufficiently smooth measurement and can be held perpendicular to the indenter. Fifty enamel blocks (3×3 mm) were gained from these teeth, were prepared by using a diamond bur, and were kept in 2% formaldehyde solution at pH 7.0. The specimens were embedded in the epoxy resin and the surface of the enamel blocks was grounded flat and polished to remove 50 μm of the surface layer with 1.2 grit waterproof silicon carbide paper and water-cooled carborundum discs. The prepared samples were submitted to the microhardness test. Molybdenum solution as sodium molybdate was evaluated and enamel blocks were randomly divided into ten groups each containing five blocks. After inducing caries-like lesions, each group was applied a daily de- and remineralization cycle period for 7 days. After pH cycling, the surface was assessed and the integrated loss of the microhardness of the subsurface was calculated. Artificial caries-like lesions were formed on specimens of intact human enamel with a demineralizing solution for 32 hours. The primary microhardness measurements were recorded by applying 100 gm force on each sample by Vickers microhardness device. The first group was treated with 0.025% sodium molybdate (Group 1), samples of the second group were treated with 0.075% sodium molybdate solution (Group 2), and the third group was treated with 0.150% (group 3). The fourth group was treated with 0.250% sodium molybdate solution (group 4), and the fifth group was treated with deionized water to be considered as a control group that did not receive any molybdenum treatment. The other groups were used in the second experiment, the sixth group and seventh group were treated with 0.05% and 0.2% sodium fluoride, respectively, and the eighth and ninth groups were treated with 0.05% and 0.2% sodium molybdate respectively, whereas the tenth group was treated with deionized water as a control. The samples were entered into a pH cycle which was creating laboratory conditions similar to the environment of the mouth. Each cycle was performed for 24 hours.

Procedure

Enamel samples were placed in a recipient containing artificial saliva at 37 °C. Microhardness was measured with a Micromet, Micro Hardness Tester (Adolph 1, Buehler INC., Optical, and Metallurgical Instruments, USA. 2120 Greenwood st. Evanston ILL, USA 60204) with a load of 100 g for 30 seconds. Microhardness was measured concerning the indentation length and microhardness number. Three measurements were considered; enamel specimens were subjected to a first measurement obtained before the demineralization process (the initial), the second was obtained after demineralization for the same samples under study by measuring the microhardness number of the demineralized enamel then the third was obtained after immersion of enamel samples in different concentrations of sodium molybdate solutions, and then after immersion in demineralization solution, specimens were subjected to another microhardness test. Three penetrations were made with a load of 100 g in the enamel. A square diamond indenter was used with a magnification of X50. Vickers microhardness measurements were obtained by dividing the gf load by the square mm area of indentation. The average (mean) of three readings of the indentation length was taken and the applied load stay 30 seconds for each reading. The Microhardness number was calculated using the following equation:

$$H = \frac{2}{d^2}$$

$$H = 1.854\frac{F}{d^2}$$

$$H. = \frac{1854.4F}{d^2}$$

Whereas H: Microhardness number, F: Load in gram, and d: The axial length resembles the arithmetic mean of the two diagonals, d_1 and d_2 in mm of indentation. The difference between the initial hardness number and the final one was represent the change in hardness (D), and (D-) represents the mean of this difference, it was calculated for each experimental group. The ratio of the difference was also calculated for each of the experimental groups which represent the rate of change in hardness of the specimens. The rate of inhibition of caries-like lesion formation was calculated by dividing the difference between the rate of change in hardness (in the experimental groups and the control groups by the rate change of hardness in control groups) according to [20], as shown in the following equations:

$$D = Hi - Hf \tag{1}$$

$$D- = \sum(D)\ 1-n/n \tag{2}$$

$$(Hi-) = (Hi)/n \tag{3}$$

$$CH = [(D-)/\ (Hi-)] \times 100 \tag{4}$$

(CH) represent the percentage of the change in hardness.

The inhibition percentage of caries progression was calculated by the following equation:

$$\%\ \text{Inhibition}\ \frac{[(CH)\ control - (CH)\ exp.]}{(CH)\ control} \times 100$$

Statistical analysis

Statistical Analysis was conducted by applying Analysis of variance ANOVA and a Dunnett t-test (2-sided) under $P < 0.05$.

Demineralization process

Subsurface artificial caries lesions were induced in each slab by immersion of these specimens for 24 hours at 37 °C in demineralization solution (pH 5.0), which consisted of 13 ml of 0.1 M lactic acid with 0.2% water-soluble resin (Carbopol C907; BF, Goodrich Chemical Co. Chemical division, 6100 Oak Tree Blvd. Cleveland, Ohio, USA) and, 50% saturated hydroxyapatite solution at pH 5.0 according to Torrado *et al.* [22], and then examined under a polarized microscope (100 X). After that slabs were treated with a repeated schedule by exposing them for one minute to 0.025%, 0.075%, 0.150%, and 0.250% sodium molybdate prepared by serial dilution by constant stirring, followed by exposure to saliva for 29 minutes, and then immersed in mineralization solution for six hours without stirring, followed by rinsing with deionized water. Slabs were subjected again to treatment with molybdenum solution followed by saliva for 16 hours. This treatment regime was repeated for four consecutive days rinsing with deionized water.

Results and Discussion

Figure 1 demonstrates the microscopic pictures showing the indentation lengths after microhardness measurements using different concentrations of molybdenum.

Table 1 presents the difference in microhardness measurements before and after immersion in different concentrations of sodium molybdate solutions as well as the percentages of the increase in the microhardness at each concentration of the molybdenum solutions and deionized water determined. Data showed that the maximum increase in microhardness number was gained from the use of 0.025 %, (54.43%) followed by the effect of 0.075% concentration (46.99%) and the minimum concentration was gained by the concentration of 0.150% even with all concentrations cause an increase in the final values of microhardness numbers with highly significant differences (p<0.000) and showed the highly significant difference between measurements after demineralization process and after immersion of the samples in the test solution. Data of this table indicates that the minimum percentage of mineral loss during microhardness measurements gained from the use of molybdenum at 0.025% concentration was 0.733% and the maximum value was gained from the use of 0.075 % (1.607%) compared with the other concentrations and all compared with that of the deionized distal water with highly significant differences (P<0.000).

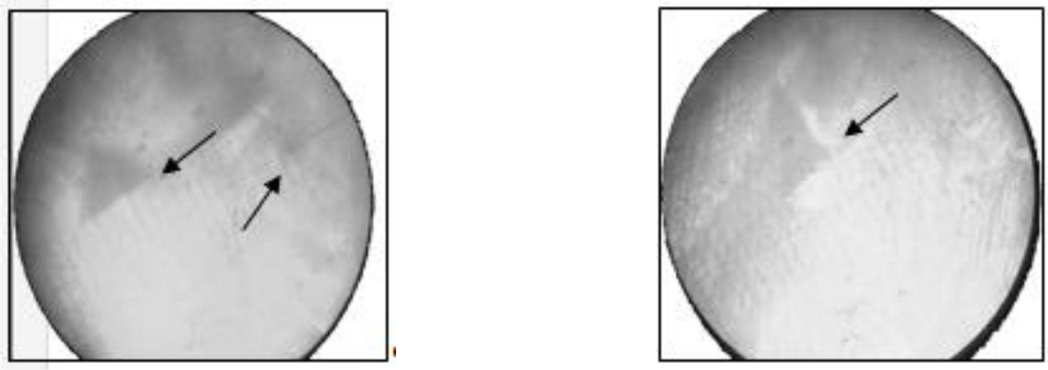

Figure 1: Flow of septic patients in the intensive care unit given with thiamine, ascorbic acid, and a combination of both with propensity score analysis

Table 1: Initial (after demineralization) and final microhardness measurements (Mean ±SD) before and after immersion in different concentrations of molybdenum including % of the difference in hardness, % volume mineral, and % mineral loss

Molybdenum concentration %	Microhardness number (mean ± SD)			% of increase	Volume mineral%	mineral loss %	P-value
	After demineralization-Initial	After immerse-oion in Mo. Final	Difference in Hardness Final-Initial				
0.025	146 ± 3.20	320.4± 2.80	174.4	54.43	22.2	0.733	0.000*
0.075	167± 2.80	332.0± 3.10	156	46.99	48.7	1.607	0.000*
0.150	211.2± 3.20	312.6± 2.90	101.4	32.43	33.8	1.115	0.000*
0.250	173.4± 3.00	293.5± 2.70	120.10	40.92	40.2	1.327	0.000*
Deionized water	107.2±2.7	118.2±2.80	11.00	9.31	8.91	0.294	N.S

** Highly significant*

Table 2 provides the effect of different concentrations of test solutions (molybdenum as sodium molybdate at 0.05% and 0.2%, sodium fluoride at 0.05% and 0.2% in comparison with deionized water) on the indentation length and microhardness number had been considered, means of microhardness number (initial and final measurements) for the samples treated with deionized water were found to be significantly lower than that of the corresponding means of the samples treated with sodium molybdate and sodium fluoride for both concentrations. Means and standard deviations of the indentation lengths and the microhardness numbers were calculated at each concentration of sodium molybdate and sodium fluoride for the initial and final measurements, as well as for the deionized water. The means of microhardness number (initial and final measurements) for the samples treated with deionized water were found to be significantly lower than that of the corresponding means of the samples treated with sodium molybdate and sodium fluoride for both concentrations. It shows that both test solutions demonstrated an increase in the microhardness numbers but the difference between final and initial measurements was higher related to sodium fluoride for both concentrations (0.05% and 0.2%), although the result showed the means ± SD of microhardness number gained from the use of sodium molybdate was higher regarding the final microhardness measurements (346.11 ± 0.287, 331.75 ± 0.361) in comparison with that of sodium fluoride (358.08 ± 0.567, 347.24 ± 0.215) for both 0.05% and 0.2% concentrations, respectively, the difference between the final and initial measurements indicated that sodium fluoride concentrations demonstrate higher effect (27.64% and 34.20%) than sodium molybdate (24.79 and 31.38%) regarding 0.05% and 0.2% respectively. Whereas that of deionized water was the lowest percentage (3.62) (243.42± 0.616) the difference was highly significant (P<0.000).

Table 3 represents the mean difference in the indentation length. In the case of sodium fluoride, the difference was about equal in both concentrations (0.05 and 0.02%), while the difference in sodium molybdate was higher in the concentration 0.2% than in 0.05% concentration. Table 4 and Figure 2 reports that the mean difference in microhardness, was significant in two concentrations for both test solutions (p = 0.000), the difference was increasing with the increasing concentration.

Data showed that a minimum increase occur at 0.025 %, this means that this concentration of molybdenum compound could cause an increase in enamel microhardness, and all concentrations of molybdenum compound solutions showed an increase in microhardness of dental enamel and all could increase the resistance of dental enamel against demineralization process.

Table 2: The effect of different concentrations of test sodium molybdate and sodium fluoride at 0.05% and 0.2% concentrations in comparison with deionized water, on the indentation length and microhardness number

Concentration of Test solution %		Indentation Length before Mean±SD	Microhardness Number before Mean ± SD	Indentation Length after Mean± SD	Microhardness number after Mean± SD	Difference in microhardness No.	%
Sodium Fluoride	0.05%	24.26 ± 0.561	250.43 ± 0.635	28.25 ± 0.360	346.11 ± 0.287	95.68	27.64
	0.2%	25.41 ± 0.010	218.29± 0.390	29.73 ± 0.035	331.75 ± 0.361	113.46	34.20
Sodium Molybdate	0.05%	25.32 ± 0.030	269.30 ± 0.576	26.232± .0700	358.08 ± 0.567	88.78	24.79
	0.2%	22.36 ± 0.020	238.26 ± 0.425	26.58 ± 0.114	347.24 ± 0.215	108.98	31.38
Deionized Water		26.45±0.372	234.59± 0.467	25.63± 0.100	243.42 ± 0.616	8.83	3.62

Table 3: The mean difference in the indentation length

Concentration of Test solution		Indentation Length (Before) Mean± SD	Indentation Length (After) Mean± SD	Mean Difference in Indentation Length
Sodium Fluoride	0.05%	24.26 ± 0.561	28.25 ± 0.360	3.99
	0.2%	25.41 ± 0.010	29.73 ± 0.035	4.32
Sodium Molybdate	0.05%	25.32 ± 0.030	26.23± .0700	0.91
	0.2%	22.36 ± 0.020	26.58 ± 0.114	4.22
Deionized Water		26.45±0.372	25.63± 0.100	-0.82

Table 4: Statistical difference in means of enamel microhardness for both test solutions

Concentration of Test solution (%)		Microhardness Number		Mean Difference in microhardness	t-test df	P-value
		Before Mean ± SD	After Mean± SD			
Sodium Fluoride	0.05%	250.43 ± 0.635	346.11 ± 0.287	95.68	-237.824	0.000
	0.2%	218.29± 0.390	331.75 ± 0.361	113.46	-376.564	0.000
Sodium Molybdate	0.05%	269.30 ± 0.576	358.08 ± 0.567	88.78	190.194	0.000
	0.2%	238.26 ± 0.425	347.24 ± 0.215	10.98	396.124	0.000

Microhardness was measured by considering the indentation length and microhardness number, by conducting two measurements; the first was obtained during the demineralization process, as shown in Table 1, and the second was obtained after demineralization for the same samples under study by measuring the microhardness number of the demineralized enamel then obtained the final measurement after immersion of enamel samples in different concentrations of molybdenum solutions. Dental enamel microhardness resembles microhardness properties of dental enamel surfaces which are determined by performing indentations by applying a diamond indenter (a Vickers indenter) into the surface of the tested agents under the average of 100 gm load measured by a microscope because the indentations are very small [5]. This *in vitro* study was conducted because it is very useful for evaluating the microhardness of different concentrations of molybdenum as sodium molybdate *in vitro* by an experimental study to determine their effects on the microhardness of dental enamel and the tooth as a whole for the sake of finding alternative elements other than fluoride that used as a preventive agent against dental caries initiation and progression. For this purpose, Vickers Microhardness Test was used which was a “standard procedure” [16, 17]. Due to the variation in mineral contents in dental enamel and their concentrations “because the outer enamel surface is highly mineralized due to continuous contact with saliva and as a result of remineralization process there was a great variation in the values of dental enamel microhardness in different teeth and different regions in the same teeth, so that enamel microhardness was greatest on the outer enamel surface and gradually decreased in hardness to its lowest value near to the dentino-enamel junction”[8], and the difference in the value of penetration between initial and final indentations will demonstrate the result of the demineralization process.

In this study, different concentrations of molybdenum compounds were used. Measurement of microhardness number of enamel samples was assessed first before the application of molybdenum solutions and after demineralization. Three measurements were conducted depending on the assessment of indentation lengths for each group. Statistically, data of results of this study showed significant differences between the initial and final measurements for all groups of enamel samples, the interpretation of these differences was due to differences in mineral contents in dental enamel and their distribution in different areas in the same tooth. Measurement was done under a load of 100 g which considered the least load was found to be necessary to obtain a well-defined indentation in the Vickers instrument; this load depended on the physical property of dental enamel, so this small load was used to avoid the plastic strain of enamel when dental enamel microhardness was measured as explained by a previous study [22-25].

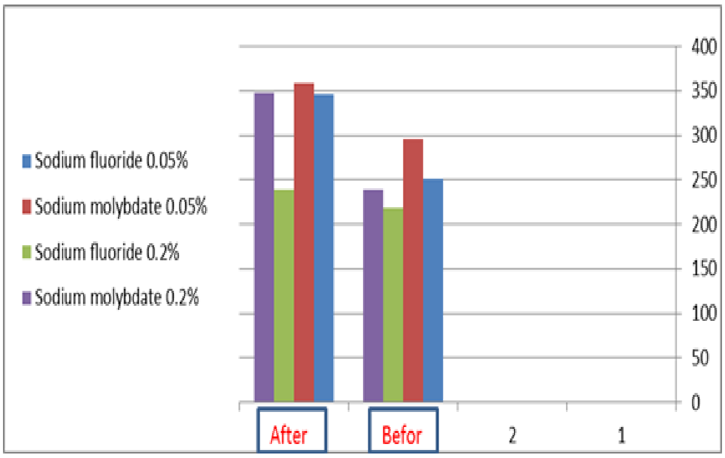

Figure 2: Means of before and after measurements of enamel microhardness using both test solutions

Table 5: Difference in means of microhardness of sodium fluoride and sodium molybdate solutions for both concentrations

Concentration of Test solution		Sodium Fluoride	Sodium Molybdate	P-value
0.05%	Initial	250.43±0.64	269.30±0.576	0.000*
	Final	346.11±0.287	358.08±0.57	0.000*
0.2%	Initial	218.29±0.39	238.26±0.43	0.000*
	Final	331.75±0.36	347.24±0.22	0.000*

**Highly significant*

Table 6: Statistical difference between different concentrations of sodium fluoride and sodium molybdate test solutions at a confidence interval of 95% using the Dunnett t-test

The concentration of test solution 0.05%				
Test Solution	Control solution	Sig.	95% Confidence Interval	
			Lower Bound	Upper Bound
Sodium Fluoride	Deionized water	.000	101.4954	103.8846
Sodium molybdate	Deionized water	.000	113.4688	115.8579
a. Dunnett t-tests (2-sided)				
The concentration of the test solution is 0.2%				
Solution	Control solution	Sig.	95% Confidence Interval	
			Lower Bound	Upper Bound
Sodium Fluoride	Deionized water	.000	-17.3085	-15.3048
Sodium molybdate	Deionized water	.000	2.6682	4.6718
a. Dunnett t-tests (2-sided)				

As revealed in this study, demineralization was responsible for the reduction of microhardness values before the application of molybdenum solutions due to the loss of minerals by the action of acid in the demineralization solution, and as shown in this study, it was provoked by the application of different concentrations of molybdenum solution by increasing of the microhardness values after demineralization. The results of this study also showed that all molybdenum concentrations tested were found to be significantly increasing the microhardness values of enamel samples by its action on the enamel surface, and there were statistical differences between these values (P<0.000). The difference in these results was attributed to the difference in enamel structure, chemical composition, variation in sample preparation, and variation in the direction of indentation in a single section, in addition to the difference in loads and the error in the indentation length. The same conclusion could be generalized to the percentage of reduction in microhardness, although the concentration of 0.025% plays an inflection point since all concentrations of molybdenum increased the microhardness of enamel samples. Results of the microhardness experiment revealed that there was a significant difference between means of indentation length and microhardness number (initial and final measurements) for both the sodium molybdate and sodium fluoride concentrations. In this study, the concentration of 0.05 and 0.2% of sodium fluoride was used depending on the same standardized concentrations of sodium fluoride used in sodium fluoride mouth rinses which caused an increase in the prevention of dental caries to determine whether sodium molybdate exhibits the same effect or not.

Table 5 demonstrated the difference in means of microhardness of sodium fluoride and sodium molybdate solutions for both concentrations.

Statistically, data of results of this study showed significant differences between the initial and final measurements for all groups of enamel samples, the interpretation of these differences was due to differences in mineral contents in dental enamel and their distribution in different areas in the same tooth.

Table 6, demonstrated the significant differences between the effect of both solutions with the deionized water which was used as a control (P< 0.000). Concentrations of sodium molybdate were chosen to mimic the proved concentrations of sodium fluoride in prevention of dental caries to ease the comparison between their means before and after measurements. Regarding sodium fluoride results of current study were in agreement with several studies [22-25]. Table 6 demonstrates the significant differences between both solutions with that of deionized water which was used as a control (P< 0.000). Concentrations of sodium molybdate were closed to mimic the proven concentrations of sodium fluoride in the prevention of dental caries and to ease the comparison between their means before and after. Regarding sodium fluoride, the results of the current studies were in agreement with several studies [21-23].

Limitations

There are no similar studies regarding the effect of molybdenum on the microhardness of dental enamel, to compare the results of this study with their results, and the concentrations used in the second experiments of the current studies were the same concentrations of sodium fluoride which used in the prevention of dental caries to determine their effects with that of sodium molybdate. Few previous and old references were used because there was a shortage and non-available of the same study about molybdenum and microhardness of dental enamel to compare with its results.

Conclusion

The mean microhardness of enamel samples after the molybdenum application was significantly greater than before application, and the microhardness of enamel was significantly increased when molybdenum was applied after the demineralization process. The mean microhardness of enamel samples treated with deionized water was found to be significantly lower than the corresponding mean with sodium molybdate and sodium fluoride. Molybdenum as a trace element could give a promising preventive effect against dental caries and be used as an alternative safe element instead of fluoride.

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Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

The author declared that they have no conflict of interest.

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