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The Effect of Concentrated Growth Factor or Oracure on Healing of Induced Oral Ulcer in Albino Rats

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ORIGINAL STUDY

The Effect of Concentrated Growth Factor or Oracure on Healing of Induced Oral Ulcer in Albino Rats

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Abstract

Purpose: The current research aimed to investigate the effect of concentrated growth factor (CGF) or Oracure on the repair of induced ulcer in the cheek mucosa in the experimental rats. **Patients and methods:** Thirty albino rats (weight 200−250 gm) are used in this study. Rats were divided into three groups, the first group (negative control), the second group (CGF treated group) and the third group (Oracure gel treated group) then according to the scarification date, the rats in all groups were divided into two groups at 4 and 10 days. The rats were anesthetized then the rat's oral cavities were opened exposing the inner aspect of the cheek (buccal mucosa) to made ulcers. The specimens were collected on days (4 and 10) to be examined for histological and statistical analysis. **Results:** Totally closure of the ulcer lesions were occurred. The epithelium in the third group was observed thicker than the epithelium in group II. Maturation of the collagen fiber and formation of new blood vessels (angiogenesis) were observed in group II while less maturation of collagen fiber and fewer numbers of blood vessels were observed in group III. **Conclusion:** It was concluded that CGF and Oracure gel accelerated the healing of mucosa compared with the control group but CGF was more effective than Oracure in accelerating maturation of collagen fiber and inducing the angiogenesis.

Keywords: Concentrated growth factor, Healing of oral ulcer, Oracure

1. Introduction

Regeneration of soft tissue is caused by platelet growth factors, which are classified into the first-generation platelet-rich plasma (PRP), the second-generation platelet-rich fibrin (PRF), and the third-generation platelet concentrated growth factor (CGF), which are considered biocompatible and is used in the body without any immune reaction. The fibrin network in the platelet growth factor acts as a scaffold and induces UMC to be differentiated into fibroblast. The fibroblast forms collagen fiber and the scaffold has role in soft tissue repair [1,2].

The introduction of the first-generation platelet PRP was in 1998, and many steps were taken to obtain PRP. The first step was adding an anticoagulant to the blood. The second step was centrifugation twice in the centrifuge device, and in the last step, a fibrin scaffold was obtained, but the disadvantage of this fibrin was that it was weak as a scaffold. The difficulty of using PRP was due to several steps that occurred [3].

The second-generation platelet PRF was discovered. PRF gels improve the repair of soft tissue, but the disadvantage of this fibrin is that it is weak as a scaffold, identical to first-generation platelets PRP. The introduction of the third generation platelet (CGF) was in 2006 by Sacco, which included the fibrin network, which appeared stronger than PRF, PRP, and more growth factors that made it perfect as a scaffold [4,5].

The third-generation platelet concentrated growth factor (CGF) consists of more fibrin and leukocytes. To make CGF, we need a digital centrifuge device with variable speeds to make more growth factors in fibrin blood clots. The simplicity of making CGF render it is used as a scaffold and is better than the first and second-generations of platelets. The fibrin network of CGF is close to its original, and it consists of many growth factors. Recently, CGF has been implanted to improve the quality of the formed bone [6].

The CGF shows stronger and higher growth factors than first- and second-generation platelet

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concentrates. It improves the osteointegration of the implant. It decreases postoperative complications such as inflammation and pain. Some investigators observed that the addition of CGFs accelerates the healing of human intrabony defects, and others found an acceleration in the healing of soft tissue defects in cases of gingival recession when using CGF [7,8].

Oracure dental gel is used to treat mouth ulcers and consists of two medicines. It also reduces the redness, swelling, pain, and burning sensation of the ulcers. This medicine is safe and does not cause any side effects.

Ulcers are considered the most common lesions that occur in the oral cavity. The mucosa of the cheek is considered the most diseased location with ulcer lesions. Some problems occurred with patients with ulcer lesions, such as speaking, eating, and swallowing. Antibiotics and corticosteroids are used to treat ulcers, but these medications lead to the occurrence of oral candidiasis as a side effect [9,10].

The nonkeratinized stratified squamous epithelium covers the mucous membrane of the cheek and consists of the basal cell layer, intermediate layer, and superficial layer, which represent the covering of the buccal mucosa of the mouth and separates by oral vestibule from the alveolar ridge [11].

In this research, the effect of CGF, or Oracure, on the repair of an induced ulcer in the cheek mucosa in experimental rats will be investigated.

2. Patients and methods

2.1. Concentrated growth factor

2.1.1. It's preparation

The male rat was used to obtain CGF from the blood of this rat. Rats were injected intraperitoneally with anesthetic solutions of chloral hydrate. Blood was obtained from his ear, and the blood was put in a test tube free of anticoagulant, and then the tube was centrifuged in a digital centrifuge device for 15 min. The fibrous phase of CGF was separated by using scissors [12].

2.2. Oracure gel

30 g. in the form of a ready-made gel. (Gardenia Pharmacy, Al Haram, Al Giza).

2.3. Animals

Thirty rats (100–150 g) were used in this research. The experiment was conducted in the animal house, faculty of medicine, Al-Azhar University. The animal housing conditions were followed by the regulations of the animal house as recommended by the research ethics committee. Put rats in separate boxes for each group at a temperature of about 30 °C in the lab room, and the boxes contained the nutrition and water of the rats.

Rats were divided into three groups. Rats (group 1, group II, and group III).

Animal groups		
Group I	Group II	Group III
Control group	Concentrated growth factor treated group that received topical treatment of CGF	Oracure gel treated group received topical treatment of Oracure gel

According to scarification's date, rats in all groups were divided into two groups at 4 and 10 days.

Every day rats were examined. For scarification, euthanasia of rats in all groups was done through anesthetic overdoses of thiopental. The buccal mucosa was separated using a scissor. Then, at days 4 and 10, the specimens of all groups were obtained, then put in the paraffin wax, and sets of serial sections were prepared using a microtome for the histological examination. The rats were disposed of after the end of the experiment in the incinerator. Ethical approval was obtained from the faculty of dental medicine for girls at Al Azhar University with the code REC-PD-24-05.

2.4. Histology examination

For the preservation of the specimens, they were put for 24 h in the formalin. Then water was used to wash the specimen from excess formalin. Then ethyl alcohol was used for the dehydration of the specimens. Then specimens were put in paraffin wax. Then a rotary microtome was used for sectioning specimens into 6 μm and fixing them on a glass slide, and H&E stain was put on them for the histological examination [13].

2.5. Ulcer induction

Anesthetization of the rats was made by intraperitoneal injection of xylazine hydrochloride (0.1–0.2 mg/kg IM) for 30 min, then induction of an ulcer was made by opening the oral cavity of the rat and exposing the inner aspect of the cheek (the buccal mucosa).

An oral mucosal ulcer was created by using a punch biopsy device with a diameter of 5 mm and a thickness of 1 mm, and then soft wax was added

inside the punch biopsy to determine the depth of the tissue. A punch biopsy was held perpendicular to the buccal mucosa, resulting in a consistent, circular incision in the cheek (buccal mucosa). Excess tissue was excised with a knife [14].

For group I, the rat's right and left cheeks were retracted, and an ulcer was made without using any materials. For group II, retraction of the cheek of the rat was done, and CGF was applied by using a microliter syringe once daily. For group III, the rat's left cheek was pulled back, and a graduated plastic syringe was used to inject 0.1 ml of Oracure gel into the ulcerated area. Every day, an injection was made into the submucosa next to the mucosal defect's edge at four different spots. Then the animals were denied access to food and water for 30 min following the procedure [15].

The specimens of each group were subdivided into two groups at (4, 10) days according to the date of euthanasia. Each group consisted of 15 rats. Animals were euthanized by anesthetic overdoses of thiopental; specimens were collected on days 4 and 10 to be examined for histological and statistical analysis.

2.6. Sample size calculation

Sample size calculation was performed using G*Power version 3.1.9.2 [14,15], University Kiel, Germany. Copyright (c) 1992-2014.

$$f = \frac{\sigma_\mu}{\sigma}$$

$$\sigma_\mu^2 = \frac{\sum_{i=1}^{k} n_j(\mu_i - \mu)^2}{N}$$

Where;

F: is the effect size; $\alpha = 0.05$; $\beta = 0.05$; Power = 1-$\beta = 0.95$.

The effect size f was 0.78 using alpha (α) level of 0.05 and Beta (β) level of 0.05, i.e., power = 95 %; the estimated sample size (n) should be 30 rats.

2.7. Statistical analysis

Version 20 of the statistical package for social science (IBM, Chicago, USA) was used for data administration and statistical analysis. The confidence interval and mean standard deviation were used to summarize the numerical data. By examining the distribution of the data and applying the Shapiro–Wilk and Kolmogorov–Smirnov tests, the normality of the data was investigated.

The one-way analysis of variance (ANOVA) test was used to compare groups about normally distributed numerical variables, and Bonferroni's post hoc test was then performed. Using the Paired *t* test, observation times within the same group were compared. The group and time factors' interaction was examined using a two-way ANOVA test. Every *P* value has two sides. *P* values less than 0.05 were regarded as significant.

3. Results

On day 4, the epithelium appeared to close the defect area overlying connective tissue in all groups. The thickness of epithelium in the CGF-treated group (group II) was larger than the other groups (Fig. 1).

The epithelium thickness in group III appeared larger than that of the control group (Fig. 2).

The keratin layer was thin in groups I and III (Figs. 3 and 2), but group II appeared thicker than the other groups (Fig. 1).

On day 10, the defect area was completely closed in all groups. The epithelium in group III appeared thicker than the other groups (Fig. 4). Epithelium appeared in group II, which was thicker than the epithelium in the control group (Fig. 5).

The keratin layer in group II appeared thicker than the other groups. The newly formed blood

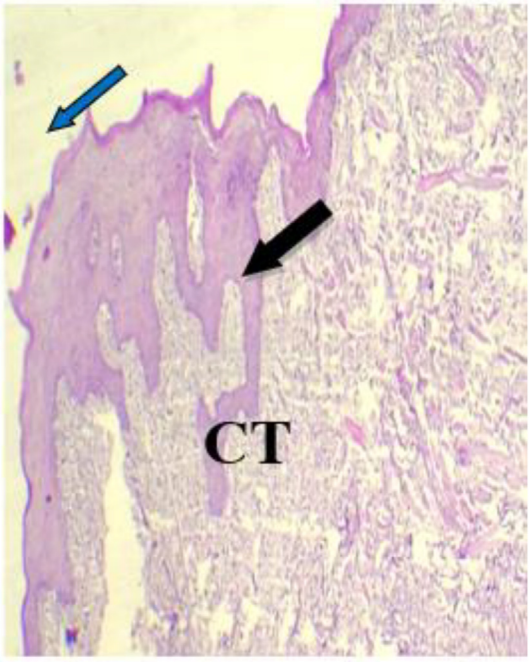

Fig. 1. Photomicrograph of group II (CGF group) (4 day postoperative) showing: newly formed thick layer of the epithelium (black arrows), Thick layer of the keratin covered the epithelium (blue arrows) and connective tissue (CT) (H&E Orig. Mag. × 100).

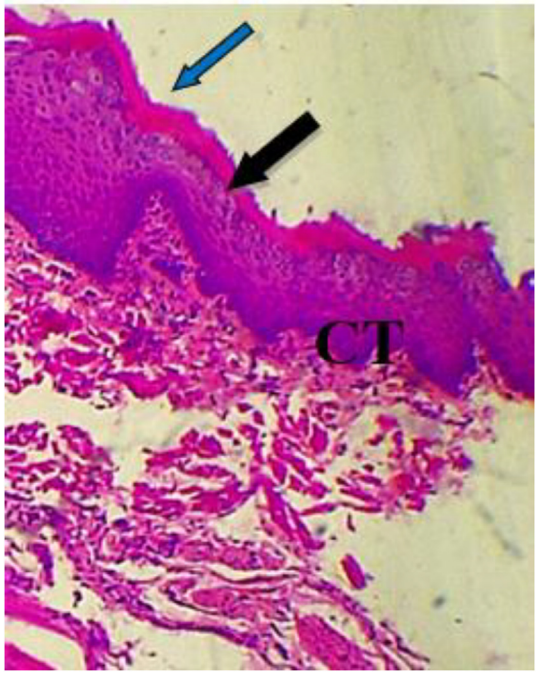

Fig. 2. Photomicrograph of group III (Oracure group) (4 day post-operative) showing: newly formed thick layer of the epithelium (black arrows), Thin layer of the keratin covered the epithelium (blue arrows) and connective tissue (CT) (H&E Orig. Mag. × 100).

vessels and collagen fiber of granulation tissue appeared mature in the CGF group (Fig. 5). The keratin layer appeared thin in groups I and III (Figs. 6 and 4).

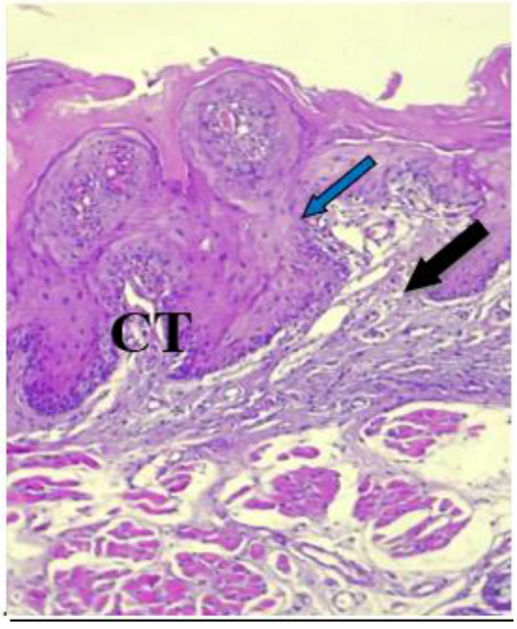

Fig. 3. Photomicrograph of the control defects (4 day postoperative) showing: newly formed thin layer of the epithelium (black arrows), Thin layer of the keratin covered the epithelium (blue arrows) and connective tissue (CT) (H&E Orig. Mag. × 100).

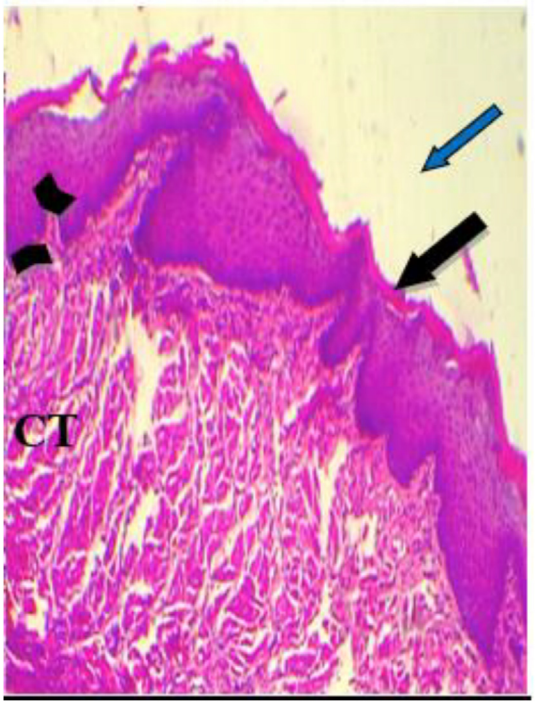

Fig. 4. Photomicrograph of group III (Oracure group) (10 days post-operative) showing: The epithelium appeared thicker than the other groups (black arrow), a thin layer of keratin covered the epithelium (blue arrow) and connective tissue (CT) with less number of blood vessel (arrows head) and less matured collagen fiber (H&E Orig. Mag. × 100).

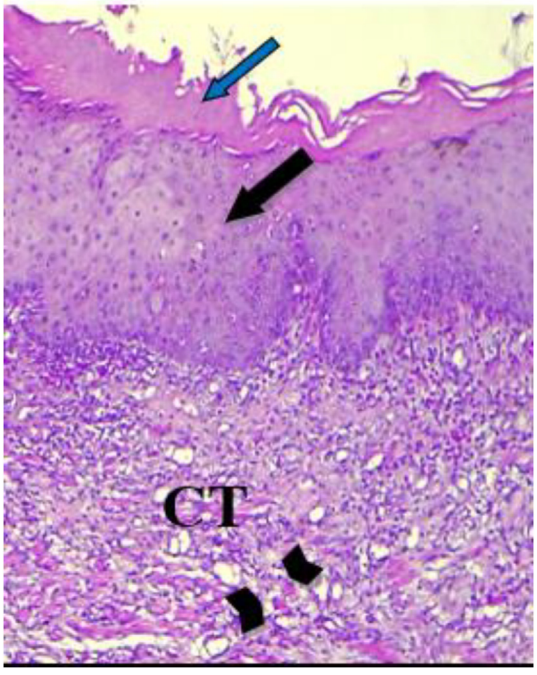

Fig. 5. Photomicrograph of group II (CGF group) (10 days postoperative) showing: The layer of the epithelium appeared thicker than a control group (black arrow), a thick layer of keratin covered the epithelium (blue arrow) and connective tissue (CT) with blood vessel (arrows head) and mature collagen fiber. (H&E Orig. Mag. × 100).

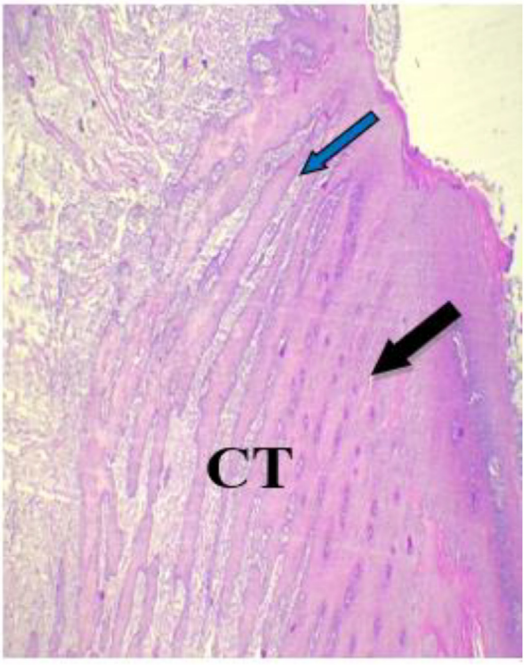

Fig. 6. Photomicrograph of the control defects (10 days postoperative) showing: The defect completely closed by a thick layer of epithelium (black arrow), a thin layer of keratin covered the epithelium (blue arrow) and connective tissue (CT) (H&E Orig. Mag. × 100).

In the Oracure group, collagen fiber appeared less mature, and fewer blood vessels were observed (Fig. 4).

3.1. Statistical results

3.1.1. Comparison between groups

Comparisons between groups were summarized in Table 1 and Fig. 3.

Group II had the highest mean value (1314.73 ± 31.39) at 4 days. Group I had the lowest value (274.47 ± 53.11), while group III had the highest value (311.20 ± 51.93). The value found in group III was substantially greater than the values found in the other two groups, according to the ANOVA and post hoc tests ($P = 0.000$).

After ten days, group II (1065.6 ± 109.94) had the lowest mean value (616.76 ± 37.99), while group III (1234.92 ± 145.82) had the greatest mean value. The results of the ANOVA and post hoc tests showed a significant difference ($P = 0.000$) between the two groups. On both dates, group II had the highest mean value (1190.16 ± 151.82), group III had the lowest value (773.06 ± 297.66), and group I had the lowest value (445.62 ± 185.58). The results of the ANOVA and post hoc tests showed a significant difference ($P = 0.000$) between the two groups.

3.1.2. Comparison within the same group

Contrasting observations made by the same group are summarized in Table 2 and Fig. 1.

Group I and group III observed a considerably greater value on day 10 compared with day 4 ($P = 0.000$). On the other hand, on day 4, group III recorded a considerably higher value ($P = 0.001$). Considering all groups together revealed a significantly higher value on day 10 in comparison to day 4 ($P = 0.001$).

Table 2. Compares day 4 and day 10 within the same group and provides descriptive statistics on distance (Paired t test).

Groups	Time	Mean	SD	Difference		t	P
				Mean	SD		
Group I	4 days	274.47	53.11	342.29	29.20	11.72	0.000*
	10 days	616.76	37.99				
Group II	4 days	1314.73	31.39	249.13	51.13	4.87	0.001*
	10 days	1065.60	109.94				
Group III	4 days	311.20	51.93	923.72	69.22	13.34	0.000*
	10 days	1234.92	145.82				
All groups	4 days	633.47	200.73	338.96	78.76	3.75	0.001*
	10 days	972.43	287.80				

Significant at a P value of less than 0.05.

Table 1. Descriptive statistics of group comparison and distance analysis of variance.

Time	Mean	SD	95 % Confidence interval for mean		Minimum	Maximum	F	P
			Lower bound	Upper bound				
4 days								
Group I	274.47[b]	53.11	208.53	340.42	206.20	346.32	803.69	0.000*
Group II	1314.73[a]	31.39	1275.75	1353.71	1261.24	1344.28		
Group III	311.20[b]	51.93	246.72	375.69	253.38	370.67		
10 days								
Group I	616.76[Q]	37.99	569.58	663.94	567.43	669.47	43.99	0.000*
Group II	1065.60[P]	109.94	929.09	1202.11	910.28	1171.68		
Group III	1234.92[◦]	145.82	1053.86	1415.98	999.94	1377.97		
Both dates								
Group I	445.62[z]	185.58	391.47	499.76	206.20	669.47	202.32	0.000*
Group II	1190.16[X]	151.82	1136.02	1244.31	910.28	1344.28		
Group III	773.06[Y]	297.66	718.91	827.21	253.38	1377.97		

Table 3. Outcomes of the two-way analysis of variance test for the interaction of groups and time variables.

Source	Type III sum of squares	Mean Square	F	*P* value	Partial Eta Squared
Groups	2785148.47	1392574.24	202.32	0.000	0.944
Time	861700.72	861700.72	125.19	0.000	0.839
Groups * time	1719507.04	859753.52	124.91	0.000	0.912

Significant at a *P* value of less than 0.05.

3.1.3. Impact of several factors and how they interact

Two-ways ANOVA test indicated a significant difference ($P = 0.000$) between the groups and between observation times ($P = 0.000$). Moreover, the group variable and time variable had a significant interaction ($P = 0.000$) (Table 3).

In a post hoc analysis of significance with a *P* value less than 0.05 when comparing identical means with the same superscript letter, there were no appreciable differences.

4. Discussion

The cheek mucosa is the most damaged location in the oral cavity, and ulcers are the most common inflammatory diseases that occur in the oral cavity. Therefore, we described in this work how to create a consistent buccal mucosa ulcer for evaluation of the effect of CGF and Oracure on mucosal repair. For this evaluation, we needed to create an ulcer of uniform depth and width through the creation of a 1 mm depth in the buccal mucosa using a punch biopsy tool (5 mm in diameter). During operation, the apparatus's application was appropriate and quick. Thus, the length of anesthesia was shortened [16].

Rats were used as an experimental model for the examination of buccal mucosa because, for ethical concerns, it would be difficult to make this examination in humans. Rats's buccal mucosa is similar to that of humans [17].

At day 4, the defect area is still quite large in the negative control group. In the CGF and Oracure groups, the defect area decreased in contrast to the preceding control group due to the formation of the epithelium in both groups. The connective tissue in the CGF group was filled with big, spindle-shaped fibroblasts, which may suggest that CGF is a mitogen that enhances the mitotic division of fibroblastic cells. In Oracure gel, connective tissue displayed a persistent inflammatory response [18].

Both CGF and Oracure-treated groups have distinct connective tissue. In the CGF group, connective tissue was filled with new blood vessels (angiogenesis). It was found that CGF has the potential to effectively induce the formation of new blood vessels (angiogenesis). Also, the presence of mature collagen fibers in the recently created granulation tissue suggests that the CGF accelerated collagen maturation more than the control group. Granulation tissue in the Oracure group was less developed than in the CGF group, so Oracure gel has a little effect on the maturation of collagen fiber [19].

4.1. Conclusion

Both CGF (group II) and Oracure gel (group III) accelerated the healing of mucosa compared with the control group. But CGF was more effective than Oracure in accelerating the maturation of collagen fiber and in stimulating angiogenesis (the formation of new blood vessels).

4.2. Recommendation

Further investigation tools were recommended for the following: To widen the range of animals, they were subjected to research for their biological response to CGF, to study the quality of the formed soft tissue, and to determine the behavior of CGF when used in the repair of buccal mucosal ulceration in humans.

Ethics information

Ethical approval was obtained from the faculty of dental medicine for girls at Al Azhar University with the code REC-PD-24-05.

Funding

No funding.

Biographical information

The study was done at Faculty of dental medicine for girls Al Azhar University.

Conflict of interest

There is no conflict of interest.

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