PROPHYLACTIC EFFECT OF CHITOSAN-COATED GINGER NANOPARTICLES VERSUS GINGER EXTRACT ON TONGUE WOUND HEALING IN RATS EXPOSED TO ELECTRONIC CIGARETTE SMOKE

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ABSTRACT

**Purpose:** The study aimed to investigate the prophylactic value of administering ginger extract versus the combined use of chitosan-coated ginger nanoparticles on tongue wound healing ability in rats exposed to electronic cigarettes (E-cigs) smoke. **Methods:** 60 rats were equally assigned into 3 experimental groups as follow: (1) Group I (untreated group), (2) Group II (ginger extract treated group) and (3) Group III (chitosan-coated ginger nanoparticles treated group). All rats were exposed to E-cigs liquid vapor 30 days then tongue incision. At the 7th and 14th days, healing was assessed using histological and immunohistochemical evaluation. **Results:** This study revealed delayed wound healing in the 7th day in group I and II, however at the 14th day, a thin covering of epithelium was seen. Group III showed marked reduction in inflammation and initial healing at the 7th day. At the 14th day, almost normal epithelium was observed. Positive Ki-67 immunostaining was observed in group III at both periods compared to the other groups. **Conclusion:** Application of chitosan coated ginger nanoparticles alleviated the effect of E-cigs smoking and enhanced wound healing.

**KEYWORDS:** E-cigarette smoke, healing, chitosan, ginger nanoparticles.

INTRODUCTION

Oral mucosa wound healing is a critical process in most surgical procedures. Wound closure is an important physiological process that halts the invasion of microorganisms or other elements invasion into the investing tissues of oral cavity [1]. Smoking was proved to have severe negative effects on all phases of wound healing such as delayed tissue oxygenation and altered immunological response. Tobacco-derived substances, including nicotine...
were proved to disturb tensile wound strength via fibroblasts and leukocytes functional alteration, resulting in inadequate collagen production, necrosis, and insufficient eradication of microorganisms [2].

Electronic cigarettes (E-cigs) are commonly spread form of nicotine usage, recognized as vaping [3]. E-cigs are devices that mainly depends on production of an aerosolized mixture from a nicotine-containing solution together with flavoring chemicals, that release vapor upon heating to be inhaled by the users [4]. Numerous studies have evaluated the effects of traditional and E-cigs on the health of oral tissues and concluded that using E-cigs result in less pathological changes than smoking conventional cigarettes [5]. However, they added that the mucosal lining of the oral cavity was exposed to histopathological damages as a result of vaping [6]. Sundar et al. [6] portrayed that E-cigs with flavoring agents caused an amplified oxidative stresses and secretion of inflammatory cytokines from both the gingival epithelium and the periodontal ligament fibroblasts. Similarly, experimental research confirmed that e-cigs aerosols might cause cytotoxicity to the oral epithelial cells [7]. Furthermore, xerostomia, irritation, and impaired healing after dental treatment were reported to be an adverse effects associated with chronic usage of E-cigs [8].

To enhance oral mucosa wound healing in smokers, some researchers tried to decrease e-cigs harmful effects by using antioxidant materials such as vitamin C [9], Royal jelly [10] and ginger [11]. Interestingly, It has been reported that ginger volatile oil possesses antioxidant and anti-inflammatory properties [11]. For these palliative role researchers used ginger for the treatment of nervous disease, gingival inflammation, tooth pain, asthma, stroke, constipation and diabetes [12]. On the molecular level, volatile oil of ginger was proved to have a unique ability to amend cellular immunological response and lymphocyte functions [13]. Additionally, ginger was used as nanoparticles that were loaded on a delivery system and was proved to be an ideal candidate for wound applications [14]. Considering drug delivery systems, chitosan was reported to be a biological material extracted from renewable resources and was recently portrayed to has a great role in wound healing [15]. More specifically, chitosan loaded with ginger nanoparticles (NPs) accelerated wound healing and enhanced healing outcomes [16]. The later formula was verified to combine the remarkable ginger antimicrobial and anti-inflammatory effects [17] together with the antibacterial effect of chitosan [18]. Recently, Refaat et al. [19] compared the potential prophylactic roles of chitosan coated ginger nanoparticles versus ginger extract against vancomycin-induced renal cytotoxicity in rats. They portrayed that both formulas ameliorated the vancomycin toxicity.

To the best of our knowledge, no previous studies have tested the effect of the combined use of ginger NPs loaded on chitosan to alleviate the effect of smoking E-cigs on wound healing. Therefore, the aim of the current study was to compare the prophylactic benefit of chitosan-coated ginger NPs versus ginger extract alone on the healing of experimentally induced tongue incisions in a rat model exposed to E-cig smoke.

**MATERIAL AND METHODS**

In this study, 60 mature male Wistar rats weighing 250–350 g were used. The sample size was calculated using G Power (version 3.1.9.4, Germany). According to the pervious study of Lee et. al. 2022 [20], a means of 102.15 and 16.67 and a difference between standard deviation of 12.35, \( \alpha=0.09 \), and a power of 0.9 was considered. Sample size have been calculated of 19.5. So, we have used twenty rats per group.

Rats were housed one week before the study as an accommodation period. Throughout the course of the study, the animals were housed in plastic cages and provided standard laboratory food and tap water as needed. All methods were carried out in accordance with relevant guidelines and regulations.
The study protocol was approved by the Ethics Committee of the Faculty of Dentistry at Tanta University, which was accepted and developed in accordance with the standards for the responsible use of animals in research.

**Experimental design**

Rats were randomly divided into three groups:

- **Group I (Smoking with incision):** Animals were exposed to E-cigs liquid vapor for 30 days followed by tongue incision. Then, tongue wound was left to heal spontaneously.

- **Group II (Smoking, incision and treatment with ginger extract):** Animals were exposed to E-cigs smoking liquid vapor together with daily oral administration of ginger extract for 30 days, followed by tongue incision. After incision, rats continued to receive daily dose of ginger extract by orogastric tube during the healing period.

- **Group III (Smoking, incision and treatment with chitosan coated ginger nanoparticles):** Animals were exposed to E-cigs liquid vapor together with the use of a daily 500 mg/kg dosage of chitosan coated ginger nanoparticles by orogastric tube for 30 days, followed by tongue incision. After wounding, rats received the same daily dose of chitosan coated ginger nanoparticles by orogastric tube during healing period.

The rats from each group were divided into two halves, with one half being euthanized on the 7th day after wounding, and the remained half being euthanized on the 14th day. The tongue of each rat was dissected and processed for qualitative histological hematoxylin and eosin and immunohistochemical staining for Ki-67. Study design was shown in Figure. 1.

**Experimental procedure**

**Exposure to e-cigs liquid vapor**

All rats were exposed to E-cig smoke by placing them into a smoking box for 30 min twice daily for 30 days before the experiment \(^{[21,22]}\). The E-cigs liquid was obtained from Egypt’s commercially accessible supplies, (Dollars Blends Company).

**Preparation of ginger extract**

Ginger was purchased from Cairo University of Medical Sciences’ Medicinal Plants Research Center, Egypt. The ginger extract was prepared following the method used in the literature of Nasri et. al. 2015 \(^{[23]}\). Ginger alcoholic extract was prepared using maceration method. The freshly prepared powdered ginger samples (10 g) were extracted with 150 mL of 99% methanol. Then, the prepared alcoholic extract was left at 37°C for 72 hours. Afterwards, the solution was filtered and the solvent was evaporated using a rotary evaporator and freeze drier. Fresh preparations of the extract were made every three days and kept at -20°C until utilized. For administration orally, rats were given daily intake of 1g/kg of ginger extract by oral gavages \(^{[24]}\) till the end of the experimental period.

**Preparation of chitosan coated ginger nanoparticles**

Chitosan coated ginger nanoparticles in liquid form was purchased from Center of Nano Technology, Faculty of Pharmacy, Tanta University. Chitosan (100mg) was left in 100 ml of acetic acid (1%) solution for 24 hours to be completely hydrated and then wholly dissolved to obtain a uniform solvent (1 mg/mL solution of chitosan). For preparation of ginger nanoparticles, the ginger extract sample was exposed to serial centrifugations start with 1,000g for 10min, then 3,000g for 20min and 10,000g for 40min. Then, after last centrifugation the sample was resuspended in distilled water for further centrifugation at 140,000g for 90min. To produce chitosan-loaded ginger nanoparticles solution, chitosan 1mg/ml were added to 50mg/2ml of the ginger nanoparticles. Then, the solution was segregated from aqueous suspension by centrifugation (30,000 g for 30 min). The daily dose was prepared following the protocol that was applied by Rafaat et al. 2021 \(^{[19]}\).
Surgical wound induction

Sodium phenobarbital (50mg/kg) was intraperitoneally administered to anesthetize the rats. A 5 mm-diameter punch was used to establish a unilateral oral mucosal wound on the right lateral tongue surface, 4 mm from the tip. The lesion was 2mm deep and restricted to the mucosa without any muscular involvement (Figure 2a).
Rat Euthanasia:

The rats from each group were euthanized on the 7, 14 days after wounding (10 rats were used for each date) with an over dose of intramuscular phenobarbital sodium salt. Rats tongues were collected and cut transversely (at the center of the wound) then were prepared for light microscopic examination.

Light microscopic examination

Tongue samples taken from the wound site were preserved in 10% formalin for two days. Samples were then processed, embedded in 4-5μm thick paraffin sections, dissected, and stained using hematoxylin and eosin. Also, sections were stained immunohistochemically using Ki-67 as a proliferating marker. The Ki-67 antigen was visualized in paraffin sections using mouse monoclonal anti-kI67 according to the instructions of the commercial supplier (Genemed; Biotechnologies) by the use of APAAP (alkaline phosphatase monoclonal anti-alkaline phosphatase) [25].

Statistical analysis

For statistical analysis, calculating the area percentage of the Ki67 immunostaining and inflammatory cells count were done. To calculate the area percentage of the Ki76 immunostaining, light microscopic images of the wound area at each follow-up period of all groups were captured under a magnification of x 400. Images were taken using LM built-in camera (Leica ICC50 HD) via image software LAS EZ version 4.0.0. Then, image J analysis software (Image J 1.42q, Wayne Rasband, USA) was used to measure the area percentage of immunohistochemical staining in the histological sections at the two follow-up periods within each group [26],[27].

Regarding the inflammatory cells count, image J analysis was also used. The cellular counting was done in a selected area equaling 4mm² that represented the area with the greatest intensity of inflammatory infiltrate. Within image J, a plug-in for analysis and cell counting were selected to count the cellular elements [28]. Five different fields in each five different captured histological sections were used to ensure the accuracy of the numerical data.

![Fig. (2) Photographs illustrating the punch-induced injury sites at different intervals; (dashed circle); (a) Lateral border of the tongue at the incision site in the 1st day, (b-d) Displaying the wound area at the 7th day, (b) Group I showing unhealed wounded area, (c) Group II viewing incompletely healed wounded area, (d) Group III superficial tongue wound was noticed, (e-g) Displaying the wound area at 14th day, (e) Group I illustrating incompletely healed wounded area, (f) Group II partially healed wounded area, (g) Group III completely healed tongue wound area.](image-url)
The data were tabulated and statistically analyzed using Statistical Package for Social Sciences (IBM SPSS Statistics version 26). Numerical variables were analyzed using descriptive statistics such as mean, standard deviation, and range. To compare groups at each time point, one-way ANOVA and post-hoc tests (Tukey’s test) were used. An independent t-test was used to compare the two follow-up periods for each group. The difference was considered significant at a p-value < 0.05 (*) and highly significant at a p-value < 0.001 (**).

RESULTS

Clinical observation

Clinically, on the 7th day all animals in group I rats presented an unhealed tongue incisional area (Figure. 2b). Groups II and III rats had incompletely healed wounds (Figure. 2 c, d). On the 14th day, Group I rats continued to exhibit an incompletely healed wound area (Figure. 2 e). However, the animals in group II showed partially healed wound areas (Figure. 2 f). Interestingly, the animals in group III exhibited completely healed tongues in the punched area (Figure. 2 g).

Histopathological results

Hematoxylin and Eosin stain

Histologically, in the incised tongue area on the 7th day, Group I tongue sections disclosed a granulation tissue covering the unhealed epithelial area (Figure. 3 a,b) together with numerous inflammatory cells infiltrating the underlying...

Fig. (3) Histological sections of the lateral border of the rat tongues at the 7th day at the wound areas, (a, b) group I, showing granulation tissue filling the wound gap (red star); unhealed epithelium (double arrow head) and inflammatory cells infiltration (white arrows). (c, d) group II, illustrates approximation of the wound edges (double arrow head), initial epithelial migration (yellow arrow). The underlying lamina propria shows inflammatory cell infiltration in a close proximity to dilated blood vessels (red arrow). (e, f) group III, displaying epithelial migration at the wound bottom with poorly defined basement membrane (yellow arrow) at the wound area. The underlying lamina propria exhibits newly formed blood vessels (red arrows) together with absence of inflammatory cells (H&E. a, c, e x 100; b, d, f x400).
connective tissue. In contrast, in the Group II incised tongue areas showed an unhealed clean-cut wound with a minimum degree of epithelial migration and discontinuation of the basement membrane. The underlying connective tissue showed few inflammatory cell infiltrates around the dilated blood capillaries (Figure 3 c,d). Interestingly, Group III sections portrayed healing initiation that manifested as epithelial migration at the base of the wound, with a poorly defined basement membrane. In addition, inflammatory cells were absent in the underlying connective tissue (Figure 3 e, f).

The histological features of the incised tongue area on day 14 in Group I showed that the wound area was covered with thin epithelial cell layers. The underlying connective tissue showed few inflammatory cells and dilated congested blood capillaries (Figure 4 a, b). However, Group II histological sections displayed a thin epithelial layer at the wound site together with initial ill-defined surface keratinization. The connective tissue was highly fibrous, organized, and filled with active fibroblasts (Figure 4 c, d). Remarkably, Group III illustrated enhanced wound healing in the form of incision coverage, with an almost normal epithelial thickness and well-defined surface keratinization. In addition, the connective tissue showed active fibroblasts together with well-organized collagen fibers (Figure 4 e, f).

Fig. (4) Histological sections of the lateral border of the rat tongues at the at the 14th day at the wound areas, (a, b) group I, shows the beginning of re-epithelization with keratin layer formation (double arrow head), thin epithelial layers arranged in a depressed manner at the wound area. The underlying tissue exhibits dilated congested blood vessel (red arrow) that is surrounded by irregularly arranged collagen fibers. (c, d) group II, illustrates closure of the wound edges (double head arrow) by keratinized relatively thin epithelial layer together with almost normal underlying lamina propria and muscular layer. (e, f) group III, displaying almost normal thickened and well-arranged epithelial covering (double head arrow) with rete pegs formation. (H&E. a, c, e x 100; b, d, f x400).
Immunohistochemical results

In Group I, negative immunohistochemical reaction for Ki-67 was observed in the epithelial cell lining of the wounded area (Figure. 5 a, b) at both 7- and 14-day intervals. Similarly, Group II tongue sections on day 7 after incision revealed negative immunostaining (Figure. 5 c). Immunohistochemical localization of Ki-67 in Group II depicted positive nuclear immunoreactivity at the site of the wound in the basal cell layer after 14 days (Figure. 5 d). Interestingly, Group III histological sections portrayed positive immunostaining at the wound site in the basal cell layer after 7 days (Figure. 5 e). In addition, after 14 days after wounding, positive nuclear immunostaining was observed within the epithelial basal and parabasal cell layers in the healed wound area (Figure. 5 f).

Statistical results:

Regarding the percentage area of immunostaining, a significant increase in the percentage area of immunostaining was reported in Group III at both intervals compared to Groups I and II. The latter two group showed a significant decline in the percentage area of immunostaining at both intervals (Figure. 6 a, b) (Table. 1, 2).

Regarding the number of inflammatory cells, a significant increase in the number of inflammatory cells was observed in Group I at both intervals compared to that in the remaining groups. Furthermore, Groups II and III showed a significant decrease in the number of inflammatory cells at both time points (Figure. 6 c, d) (Table. 3, 4).

Fig. (5): Photomicrographs displaying immunolocalization of anti-Ki 67 antibodies at the wound areas, (a, b) group I, a. the wound at the 7th day shows unhealed wound margins (double arrow head), negative Ki67 immunostaining within the epithelial layers at the wound area. b. the wound at the 14th day reveals gapped wound (double head arrow) with few intercellular immunostainings between the epithelial cells. (c, d) group II, c. the wound at the 7th day illustrates unhealed wound edges (double head arrow) together with a relatively smaller number of positive nuclear immunostaining at the basal cell layer (white arrows) of the epithelium. (e, f) group III, e. the wound at the 7th day shows partially-healed wound margins, positive nuclear immunostaining within the basal cell layer at the wound area (white arrow). f. the wound at the 14th day discloses positive nuclear immunostaining within the basal and parabasal cell layers (white arrows) of the epithelium at the healed wound area, (Ki 67 immunostaining. a, b, c, d, e, f x 400).
TABLE (1) Comparison between Mean ±SD values of the area percentage of immunostaining between group I, II and III at each duration and between different durations within the same group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Area after 7 days</th>
<th>Area after 14 days</th>
<th>paired t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>±S.D</td>
<td>Range</td>
<td>±S.D</td>
</tr>
<tr>
<td>---------</td>
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</tr>
<tr>
<td>Group I</td>
<td>0.800.16±</td>
<td>0.60—1.00</td>
<td>1.860.47±</td>
</tr>
<tr>
<td>Group II</td>
<td>0.800.16±</td>
<td>0.60—1.00</td>
<td>8.620.92±</td>
</tr>
<tr>
<td>Group III</td>
<td>9.850.85±</td>
<td>9—11.08</td>
<td>19.621.61±</td>
</tr>
<tr>
<td>ANOVA test</td>
<td>527.543</td>
<td></td>
<td>327.673</td>
</tr>
</tbody>
</table>

(*) means there is a significant difference when P. value < 0.05, (**) means a highly significant difference with p-value < 0.001.

**TABLE (2)** Multiple comparison (Tuckey test) was used to show the difference at the area percentage of immunostaining between each two groups.

<table>
<thead>
<tr>
<th>Durations</th>
<th>G1 vs. GII</th>
<th>G1 vs. GIII</th>
<th>G1 vs. GIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 vs. GII</td>
<td>1.000</td>
<td>0.000**</td>
<td>0.000**</td>
</tr>
<tr>
<td>G1 vs. GIII</td>
<td>0.000**</td>
<td>0.000**</td>
<td>0.000**</td>
</tr>
</tbody>
</table>

There is a significant at P-value<0.05 (*), and highly significant at P-value<0.001 (**).

**TABLE (3)** Comparison between Mean ±SD values of the inflammatory cells count between group I, II and III at each duration and between different durations within the same group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of inflammatory cells</th>
<th>After 7 days</th>
<th>After 14 days</th>
<th>paired t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>±S.D</td>
<td>±S.D</td>
<td>t   (p-value)</td>
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<td>---------</td>
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</tr>
<tr>
<td>Group I</td>
<td>12.201.92±</td>
<td>10—15</td>
<td>9.601.52±</td>
<td>8—11</td>
</tr>
<tr>
<td>Group II</td>
<td>71.58±</td>
<td>5—9</td>
<td>3.401.14±</td>
<td>2—5</td>
</tr>
<tr>
<td>Group III</td>
<td>2.401.14±</td>
<td>1—4</td>
<td>2.401.67±</td>
<td>1—5</td>
</tr>
<tr>
<td>ANOVA test</td>
<td>48.080</td>
<td></td>
<td>35.656</td>
<td></td>
</tr>
</tbody>
</table>

There is a significant at P-value<0.05 (*), and highly significant at P-value<0.001 (**).

**TABLE (4)** Multiple comparison (Tuckey test) was used to show the difference at the inflammatory cells number between each two groups.

<table>
<thead>
<tr>
<th>Durations</th>
<th>G1 vs. GII</th>
<th>G1 vs. GIII</th>
<th>G11 vs. GIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>After 7 days</td>
<td>0.001*</td>
<td>0.000**</td>
<td>0.001*</td>
</tr>
<tr>
<td>After 14 days</td>
<td>0.000**</td>
<td>0.000**</td>
<td>0.542</td>
</tr>
</tbody>
</table>

There is a significant at P-value<0.05 (*), and highly significant at P-value<0.001 (**).
DISCUSSION

The present study highlights the prophylactic role of chitosan-coated ginger NPs in alleviating the effects of e-cig smoking on wound healing. Regarding the previously reported rapid epithelial turnover and maturation rates of oral mucosal membranes, oral mucosa was selected as an appropriate site for studying the process of healing in our study.

Tongue was selected as a model for the present experiment. The tongue is the preferred location for oral squamous cell carcinomas, which account for more than 90% of malignant tumors diagnosed in the oral cavity\[^{29}\]. Hence, many studies used tongue as a model to study wound healing as, Moawad and Abobaker\[^{30}\] studied the effect of hyaluronic acid on induced tongue ulcer in diabetic rats. Also, tongue was widely used as an experimental model of oral mucositis that was induced by the combined use of chemotherapeutic agents and radiation\[^{31}\]. Although, palatal excisional wound model has been also used previously in several studies to investigate intraoral wound healing in rats and was considered as a reproducible wound that could be followed clinically and histologically\[^{32\text{-}33}\]. However, tongue was considered simple, effective and easily reproducible, model that can be used to assess the prophylactic effects of different agents in the healing process of oral mucosa.

![Graphs showing area percent and inflammatory cell count comparisons between groups I, II, and III at different intervals.](image-url)
The follow-up periods were chosen according to the previously studied spontaneous healing of rat incisional wounds that was reported to occur within two weeks after induction [36]. Consequently, follow-up periods of 7 and 14 days were selected to track the healing process.

To reach homeostasis after injury, oral mucosa acquires complex sequences of biological healing processes manifested by highly integrated programmed stages; hemostasis, inflammation, proliferation and tissue remodeling. Immediately after wounding, vascular constriction and fibrin clot formation cause hemostasis, which induce the release of pro-inflammatory cytokines and growth factors such as transforming growth factor (TGF)-β, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and epidermal growth factor (EGF). Inflammatory cells, then, migrate into the wound and promote the inflammatory phase, which is characterized by the sequential infiltration of neutrophils, macrophages, and lymphocytes. Within a week, the proliferative phase overlaps with the inflammatory phase, demarcated by formation of granulation tissue, re-epithelialization and angiogenesis. Finally, tissue remodeling occurs via protease activity, and the granulation tissue begins to remodel the wound site as it gradually returns to homeostasis [34, 35].

Interestingly, in our study, we added E-cigs as an obstacle that interrupts normal tissue homeostasis during the healing process. Previous studies have shown that nicotine, flavoring agents, and solvents included in most E-cig liquids produce toxic and dangerous aerosols upon heating [36]. Regarding their hazardous effects on wound healing, the deteriorating effect of E-cigs liquid exposure was obvious in Group I at both the 7th and 14th days of wounding. Researchers have attributed these changes to the ability of nicotine to inhibit epithelialization and decrease keratinocyte, fibroblast, and macrophage proliferation. Reactive oxygen species (ROS) are produced by the aerosols during e-liquid heating. ROS have been reported to affect cell proliferation and survival rate [37]. A study was done in 2021 verified that the antioxidant capacity of saliva has been affected in the E-cigs users in a similar degree as in cigarette smokers when compared to the saliva of non-smokers [37]. The impaired antioxidant function of saliva can also stimulate the formation of free radicals and ROS, which play a role in the progression of tissue destruction.

In this study, ginger was used as a palliative agent against the harmful effects of E-cigs on wound healing. The histological results of Group II showed better wound healing compared with that in Group I. These findings are in line with those of a prior studies that showed that ginger administration at various time intervals significantly reduced the number of inflammatory cells, increased the number of fibroblast cells, and improved incisional wound epithelialization [38, 39]. Our results can further be explained by the fact that ginger, besides its ability to treat a variety of conditions, such as cancer, diabetes mellitus, degenerative diseases and cardiovascular diseases like hypertension and atherosclerosis, it also has a unique anti-inflammatory and antioxidative properties [40].

Remarkably, histological analysis of the wound area in group III revealed initial healing that was marked by epithelial migration at the base of the wound with the absence of inflammatory cells after 7 days of wounding. The wound area on day 14 after incision was covered by an almost normally thick epithelial layer with well-defined surface keratinization, and the connective tissue showed active fibroblasts together with well-organized collagen fibers. These results were previously reported in a study in which chitosan was highly effective in reducing the time required for wound healing, improving the appearance of the wound after treatment, and significantly decreasing pain [41]. Considering the proven biocompatibility and bioactivity of chitosan, it was the preferred choice for our experiments in wound care. It exerts its wound healing effects by promoting hemostasis, antimicrobial activity, inflammatory response.
regulation, and free radical scavenging activity. The latter interferes with ROS overproduction around the wound site, especially in cases exposed to smoking[44].

Moreover, Moreover, the ginger as an NP loaded on chitosan in Group III displayed enhanced healing. Gingerols and shogaols may be the main pharmacologically active components in ginger, which are responsible for their anti-inflammatory, anticarcinogenic, and antioxidant properties [42,43]. The anti-inflammatory properties were further proven in our study by counting inflammatory cells. The latter exhibited a marked decrease in the cellular count in Group III compared to that in Group I. These results were in agreement with those of researchers who reported that the combined use of chitosan with other products enhanced the healing process by regulating inflammation and cellular viability[44].

Regarding Ki-67, it is a nuclear marker expressed in proliferating cells during wound healing [45]. The Ki-67 immunohistochemical results of the present study emphasized a significant increase in immunostaining percent area in group III compared to the remaining groups for both follow up periods. Thus, active cellular proliferation and enhanced wound healing were observed at both intervals in group III compared to those in groups I and II.

In conclusion: both ginger extract and chitosan-coated ginger NPs have a potential protective role against the effect of E-cigs smoking on wound healing. However, the greatest enhancement in wound healing was observed with chitosan-coated ginger NPs. Considering the improvement in histological features and immunohistochemical evaluation of wound healing after using chitosan-coated ginger NPs, it could be advantageous when used as an adjuvant therapy in wound healing in the oral cavity injuries. Some of the limiting factors associated with the available animal models of the current work was owed to the highly keratinized nature of the rat tongue, which caused difficulty in inducing surgical incision. Also, tongue is a motile organ that could be exposed to further injury to the punched area during food swallowing and chewing. Thus, further studies should be conducted to test the therapeutic effect of ginger and chitosan on intraoral soft and hard tissues injuries.

Declarations section:

Conflict of Interest: The authors affirm that they have no known financial or interpersonal conflicts that would have seemed to have an effect on the findings presented in this study.

Funding: No organization provided funding to the writers for the work they submitted. Availability of information and resources, the research data are accessible upon request from the corresponding author.

Ethics approval: This work was carried out in line with the ethical guidelines established by Tanta University’s faculty of Dentistry for the use of experimental animals in research.

ARRIVE guidelines: The ARRIVE guidelines for the documentation of in-vivo studies in animal research were followed in the conduction of this work.

Data availability: All data generated and analysed during the current study are available from the corresponding author on reasonable request.

Author contribution:

MB Helal: contributed to conception, design and acquisition. Drafted and critically revised paper and gave final approval. AE Abdelhamied: contributed to conception, design and acquisition. Drafted and critically revised paper and gave final approval. NT Elkazzaz: contributed to conception, design and acquisition. Drafted and critically revised paper and gave final approval. All authors agree to be responsible for all aspects of work confirming integrity and accuracy.
REFERENCES


