



## Effect of Actilux - Activated Whitening Toothpaste and Marine Salts on Color Change and Microhardness of Bovine Enamel

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### ABSTRACT

**Purpose:** To evaluate the effect of Actilux-activated whitening toothpaste and Marine salts powder on color change and microhardness of bovine enamel. **Methods:** Thirty intact bovine incisors were used in this study to produce enamel specimens. The specimens were randomly distributed into three groups (n= 10) according to the tested whitening agents as follows: Group I: BW; BlanX LED White Shock (Actilux-activated), Group II: MS; Marine salts whitening powder and Group III: CR; Crest regular fluoridated toothpaste. The specimens were stained using black tea solution for 5 days. Each specimen was brushed twice daily for 8 then 15 days. The color was assessed according to CIE (Lab) color system using a VITA Easyshade spectrophotometer. The microhardness was assessed using Digital Display Vickers Microhardness Tester at baseline, after staining, after 8 days, and 15 days brushing. Data were tabulated and statistically analyzed. **Results:** After 8 days brushing, BW showed the highest mean ( $\Delta E$ ) while MS and CR both showed lower mean ( $\Delta E$ ). BW and MS showed the highest mean ( $\Delta E$ ) after 15 days brushing with no statistically significant difference between them. After 15 days; BW showed the highest mean microhardness followed by MS while CR showed the lowest mean microhardness. **Conclusion:** Both Actilux-activated toothpaste combined with LED device and Marine salts powder were effective in tooth whitening as well as increasing enamel microhardness. The whitening efficacy and microhardness is time dependent.

### INTRODUCTION

Nowadays, people are interested in cosmetic dentistry to obtain a white bright smile. Tooth discoloration represents an esthetic problem either due to intrinsic or extrinsic factors <sup>(1)</sup>. Extrinsic discoloration caused by organic and inorganic chromophores adsorbed directly to the tooth surface especially if it has rough surface <sup>(2)</sup>. The origin of stains

### KEYWORDS

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is mainly from smoking or chromophore-containing foods as coffee and tea <sup>(3)</sup>. Moreover, indirect discoloration may occur due the components of oral care products. These ingredients cause tooth staining such as stannous fluoride, chlorhexidine and other stannous salts <sup>(4)</sup>. Therefore, teeth whitening has become a needed feature to improve dental esthetics <sup>(5)</sup>.

Teeth whitening is an effective method for removal of tooth stains. It can be done with different methods and systems such as tooth bleaching and routine prophylactic procedures as brushing with whitening toothpaste <sup>(6)</sup>. Whitening toothpaste appeared as a low-cost alternative to white the discolored teeth without dentist supervision. They are available in supermarkets, pharmacies, and through online shopping <sup>(7)</sup>. The bleaching and abrasive components are usually the most common chemically active compounds in whitening dentifrices <sup>(8)</sup>. The adverse effects of bleaching components are the released free oxygen radicals that may act on the organic matrix of dental structures and breaking up the lipids and proteins of dental tissues <sup>(9)</sup>.

Furthermore, the abrasive components may lead to an undesirable effect on teeth surface as increasing surface wear of enamel <sup>(10)</sup>. So, an ideal whitening toothpaste should remove stains without affecting teeth surface properties <sup>(6)</sup>. Recently, whitening toothpastes change the color of teeth through adsorbing stains as activated charcoal/carbon <sup>(11)</sup> or optical modified by deposition of a thin, semitransparent film of bluish pigment on the surface such as blue covarine <sup>(12)</sup>.

Nowadays, utilization of natural products has been increased in different dental fields <sup>(13)</sup>. Therefore, evolution of new whitening formulations with alternative natural ingredients and additions supposed to have a minimal effect on tooth surface properties. Actilux® (Arctic Lichen) is non-abrasive, peroxide free new toothpaste formula which restores a natural white smile. It is activated through LED accelerator or natural sun light. Consequently, the more you smile, the whiter your teeth get (Coswell, Italian Innovators since 1961- BlanX

White Shock toothpaste - Actilux® micro-crystals). Moreover, Marine salts powder is one of the natural alternatives to commercial available toothpaste in the Egyptian market. It contains the coral that is promising area of research regarding this untapped resource <sup>(14)</sup>.

Microhardness is one of the important surface properties that clarify the loss or gain of mineral content in tooth tissues <sup>(15)</sup>. Up till now, there is no research concerning the whitening effect of Marine salts powder on enamel color and microhardness. Therefore, this study was conducted to evaluate the effect of Actilux-activated whitening toothpaste and Marine salts powder on color change and microhardness of bovine enamel.

## MATERIALS AND METHODS

### Specimens' preparation and grouping

Thirty intact bovine incisors were used in this study. The teeth were washed under running water, scaled from adhering soft tissue, plaque, and then stored at 4°C in distilled water for not more than one month. The radicular portion of the premolars were cut 2 mm below the cemento-enamel-junction. The coronal portion was mounted in self-cured acrylic resin blocks using metal molds (2cm x3 cm) with the labial surface facing upward. Enamel was wet ground using 80 grit sandpaper discs to achieve flat enamel surfaces. Enamel surfaces were abraded with 400 and 600 grit sandpaper discs, and polished with rubber cups and paste. After polishing, the specimens were cleaned in an ultrasonic cleaning device with deionized water for 15 minutes in order to remove the debris.

The specimens were randomly distributed into three groups (n= 10) according to the tested whitening agents as follows: Group I: BW; BlanX LED White Shock (Actilux-activated) Group II: MS; Marine salts whitening powder and Group III: CR; Crest regular fluoridated toothpaste. The composition of all tooth whitening agents is represented in table (1).

**Table (1):** *Composition of whitening agents used in the study*

| Material (Brand name and manufacturer)   | Composition  |
|--|--|
| BlanX White Shock with Led Accelerator Toothpaste (Actilux-activated - none abrasive - no peroxide whitening toothpaste, Coswell, Italian Innovators, Italy) | Aqua, sorbitol, hydrated silica, glycerin, silica, sodium lauryl sulfate, cellulose gum, aroma, hydroxyapatite*, isopropyl alcohol, phenoxyethanol, sodium monofluorophosphate, sodium benzoate, benzyl alcohol, CI 77891, CI 42090, PVM/MA copolymer, cetraria islandica extract, sodium saccharin. |
| Marine salts whitening powder (Coral based powder, local market, Egypt)  | Calcium carbonate, sodium chloride, calcium, magnesium, phosphorus, silica, and small particles of iron. <sup>(14)</sup>   |
| Crest regular fluoridated toothpaste (Procter & Gamble, Groß-Gerau, Germany).  | Sodium fluoride, sorbitol, aqua, hydrated silica, sodium lauryl sulfate, cellulose gum, carbomer, CI 74260, aroma, trisodium phosphate, sodium saccharin, polysorbate 80, CI 77891, limonene.  |

The primary color (baseline) was assessed according to CIE (Lab) color system (Commission Internationale de L'Eclairage) by using a VITA Easyshade spectrophotometer (Advance 4.01, VITA Zahnfabric, Bad Sackingen, Germany) against a white background. According to this system the three different color parameters L\*, a\* and b\* were calculated as follow: L\* values denotes darkness–brightness (range from 0 to 100); a\* value represents the green–red component (ranging from –80 green to +80 red); and b\* represents the blue–yellow component (values ranging from –80 blue to +80 yellow). The middle part of the buccal aspect middle one third was recorded as mean measurement <sup>(16)</sup>.

The initial microhardness was assessed using Digital Display Vickers Microhardness Tester (Model HVS-50, LaizhouHuayin Testing Instrument Co., Ltd. China) with a Vickers diamond indenter and a 20X objective lens. A load of 200g was applied to the surface of the specimens for 10 seconds. On each specimen, three indentations were made on the surface and were equally placed over a circle. These indentations were not closer than 0.5 mm to the adjacent one. A built in scaled microscope was used to measure the diagonals length of the indentations then Vickers values were converted into microhardness values.

### Staining of the specimens:

The specimens were stained using black tea solution for 5 days (Yellow Label, Lipton black tea, London, United Kingdom). The solution was prepared by immersing two tea bags (2 x 2.0 g) into 200 mL of boiling water for 5 minutes then filtered with a piece of gauze. The specimens were incubated at 37°C, and tea solution was changed every 24 hours. The five days immersion represents one year of drinking supposed that one dose 200 ml of tea consumption lasts 5 minutes, given that four doses are consumed daily by regular tea drinkers <sup>(17)</sup>. At the end of the staining period, all the specimens were again subjected to color and microhardness measurements.

### Brushing procedures:

Each specimen in its corresponding group were brushed twice daily (morning and evening) for 2 minutes using Oral Oral-B Vitality Plus (cross action electronic toothbrush powered by Braun, Germany). According to the manufacturer instructions, the BlanX White Shock group, BlanX LED was screwed onto the tube instead of the screwing cap. This special and patented BlanX LED light was shined on the toothpaste while it is being placed onto the toothbrush immediately to activate the whitening action of ActiluX (Fig.1). It was used directly on the specimen to increase its whitening



Figure (1) BlanX White Shock toothpaste and BlanX LED screwed onto the tube instead of the screwing cap to activate the whitening action of ActiluX

effect. Regarding the MS group, the powder was mixed with distilled water (a ratio of 2 powder: 1 water)<sup>(16)</sup>. Each specimen was washed with distilled water then stored in artificial saliva after brushing. This process was repeated for a total of 15 days, and the artificial saliva for each group was changed every day. Artificial saliva composed of 1.5 mM Ca, 0.9 mM P, 150 mM KCL, 0.05 lg F/mL, and 0.1 M Tris buffer, set to a pH of 7.0<sup>(18)</sup>. The color and microhardness changes were observed at different time intervals; 8 days and 15 days. The color change ( $\Delta E$ ) was calculated from the following formula:  $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$ .

### Statistical Analysis

Numerical data were explored for normality by checking the distribution of data and using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). All data showed parametric (normal) distribution except for ( $a^*$ ) values data which showed non-parametric (non-normal) distribution. Data were presented as mean and standard deviation (SD) values. For parametric data; repeated measures Analysis of Variance (ANOVA) was used to compare between the groups as well as to study the changes by time within each group. Bonferroni's post-hoc test was used for pair-wise comparisons when ANOVA test is significant. For non-parametric data; Friedman's test was used to study the changes

by time in ( $a^*$ ) values. Kruskal-Wallis test was used to compare between ( $a^*$ ) values in the three groups. Dunn's test was used for pair-wise comparisons when Friedman's test is significant. The significance level was set at  $P \leq 0.05$ . Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

## RESULTS

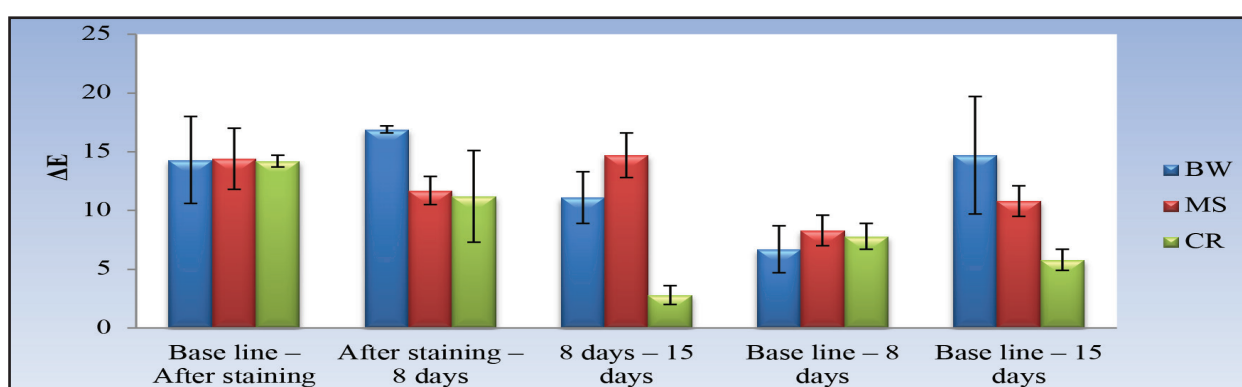
### Results of color change

Comparison between color changes ( $\Delta E$ ) of the three groups is shown in table (2) (Fig. 2), from base line to after staining; there was no statistically significant difference between mean ( $\Delta E$ ) values of the three tested groups. From after staining to 8 days; there was a statistically significant difference between the mean ( $\Delta E$ ) values in the three groups. **Pair-wise comparison** between the groups revealed that there was no statistically significant difference between **MS** and **CR** groups; both showed statistically significant lower mean ( $\Delta E$ ) than **BW** group. There was a statistically significant difference between the mean ( $\Delta E$ ) values in the three groups from 8 to 15 days. Pair-wise comparison between the groups revealed that there was no statistically significant difference between **BW** and **MS** groups; both showed statistically significant higher mean ( $\Delta E$ ) than **CR** group.

**Table (2):** Mean, standard deviation (SD) values and results of repeated measures ANOVA test for the comparison between ( $\Delta E$ ) values in the three groups

| Time                       | BW                |     | MS                |     | CR                |     | P-value | Effect size (Partial $\eta^2$ ) |
|----------------------------|-------------------|-----|-------------------|-----|-------------------|-----|---------|---------------------------------|
|                            | Mean              | SD  | Mean              | SD  | Mean              | SD  |         |                                 |
| Base line – After staining | 14.3              | 3.7 | 14.4              | 2.6 | 14.2              | 0.5 | 0.992   | 0.001                           |
| After staining – 8 days    | 16.9 <sup>A</sup> | 0.3 | 11.7 <sup>B</sup> | 1.2 | 11.2 <sup>B</sup> | 3.9 | 0.001*  | 0.677                           |
| 8 days – 15 days           | 11.1 <sup>A</sup> | 2.2 | 14.7 <sup>A</sup> | 1.9 | 2.8 <sup>B</sup>  | 0.8 | <0.001* | 0.886                           |
| Base line – 8 days         | 6.7               | 2   | 8.3               | 1.3 | 7.8               | 1.1 | 0.229   | 0.203                           |
| Base line – 15 days        | 14.7 <sup>A</sup> | 5   | 10.8 <sup>A</sup> | 1.3 | 5.8 <sup>B</sup>  | 0.9 | 0.003*  | 0.585                           |

\*: Significant at  $P \leq 0.05$ , Different superscripts in the same row are statistically significant different

Figure (2) Bar chart representing mean and standard deviation values for  $\Delta E$  in the three groups

Comparing the color changes ( $\Delta E$ ) from base line to 8 days; there was no statistically significant difference between mean ( $\Delta E$ ) values in the three groups. There was a statistically significant difference between mean ( $\Delta E$ ) values in the three groups from base line to 15 days. Pair-wise comparison between the groups revealed that there was no statistically significant difference between **BW** and **MS** groups; both showed statistically significantly

higher mean ( $\Delta E$ ) than **CR** group.

Comparing the color changes ( $\Delta E$ ) between the three groups regardless of time is shown in table (3). There was a statistically significant difference between mean ( $\Delta E$ ) values in the three groups. Pair-wise comparisons between the groups revealed that there was no statistically significant difference between **BW** and **MS** groups; both showed statistically significantly higher mean ( $\Delta E$ ) than **CR** group.

**Table (3):** The mean, standard deviation (SD) values and results of repeated measures ANOVA test for the comparison between ( $\Delta E$ ) values in the three groups regardless of time

| BW                |     | MS                |     | CR               |     | P-value | Effect size (Partial $\eta^2$ ) |
|-------------------|-----|-------------------|-----|------------------|-----|---------|---------------------------------|
| Mean              | SD  | Mean              | SD  | Mean             | SD  |         |                                 |
| 14.1 <sup>A</sup> | 3.4 | 13.6 <sup>A</sup> | 2.3 | 9.4 <sup>B</sup> | 5.5 | <0.001* | 0.763                           |

\*: Significant at  $P \leq 0.05$ , Different superscripts in the same row are statistically significant different



Changes in color parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ) for the three tested toothpastes are shown in table (4). Regarding **BW** group; there was a statistically significant change in mean ( $L^*$ ) values during the study procedure. There was a statistically significant decrease in mean ( $L^*$ ) value after staining followed by a statistically significant increase after 8 days as well as from 8 to 15 days. The mean ( $L^*$ ) value after 15 days showed statistically significant higher value than baseline. There was a statistically significant change in mean ( $a^*$ ) values during the study procedure. There was a statistically significant increase in mean ( $a^*$ ) value after staining followed by a statistically significant decrease after 8 days. From 8 to 15 days; there was no statistically significant change in mean ( $a^*$ ) value. The mean ( $a^*$ ) value after 15 days showed statistically significantly lower value than baseline. There was a statistically significant change in mean ( $b^*$ ) values during the study procedure. There was a statistically significant increase in mean ( $b^*$ ) value after staining followed by a statistically significant decrease after 8 days as well as from 8 to 15 days. The mean ( $b^*$ ) value after 15 days showed statistically significant lower value than baseline.

Regarding **MS** group; there was a statistically significant change in mean ( $L^*$ ) values during the study procedure. There was a statistically significant decrease in mean ( $L^*$ ) value after staining followed by a statistically significant increase after 8 days as well as from 8 to 15 days. The mean ( $L^*$ ) value after 15 days showed statistically significant higher value than baseline. There was a statistically significant change in mean ( $a^*$ ) values during the study procedure. There was a statistically significant increase in mean ( $a^*$ ) value after staining fol-

lowed by a statistically significant decrease after 8 days as well as from 8 to 15 days. The mean ( $a^*$ ) value after 15 days showed statistically significant lower value than baseline. There was a statistically significant change in mean ( $b^*$ ) values during the study procedure. There was a statistically significant increase in mean ( $b^*$ ) value after staining followed by non-statistically significant change after 8 days. From 8 to 15 days; there was a statistically significant decrease in mean ( $b^*$ ) value. The mean ( $b^*$ ) value after 15 days showed statistically significant lower value than baseline.

Regarding **CR** group; there was a statistically significant change in mean ( $L^*$ ) values during the study procedure. There was a statistically significant decrease in mean ( $L^*$ ) value after staining followed by a statistically significant increase after 8 days. From 8 to 15 days; there was no statistically significant change in mean ( $L^*$ ) value. The mean ( $L^*$ ) value after 15 days showed statistically insignificant difference from baseline. There was a statistically significant change in mean ( $a^*$ ) values during the study procedure. There was a statistically significant increase in mean ( $a^*$ ) value after staining followed by a statistically significant decrease after 8 days. From 8 to 15 days; there was no statistically significant change in mean ( $a^*$ ) value. The mean ( $a^*$ ) value after 15 days showed statistically significantly higher value than baseline. There was a statistically significant change in mean ( $b^*$ ) values during the study procedure. There was a statistically significant increase in mean ( $b^*$ ) value after staining followed by statistically insignificant change after 8 days as well as from 8 to 15 days. The mean ( $b^*$ ) value after 15 days showed statistically significant higher value than baseline.

**Table (4):** Mean, standard deviation (SD) values and results of repeated measures ANOVA test and Friedman’s test for the changes in (L\*, a\*, b\*) values during the study procedure

| Group | Parameter | Base line         |     | After staining    |     | 8 days            |      | 15 days           |     | P-value  | Effect size              |
|-------|-----------|-------------------|-----|-------------------|-----|-------------------|------|-------------------|-----|----------|--------------------------|
|       |           | Mean              | SD  | Mean              | SD  | Mean              | SD   | Mean              | SD  |          |                          |
| BW    | L*        | 76.4 <sup>B</sup> | 1.3 | 66.8 <sup>C</sup> | 2   | 77.9 <sup>B</sup> | 1.3  | 84.4 <sup>A</sup> | 0.7 | <0.001*  | Partial $\eta^2 = 0.980$ |
|       | a*        | 1.3 <sup>B</sup>  | 0.3 | 3.6 <sup>A</sup>  | 0.5 | 0.6 <sup>C</sup>  | 0.01 | -0.7 <sup>C</sup> | 0.8 | <0.001*† | w = 1.000                |
|       | b*        | 24.1 <sup>B</sup> | 4.6 | 31.8 <sup>A</sup> | 5.1 | 23 <sup>B</sup>   | 1.3  | 13.1 <sup>C</sup> | 2.9 | <0.001*  | Partial $\eta^2 = 0.994$ |
| MS    | L*        | 75.2 <sup>B</sup> | 1.4 | 65.2 <sup>C</sup> | 3.1 | 75.2 <sup>B</sup> | 0.2  | 85 <sup>A</sup>   | 1.5 | <0.001*  | Partial $\eta^2 = 0.960$ |
|       | a*        | 2.3 <sup>B</sup>  | 0.1 | 3.5 <sup>A</sup>  | 0.4 | 1.5 <sup>C</sup>  | 0.4  | 0.5 <sup>D</sup>  | 0.7 | 0.001*†  | w = 0.911                |
|       | b*        | 18.4 <sup>B</sup> | 1.8 | 28.1 <sup>A</sup> | 5.6 | 26.6 <sup>A</sup> | 1    | 15.8 <sup>C</sup> | 2.4 | <0.001*  | Partial $\eta^2 = 0.998$ |
| CR    | L*        | 73.9 <sup>A</sup> | 1.3 | 62.6 <sup>B</sup> | 1   | 71.9 <sup>A</sup> | 1.3  | 71.3 <sup>A</sup> | 2   | <0.001*  | Partial $\eta^2 = 0.976$ |
|       | a*        | 1.6 <sup>C</sup>  | 0.5 | 3.9 <sup>A</sup>  | 0.1 | 2.6 <sup>B</sup>  | 0.03 | 2.8 <sup>B</sup>  | 0.8 | 0.013*†  | w = 0.600                |
|       | b*        | 24.1 <sup>B</sup> | 1.7 | 28.4 <sup>A</sup> | 6.1 | 31.6 <sup>A</sup> | 0.5  | 29 <sup>A</sup>   | 1.2 | <0.001*  | Partial $\eta^2 = 0.984$ |

\*: Significant at  $P \leq 0.05$ , †: Friedman’s test,

Different superscripts in the same row are statistically significant different

**Results of Microhardness:**

Comparison between microhardness of the three groups is shown in table (5) (Fig.3). At baseline; there was no statistically significant difference between the mean microhardness values of the three groups. After staining; there was no statistically significant difference between the mean microhardness values of the three groups. After 8 days; there was no statistically significant difference between

the mean microhardness values of the three groups.

**After 15 days;** there was a statistically significant difference between the mean microhardness values of the three groups. Pair-wise comparison between the groups revealed that **BW** group showed the statistically significant highest mean microhardness. **MS** group showed statistically significantly lower mean value, whereas **CR** group showed the statistically significant lowest mean microhardness.

**Table (5):** Mean, standard deviation (SD) values and results of repeated measures ANOVA test for the comparison between microhardness values of the three groups

| Time           | BW                 |      | MS                 |      | CR                 |      | P-value | Effect size<br>(Partial $\eta^2$ ) |
|----------------|--------------------|------|--------------------|------|--------------------|------|---------|------------------------------------|
|                | Mean               | SD   | Mean               | SD   | Mean               | SD   |         |                                    |
| Base line      | 300.3              | 8.2  | 299.3              | 11.2 | 297.8              | 21.9 | 0.958   | 0.006                              |
| After staining | 297.8              | 9.9  | 304.3              | 9.4  | 292.6              | 16.5 | 0.290   | 0.152                              |
| 8 days         | 302.9              | 10.3 | 306.4              | 35.2 | 306.8              | 9.6  | 0.124   | 0.243                              |
| 15 days        | 415.3 <sup>A</sup> | 13.3 | 388.8 <sup>B</sup> | 7.2  | 309.9 <sup>C</sup> | 5.8  | <0.001* | 0.908                              |

\*: Significant at  $P \leq 0.05$ , Different superscripts in the same row are statistically significant different

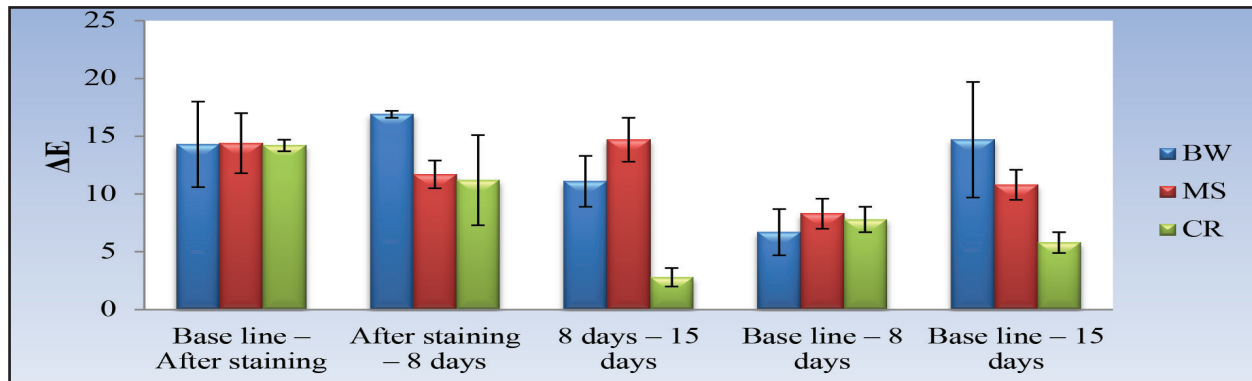


Figure (3) Bar chart representing mean and standard deviation values for microhardness of the three groups

Effect of time on microhardness of the three groups is shown in table (6). **BW group;** there was a statistically significant change in mean microhardness values during the study procedure. There was no statistically significant change in mean microhardness value after staining as well as after 8 days followed by a statistically significant increase after 15 days. The mean microhardness value after 15 days showed statistically significant higher value than baseline.

**Regarding MS group;** there was a statistically significant change in mean microhardness values during the study procedure. There was no statistically significant change in mean microhardness value after staining as well as after 8 days followed by a statistically significant increase after 15 days. The mean microhardness value after 15 days showed statistically significant higher value than baseline. **Regarding CR group;** there was no statistically significant change in mean microhardness values during the study procedure.

**Table (6):** The mean, standard deviation (SD) values and results of repeated measures ANOVA test for the changes in microhardness values during the study procedure

| Group     | Base line          |      | After staining     |      | 8 days             |      | 15 days            |      | P-value | Effect size (Partial η <sup>2</sup> ) |
|-----------|--------------------|------|--------------------|------|--------------------|------|--------------------|------|---------|---------------------------------------|
|           | Mean               | SD   | Mean               | SD   | Mean               | SD   | Mean               | SD   |         |                                       |
| <b>BW</b> | 300.3 <sup>B</sup> | 8.2  | 297.8 <sup>B</sup> | 9.9  | 302.9 <sup>B</sup> | 10.3 | 415.3 <sup>A</sup> | 13.3 | <0.001* | 0.970                                 |
| <b>MS</b> | 299.3 <sup>B</sup> | 11.2 | 304.3 <sup>B</sup> | 9.4  | 306.4 <sup>B</sup> | 35.2 | 388.8 <sup>A</sup> | 7.2  | <0.001* | 0.943                                 |
| <b>CR</b> | 297.8              | 21.9 | 292.6              | 16.5 | 306.8              | 9.6  | 309.9              | 5.8  | 0.066   | 0.185                                 |

\*: Significant at P ≤ 0.05, Different superscripts in the same row are statistically significant different



## DISCUSSION

The current study was carried out to evaluate the effect of Actilux - activated toothpaste and Marine salts powder on the color changes and microhardness of bovine enamel. The bovine teeth were used in this study as it was found that their physical and chemical properties are very similar to those of human teeth <sup>(18)</sup>. Each specimen was brushed twice daily (morning and evening) for 2 minutes. A typical tooth surface in vivo brushed on average 5 seconds, twice a day <sup>(6)</sup>. Therefore, brushing the specimens for 8 and 15 days equals 1920 and 3600 seconds respectively. Accordingly, the tooth brushing performed in this study was equal to 6 and 12 months.

In this study, all the tested whitening agents have shown an increase in  $L^*$  values and decrease in  $a^*$  and  $b^*$  values at both time intervals; 8 and 15 days. Whereas, a positive  $L^*$  value after whitening which means that the teeth tend towards white, while the negative values of  $a^*$  and  $b^*$  indicate that the teeth tend to be less yellow and less red, respectively. In the present study, the color change was measured using a Vita Easyshade spectrophotometer that proved to be more accurate than visual tools <sup>(19-20)</sup>.

BW and MS groups showed the statistically significant highest mean  $\Delta E$  values from after staining to 8 days as well as after 15 days. Also, both groups showed statistically significant higher mean ( $\Delta E$ ) from baseline to 15 days. There was also statistically significant increase in the mean  $L^*$  value after 8 days as well as from 8 to 15 days. Similarly, the mean  $L^*$  value of BW and MS groups showed statistically significant higher value after 15 days than at baseline.

The whitening effect of Blanx White shock is attributed to the presence of Actilux micro-crystals as it does not contain peroxide ingredients or abrasive particles. These micro-crystals bind to the enamel during regular teeth brushing, creating an invisible barrier that obstruct the causes of discoloration. Actilux formula is the only technology unleash the whitening power. Actilux is a patented combination

of titanium dioxide and hydroxyapatite microcrystals. Titanium dioxide is known as intense white pigment that is widely used in toothpaste formulations because it has a high refractive index. Moreover, the LED light accelerator activates the photocatalytic active ingredient of Actilux that destroy the stain molecules and lasts all the day to naturally whiten the teeth (Coswell, Italian Innovators since 1961-BlanX White Shock toothpaste - Actilux® microcrystals). These results were in agreement with previous study <sup>(21)</sup> reported that Blanx White shock toothpaste alone or with LED device had a significant tooth whitening effect which had a faster onset when combined with an LED device. Therefore, they confirmed the synergistic effect of the LED device and the nonabrasive, activator-containing toothpaste.

As for the whitening effect of Marine salts, it may be attributed to the presence of calcium carbonate and small particles of silica which are similar to the abrasive contents of the whitening toothpaste. The mechanical abrasive in whitening toothpaste that contains calcium carbonate together with the brush bristles are able to remove the extrinsic stains <sup>(6)</sup>. Previous study reported that toothpastes containing silica were able to remove the extrinsic surface stains by abrasive action with high cleaning performance <sup>(22)</sup>. As there are no available studies in the literature about the effect of Marine salt on color of enamel, this result cannot be directly compared.

On the other hand, CR group showed the lowest statistically significant mean  $\Delta E$  values after 8 days as well as after 15 days. There was a statistically significant increase in mean  $L^*$  value after 8 days, while there was no statistically significant change in mean  $L^*$  value from 8 to 15 days. After 15 days; the mean  $L^*$  value showed statistically insignificant difference from baseline. Crest is considered regular non-whitening toothpaste but it can remove the surface stains mechanically through the abrasive ingredients. This slight whitening effect may be related to the presence of hydrated silica which has greater ability to remove stains from enamel surface

compared with other abrasives<sup>(4)</sup>. This result is consistent with a recent study reported that the toothpastes containing mechanical abrasive were effective in modifying the color of bovine enamel<sup>(23)</sup>. However, these results were in disagreement with another study that demonstrated that the traditional abrasive toothpaste was not able to improve the tooth color<sup>(13)</sup>. This difference may be attributed to the assessment method as they analyzed whitening efficacy by visual comparison using the Vita Classical scale.

Regarding the results of microhardness, the BW group showed the statistically significant highest mean microhardness after 15 days followed by MS group that showed statistically significant lower mean value. Whereas, CR group showed the statistically significant lowest mean value.

BW toothpaste showed no statistically significant change in mean microhardness value after 8 days followed by a statistically significant increase after 15 days of brushing that equal to one year brushing in vivo. This increase in enamel microhardness could be attributed to hydroxyapatite contents in Blanx White shock toothpaste that have similar structure to the main mineral component of teeth and bone. Moreover, it provides a source of calcium and phosphate ions that enhance enamel remineralization<sup>(24)</sup>.

Furthermore, Blanx White shock toothpaste contain sodium monofluorophosphate that may accelerate the remineralization process through fluorapatite formation<sup>(25)</sup>. The results of the present study corroborated with the findings of another study that reported fluoridated toothpaste containing nano-hydroxyapatite was more effective in enamel remineralization than the toothpaste without nano-hydroxyapatite<sup>(26)</sup>. However, this is contradicting with previous study demonstrated that enamel remineralization using nano-hydroxyapatite fluoride dentifrice was not able to gain their original baseline level of microhardness<sup>(27)</sup>. This difference may be attributed to difference in toothpaste composition.

Marine salts showed also statistically significant increase in mean microhardness value after 15 days brushing as compared to the baseline value. It is a coral based powder containing naturally occurring calcium within the aragonite found in hard corals. The potential benefit of coral skeleton may remain in refined coral that is modified and chemically strengthened to increase its longevity and integrity<sup>(28)</sup>. Also, it contains calcium carbonate and other elements such as calcium, phosphorus, which have a remineralizing effect on tooth structure. This goes also with the same line with another study reported that calcium carbonate and calcium contents of the egg shell extract solution was able to increase enamel microhardness<sup>(29)</sup>. Therefore, enamel microhardness increased after brushing with Marine salt powder considering its composition.

On comparing the mean enamel microhardness values of the tested groups, Crest toothpaste showed the statistically significant lowest mean microhardness after 15 days brushing. However, there was no statistically significant change in mean microhardness values after 8 days as well as after 15 days brushing. Crest is a regular toothpaste for daily application contains sodium fluoride that is essential for enamel remineralization. Fluoride enhance formation of fluorapatite that is less soluble providing additional protection from demineralization<sup>(30)</sup>. Therefore, the explanation of these results may be due to the remineralization rate in Crest toothpaste was equal to their demineralization rate caused by abrasives.

This correlates with previous studies<sup>(25,30,31)</sup> that reported the ability of fluoridated toothpaste to increase enamel microhardness. However, this was conflicting with another in vitro study<sup>(32)</sup> reported that amine fluoride markedly increases enamel microhardness in comparison to sodium fluoride which could be related to the difference in fluoride formulations.

## CONCLUSIONS

On the basis of the findings of this study, Actilux-activated toothpaste combined with LED device and Marine salts powder were effective in tooth whitening as well as increasing enamel microhardness in comparison to fluoridated toothpaste. The increase in whitening efficacy and microhardness is time dependent.

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