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ULTRA STRUCTURAL AND IMMUNOHISTOCHEMICAL STUDY ON THE HEALING EFFECT OF NANO SILVER VERSUS NANOZINC ON INDUCED TRAUMATIC ULCER IN THE BUCCAL MUCOSA OF ALBINO RATS

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KEYWORDS

Buccal mucosa, nano zinc, nano silver, nanotechnology, silver nitrate, zinc oxide

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ABSTRACT

Introduction: Nanotechnology is becoming of increasing interest in medicine. Nanoparticles have a large surface area-to-volume ratio which increases their interaction with the tissues and offers improved penetration into wounds. **Aim:** This research aimed to evaluate and compare the effect of silver nitrate, zinc oxide, Nano silver and Nano zinc oxide on promoting healing of induced traumatic ulcer in the buccal mucosa of Albino rats. **Materials and methods:** Sixty adult male albino rats were divided randomly into six groups, 10 rats for each group: Group I: was a normal group. Group II: left without treatment. Group III: twice daily topical application of silver nitrate solution. Group IV: twice daily topical application of nano silver solution. Group V: twice daily topical application of zinc oxide solution. Group VI: twice daily topical application of nano zinc solution. Each group except Group I divided into two subgroups: subgroup 1 sacrificed after 7 days and subgroup 2 sacrificed after 14 days. **Results:** Histological sections of buccal mucosa of rats showed after 7 days loss of epithelium, lamina propria showed degeneration in collagen fibers, inflammatory cells infiltrations and dilation in blood vessels with engorgement of blood inside them. After 14 days there was improvement in healing. While nanomaterials showed the best wound healing. **Conclusions:** Infections may delay and deteriorate wound healing, so antimicrobial agents should be used. Nano silver improve healing faster than silver nitrate, zinc oxide and nano zinc.

INTRODUCTION

The most common mucosal illnesses in the oral cavity are oral ulcers. Oral mucosal ulcers are a frequent ailment that can have a serious impact on quality of life. A rupture in the oral mucosa that penetrates the lamina propria and spreads into the submucosa is referred to as an oral ulcer [(1)].

Many circumstances, such as trauma, infections, systemic disease, unfavorable drug reactions, or allergic reactions, can result in oral ulcers. Accidental damage is the primary cause of traumatic ulcers, which are most common in the oral cavity. The cheek mucosa is the most commonly afflicted location when ulcerations occur. The intraoral mucosal coating of the inner cheeks that covers the buccal space and attaches both superiorly and inferiorly to the mucosa of the alveolar ridges is known as the buccal mucosa [(2)].

While the body's defensive mechanism of wound healing priorities speedy recovery, the process of regeneration in a hostile environment requires more time. Specifically, the oral cavity is an amazing place where millions of microorganisms are present in warm oral fluid and wound healing takes place [3].

The human body heals wounds on a regular biological basis through a number of intricate processes. It is made up of precisely timed stages that include angiogenesis, wound contraction, cell proliferation, inflammation, remodeling of the matrix, and epithelialization. Although a variety of topical medications, including antibiotics, antihistamines, local anesthetics, and non-steroidal anti-inflammatory drugs, are used to relieve symptoms, their precise effectiveness has not been evaluated [4].

The field of medicine is growing more and more interested in nanotechnology. The enormous surface area-to-volume ratio of nanoparticles, ranging in size from 1 to 100nm, enhances their ability to interact with tissues and improves their penetration into wounds [5].

Metallic nanoparticles' low cost, high surface-to-volume ratio, stability, and safety have made them attractive for clinical use in the medical and wound healing fields. Nanoparticles that serve as delivery systems, aid in the healing process, and exhibit antimicrobial activity meet three primary requirements for their application in wound healing. These characteristics make nanoparticles (NPs) of metals including gold, silver, copper, and zinc excellent choices for integration into dressings and application in the wound bed [6].

Treatment with silver offers numerous benefits, such as lowering the risk of bacteria becoming resistant, being effective against pathogens resistant to multiple drugs, and having minimal systemic toxicity. Since ancient times, silver nitrate, a well-known antibacterial agent, has been utilized for a variety of medical applications, including the treatment of chronic wounds. Some drawbacks of silver nitrate include the possibility that it will be neutralized by anions (protein, chloride, and bicarbonate) in bodily fluids, that it may cause argyria (blue grey coloring) as a cosmetic aberration after prolonged use, and that it may impede healing due to fibroblast and epithelial cell toxicity [7,8].

Silver nanoparticles, or AgNPs, are compatible with wound dressing materials and antibiotic alternatives due to their demonstrated anticancer, antioxidant, antibacterial, and anti-scratch properties [9].

In epidermal and dermal tissues, zinc is found intracellularly and in the extracellular matrix (ECM) as protein complexes. Zinc plays a crucial role in mitosis, migration, and maturation, as well as acting as a stabilizer of cell membranes and a vital cofactor[10].

Through re-epithelialization, keratinocyte migration coupled with collagen fibers deposition, and tissue granulation, zinc oxide nanoparticles (ZnONPs) have demonstrated extraordinary regeneration abilities in vivo (rat model). They are also biocompatible and permeable to the dermis and epidermis [11].

Since no other research had made this comparison, the purpose of this study was to assess and compare the effectiveness of silver, zinc oxide, and their nano formulations in encouraging healing of traumatic ulcers that were generated in the buccal mucosa of albino rats.

MATERIALS AND METHODS

The present study was conducted after the approval of the Research Ethics Committee (REC) of the Faculty of Dentistry, Suez Canal University (249 /2019), following the ethical guidelines of animal care.

I. Materials (Table 1):

Table (1) *Includes the materials used in this study:*

1) Silver nitrate solution	5% Silver nitrate solution: • Silver nitrate: 5 g • Distilled water: 100 mL
2) Nano silver solution	• The content of metallic silver is 12 mg/ml • Polyvinylpyrrolidone (PVP) {Stabilizer}.
3) Zinc oxide solution	Zinc-containing fluids (regular crystalline insulin or aqueous zinc chloride solutions at 0.2 mg/100mL)
4) Nano zinc solution	The minimal inhibitory concentration of ZnO nanoparticles was determined 125 µg/ml

Instruments:

Soft tissue punch drill, mounted on low-speed surgical hand piece

II. Methods:

II.1-Study design:

Sixty adult male albino rats with body weight 160-180 grams at the beginning of the experiment (obtained from Faculty of Veterinary Medicine, Suez Canal University). The animals were kept each five in one cage at the animal house of faculty of dentistry, Suez Canal University. They were fed natural diet and given drinking water ad libitum. They were kept under proper conditions of temperature and ventilation.

II.2- Animal grouping:

The animals were divided randomly into six groups, 10 rats for each group

Group I Normal rats (-ve control)

Group II Have no treatment (+ve control)

Group III Twice daily topical application of silver nitrate solution

Group IV Twice daily topical application of nano silver solution

Group V Twice daily topical application of zinc oxide solution

Group VI Twice daily topical application of nano zinc solution

Each group is divided into two subgroups except group I: Subgroup 1 is sacrificed after 7 days and subgroup 2 is sacrificed after 14 days.

II.3- Ulcer Induction:

Prior to the creation of the ulcers, all animals anesthetized with a mixture of 13 mg/ kg of xylazine (2%) and 33 mg/kg of ketamine via intramuscular injection. After anesthesia, an ulcer was inflicted in the central region of the buccal mucosa on the right and left sides using a punch of 5 mm in diameter. The lesion was limited to the mucosa without muscular involvement [12].

II.4- Euthanization method:

Half of the animals in each group (5 animals) were scarified after euthanization by overdose of ether in the 7th day, while the other half (5 animals) were scarified after 14 days. After sacrificing, the buccal mucosa specimens were taken and prepared for light and TEM investigations. The dead experimental animals were disposed of by burning in the Animal Ashing Unit of Faculty of Medicine, Suez Canal University.

II.5- Evaluation:

Histological Evaluation: In our research, the stains have been used are:

1. Hematoxylin and eosin for histological examination.

2. Immunohistochemical localization of Proliferating cell nuclear antigen (PCNA) to detect the rate of cell proliferation.
3. Ultrastructural evaluation: transmission electron microscope

Statistical Analysis:

All data was calculated, tabulated, statistically analyzed and compared via suitable statistical tests. Statistical analysis was performed using the computer SPSS software (Statistical Package for Social Science).

RESULTS

Histological results using: Hematoxylin and Eosin stain.

Histological examination of the buccal mucosal sections of group I showed normal histological features of the surface epithelium, lamina propria and submucosal layers. Group 2.1 showed complete loss of epithelium, lamina propria showed degeneration in collagen fibers, inflammatory cells infiltrations and dilation in blood vessels with engorgement of blood inside them while group 2.2 showed a slight healing improvement. Group III showed better healing than group II **(Fig.1)**. Group 4.1 showed healing in epithelium. The epithelial ridges become irregular. The basal cells lacked their normal architecture, the epithelial-connective tissue interface showed areas with loss of basal cells adhesion. There are binucleated cells in the prickle cell layer were shown. Lamina propria with infiltration of inflammatory cells and dilation in blood vessels while in group 4.2 nearly a complete healing was happened. Group 5.1 showed slight healing in the epithelium with irregular epithelial ridges. Lamina propria showed degeneration of collagen fibers, infiltration of inflammatory cells and dilation in blood vessels with engorgement of blood inside them. Group V2 showed healing in the epithelium with little improvement. Group VI showed better healing than group V **(Fig.2)**.

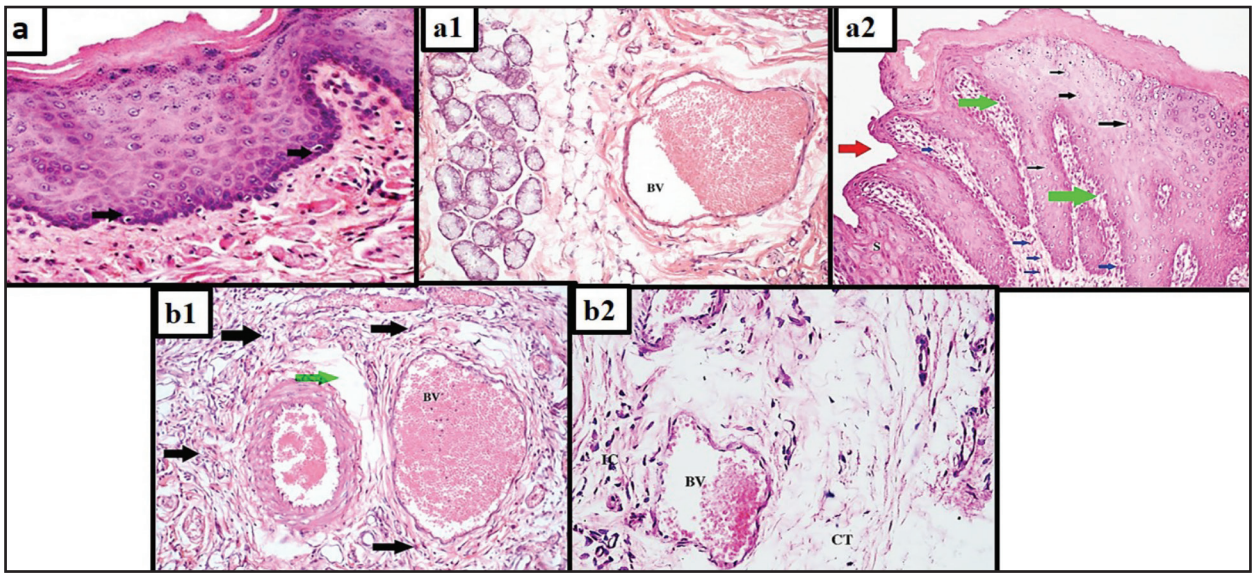

Fig. (1) A photomicrograph of group I normal buccal mucosa **(a)** showed epithelium with clear cells (arrows). **(a1)** a photomicrograph of 2.1 subgroup buccal mucosa a dilated blood vessel engorged with blood (BV). **(a2)** group 2.2 showed increase in epithelium thickness with long epithelial ridges with no keratin (red arrow), clear cells scattered along epithelial layers (black arrows), inflammatory cells scattered in lamina propria (blue arrows), damaged basement membrane (green arrows) and mild acanthosis with oedema and swelling of cells of prickle layer (S) while **(b1)** group 3.1 showed dilated blood vessel (BV), degeneration and loss of collagen fibers (green arrow) and infiltration of inflammatory cells (black arrows). **(b2)** group 3.2 showed inflammatory cells (IC), destruction in connective tissue (CT) and dilated blood vessels (BV). (H & E. orig. mag. 400)

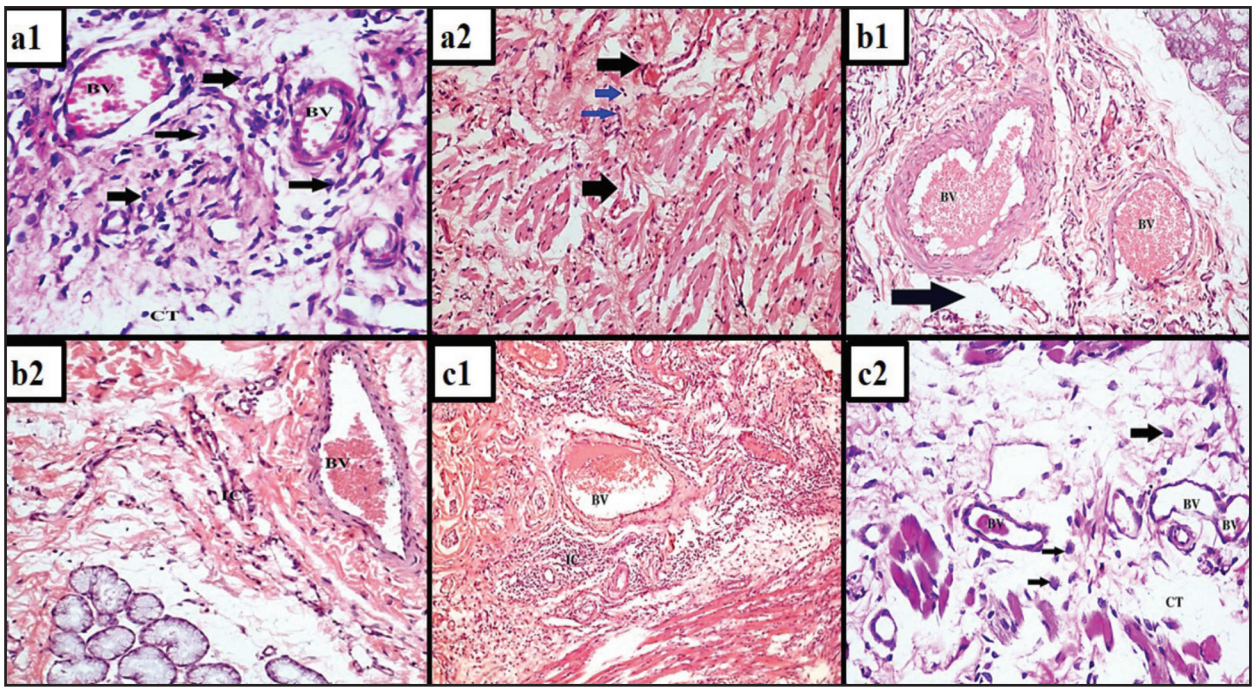

Fig. (2) A photomicrograph **(a1)** showed dilation in blood vessels (BV), inflammatory cells (black arrows) and a slight degeneration of collagen fibers (CT). **(a2)** showed inflammatory cells (blue arrows) and slightly dilated blood vessels (black arrows) while **(b1)** showed dilated blood vessels (BV), inflammatory cells (IC) and degeneration of collagen fibers (black arrow). **(b2)** and **(c1)** showed dilation in blood vessels (BV) and infiltration of inflammatory cells (IC). **(C2)** showed degeneration in collagen fibers, infiltration of inflammatory cells (black arrows) and dilated blood vessels (BV). (H & E. orig. mag. 200)

Immunohistochemical results:

Negative control: Section taken from the buccal mucosa of rats as a negative control were incubated with non-specific serum and color developed by DAB revealed negative staining reaction of all elements forming the buccal mucosa **(Fig. 3)**

Immunohistochemical localization of Proliferating Cell Nuclear Antigen (PCNA)

Group I showed the basal and suprabasal cells of the surface epithelium of the control animals were presented with moderate to strongly positive nuclear staining reactivity for PCNA. Group 2.1 loss in epithelium while group 2.2 revealed negative to weakly positive nuclear staining reactivity for PCNA in almost all layers forming the buccal mucosa**.** Group III showed better results than group II **(Fig. 4)**. While group IV showed better results than group III**.** Group 5.1 presented with weak to moderately positive nuclear staining reactivity for PCNA while group 5.2 showed improvement. Group VI represented better results than group V **(Fig. 5)**.

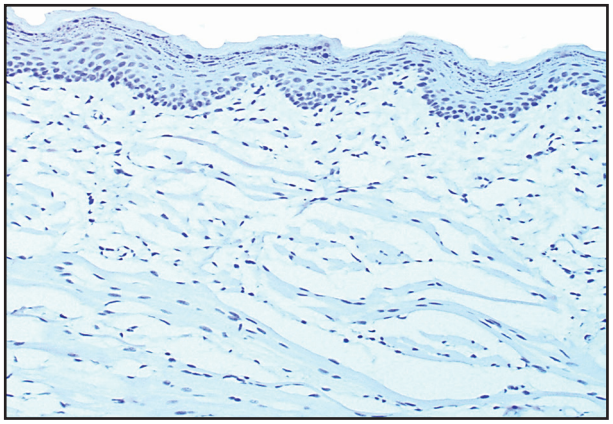

Fig. (3) A photomicrograph of negative control buccal mucosa of animal incubated with non-specific serum and color developed by DAB showed negative staining reaction of the buccal mucosa. (Orig.mag.100)

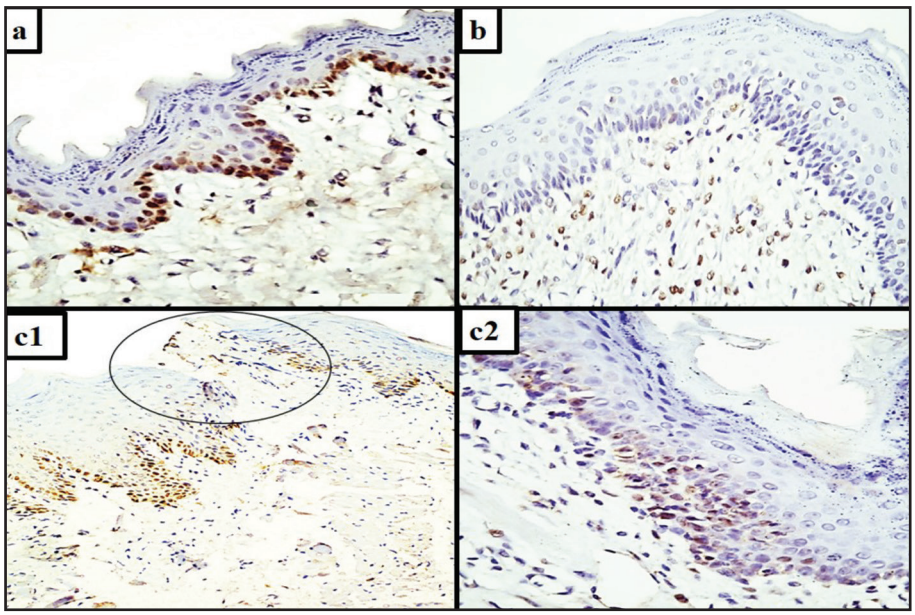

Fig. (4) A photomicrograph of group I buccal mucosa showing **(a)** moderate to strongly positive nuclear staining reactivity of the basal and suprabasal cells to PCNA. **(b)** group 2.2 showed negative to weekly positive nuclear staining reactivity of the basal and suprabasal cells to PCNA. **(c1)** group 3.1 showed the basal cells of the surface epithelium presented with weakly positive nuclear staining reactivity for PCNA (circle) **(c2)** group 3.2 showed weak to moderately positive nuclear staining reactivity of the basal and suprabasal cells to PCNA. (PCNA orig. mag. 200)

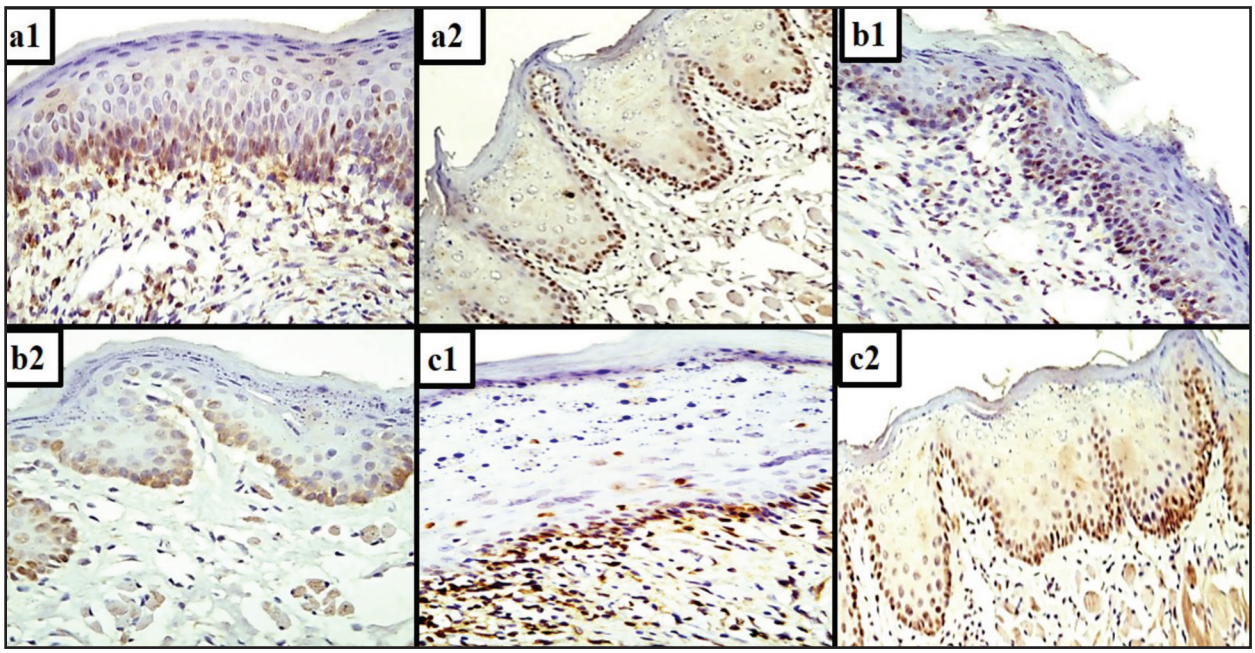

Fig. (5) A photomicrograph of group I buccal mucosa showed **(a1)** moderately positive nuclear staining reactivity of the basal and suprabasal cells to PCNA. **(a2)** group 4.2 showed moderate to strongly positive nuclear staining reactivity of the basal and suprabasal cells to PCNA. **(b1)** group 5.1 showed weak to moderately positive nuclear staining reactivity of the basal and suprabasal cells to PCNA. **(b2)** group 5.2 showed moderately positive nuclear staining reactivity of the basal and suprabasal cells to PCNA. **(c1)** group 6.1 showed moderately positive nuclear staining reactivity of the basal and suprabasal cells to PCNA. **(c2)** group 6.2 showed moderate to strongly positive nuclear staining reactivity of the basal and suprabasal cells to PCNA. (PCNA orig. mag. 400)

Statistical analysis of PCNA expression:

The results in Table (2): showed that there is clear significant difference between the studied groups for the PCNA using one way ANOVA (F= 132.582, P<0.0001). Pair wise comparison showed significant difference between all studied groups except GIV with GVI. The high mean values were recorded in GI (234.82±5.01) followed by GIV (202.86±4.43), GVI (192.38±3.49), GV (181.37±4.66), while GII and GIII were the lowest with (159.20±3.11) and (177.23 ±1.66) respectively (Fig. 6).

Table (2) *Comparison between different groups for PCNA*

Groups	Mean	SD	Min.	Max.	F test	P values
GI	234.82 [a]	5.01	231.70	240.60	132.582	<0.0001**
GII	159.20 [e]	3.11	156.16	162.37		
GIII	177.23 [d]	1.66	175.38	178.59		
GIV	202.86 [b]	4.43	198.54	207.40		
GV	181.37 [d]	4.66	177.20	186.40		
GVI	192.38 [c]	3.49	188.79	195.76		

Pair wise comparison used Bonferroni Post Hoc Tests for PCNA

	Mean difference	P value	95%CI	
			Lower	upper
GI *Vs* **GII**	75.61	<0.0001**	64.00	87.22
GI *Vs* **GIII**	57.58	<0.0001**	45.97	69.19
GI *Vs* **GIV**	31.96	<0.0001**	20.35	43.57
GI *Vs* **GV**	53.44	<0.0001**	41.83	65.05
GI *Vs* **GVI**	42.43	<0.0001**	30.82	54.04
GII *Vs* **GIII**	-18.03	<0.0001**	-29.64	-6.42
GII *Vs* **GIV**	-43.65	<0.0001**	-55.26	-32.04
GII *Vs* **GV**	-22.17	<0.0001**	-33.78	-10.56
GII *Vs* **GVI**	-33.18	<0.0001**	-44.79	-21.57
GIII *Vs* **GIV**	-25.62	<0.0001**	-37.23	-14.01
GIII *Vs* **GV**	-4.14	0.997	-15.75	7.47
GIII *Vs* **GVI**	-15.15	0.01**	-26.76	-3.54
GIV *Vs* **GV**	21.48	<0.0001**	9.87	33.09
GIV *Vs* **GVI**	10.47	0.10	-1.14	22.08
GV *Vs* **GVI**	-11.01	0.07**	-22.62	0.60

***, and different supper script letters means significant difference using one way ANOVA at P<0.05*
CI; **Confidence Interval at 95%**

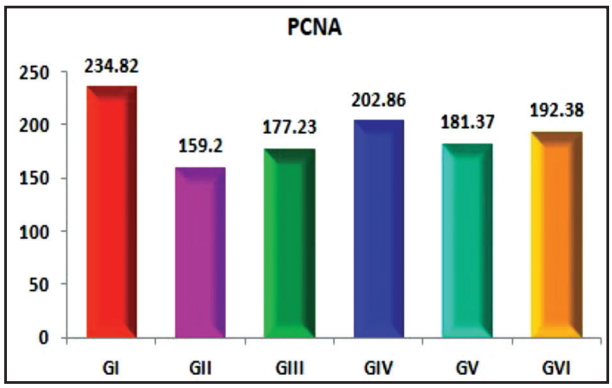

Fig. (6) A statistical chart illustrates the staining reactivity of buccal mucosa of different groups to PCNA.

Investigations under transmission electron microscope

Group I is a normal one. 2.1 sub-group showed loss of epithelial cells and in lamina propria; fibroblasts presented with signs of degeneration manifested as cytoplasmic vacuolization, ruptured mitochondria, marked decrease in rough endoplasmic reticulum and Golgi apparatus. Macrophages and mast cells with cytoplasmic vacuoles were also detected. A morphological alteration was also detected among submucosal layer presented in muscular cells with obvious vacuolization with shrinked fibroblast and dilated blood vessel while 2.2 sub- group slight healing in epithelial cells **(Fig.7)**. Group III showed better results than group II **(Fig.8),** but group IV showed a great improvement than group III **(Fig.9)**. 5.1 sub-group revealed loss of epithelial cells and dissociation of collagen fibers in lamina propria, damaged fibroblasts were seen. Macrophages and mast cells with cytoplasmic vacuoles were also detected. Submucosal layer presented in muscular cells with obvious vacuolization and dilated blood vessel while, 5.2 sub-group showed slight healing in epithelial cells **(Fig. 10).** Group VI showed better healing than group V **(Fig. 11).**

Fig. (7) An electron micrograph of group normal I buccal mucosa showed **(a)** fibroblast (F) having large nuclei that usually showed peripheral chromatin condensation. The fibroblasts are surrounded by collagen fibers. **(b1)** group 2.1 buccal mucosa showed multiple vacuolization between dark and light bands of myofibrils striations. **(b2)** group 2.2 showed basal cells resting on ill-defined basal lamina with widened intercellular spaces and prickle cells with decrease in the desmosomal junction in between. (Uranyl acetate & Lead citrate x5000)

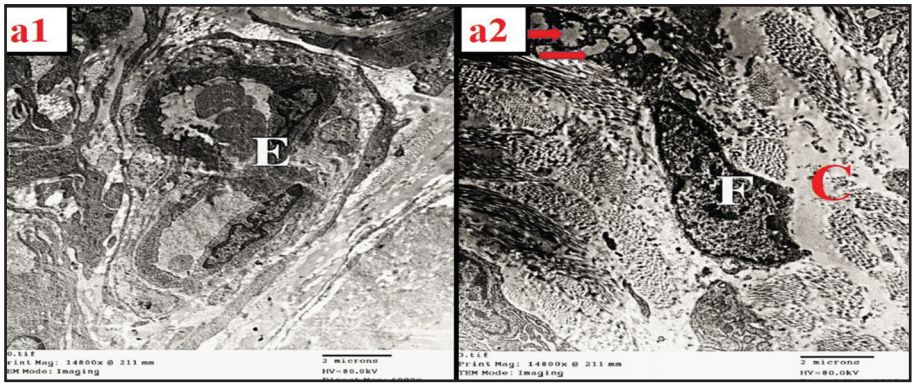

Fig. (8) An electron micrograph of group 3.1 buccal mucosa showed **(a1)** congested blood capillary with ill-defined endothelial cell lining (E). **(a2)** group 3.2 buccal mucosa showed degenerated fibroblast (F) with multiple vacuoles (arrows) and dissociation of collagen fibers (C). (Uranyl acetate & Lead citrate x6000)

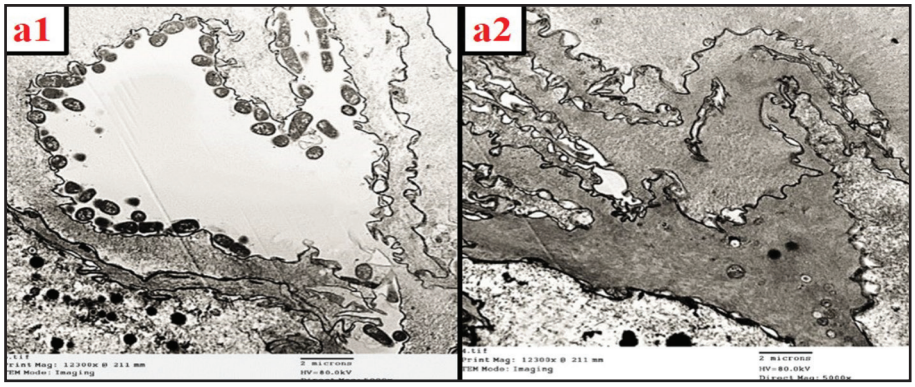

Fig. (9) An electron micrograph of buccal mucosa **(a1)** group 4.1 showed granular layer with variable sizes of keratohyalin granules and hornified cells showed marked increase in the inter cellular spaces. **(a2)** group 4.2 buccal mucosa showed granular cell with few keratohyalin granules and hornified layer with intercellular spaces between cells. (Uranyl acetate & Lead citrate x5000)

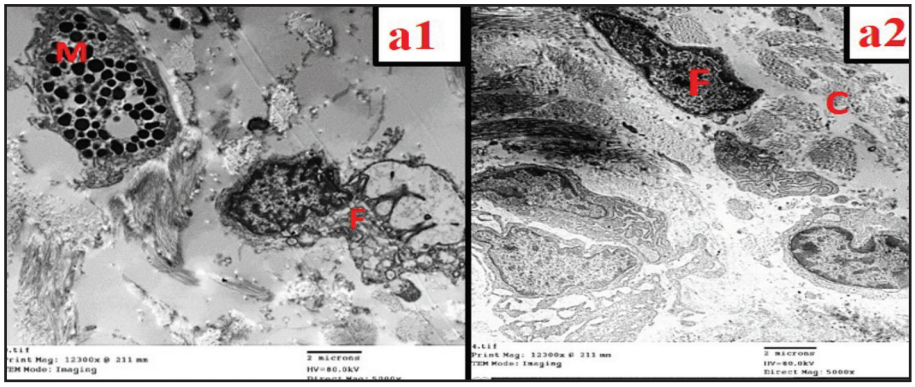

Fig. (10) An electron micrograph of buccal mucosa **(a1)** group 5.1 showed damaged fibroblast (F) and mast cell with multiple secretory granules (M). **(a2)** group 5.2 buccal mucosa showed fibroblast (F) associated with destructive collagen fibers (C). (Uranyl acetate & Lead citrate x5000)

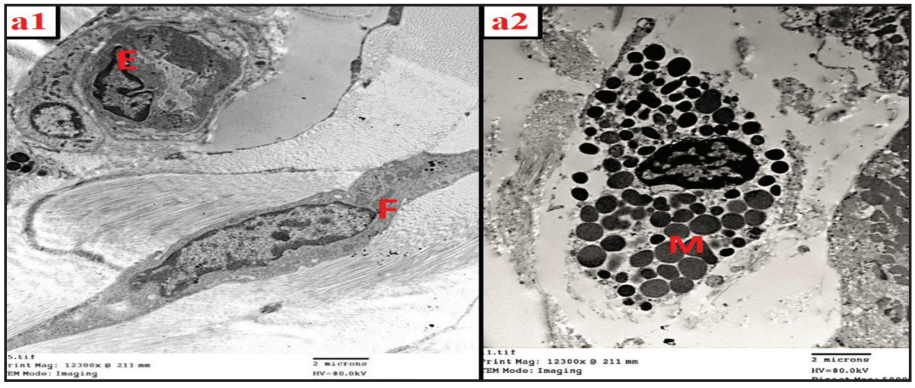

Fig. (11) An electron micrograph of buccal mucosa **(a1)** group 6.1 showed lamina propria with fibroblast (F), endothelial cell (E). **(a2)** group 6.2 buccal mucosa showed lamina propria with mast cell (M) was shown with multiple secretory granules. (Uranyl acetate & Lead citrate x5000)

DISCUSSION

This study was outlined to assess the helpful impact of AgNPs on verbal ulcers and wounds in comparison with silver nitrate and zinc nanoparticle in comparison with zinc oxide.

Nano materials have special physicochemical and natural properties as compared to their bigger partners. The properties of nano materials can enormously impact their intelligence with bio particles and cells, due to their impossible to miss estimate, shape, chemical composition, surface structure, charge, solvency and agglomeration [13].

The antimicrobial impact of a silver-based compound depends not only on the sort of compound (silver nitrate, silver-sulfadiazine, etc.) and discharged species (Ag+, Ag0, etc.), but also on the morphology (measure and shape) of the compound. As the estimate of the compound diminishes, the surface range increments. A huge surface zone leads to a high efflux rate of silver species, coming about in improved antimicrobial action. The antimicrobial action of the silver nanorods was higher than that of bulk silver for most microscopic organisms [14].

It was detailed that topicala zinc treatment decreases wound flotsam and jetsam and propels epithelialization in surgical wounds within the rodent. Zinc oxide and the lower zinc sulfate concentrations had a gentle anti-inflammatory impact. Zinc particles mirror the activity of development variables by improving intracellular mitogenic signaling pathways and zinc oxide is competent of up-regulating endogenous development variables, strikingly insulin-like development factor-I which may increase epithelialization [15].

Multiplying cell atomic antigen (PCNA) is a valuable immunohistochemical marker of multiplying cells since its expression and conveyance connect with cellular multiplying rate, so it is considered a profitable instrument for surveying cell multiplication all through wound mending. Immunohistochemical expression of PCNA increments amid Gl- stage, crests at the S-stage and decays amid the G2/M stage of the cell cycle [16].

Anti-PCNA antibodies give a suitable strategy for clarifying all stages of the cell cycle of multiplying cells. PCNA is being included in DNA repair, proposing that it may be communicated by cells that are not cycling. In spite of the fact that PCNA positive cells were observed in both basal and suprabasal layers, it is broadly acknowledged that multiplication of gingival epithelial cells is caused by mitosis in basal layer and less habitually in suprabasal layers [17].

In this examination, areas taken from the buccal mucosa of rats of group II with actuated traumatic ulcers cleared out without treatment for 14 days uncovered negative to feebly positive recoloring reactivity for PCNA in nearly all layers shaping the buccal mucosa.

Re-epithelialization is resurfacing a wound with modern epithelium to reestablish the total thickness of the epidermis. It includes two imperative forms, expansion and movement of keratinocytes. Cell expansion is a basic occasion amid re-epithelization. Multiplying cell atomic antigen (PCNA) has been considered as a well-known marker of cell multiplication. Multiplying keratinocytes guarantee a satisfactory supply of cells that emigrate into and cover the wound, at that point cellular relocation plays an awfully vital role in reestablishing epidermal progression[18].

Our examinations are exceptionally near to the comes about accomplished by **Rania *et al,*** [19] when 30 male mice and a cutaneous wound was made on the shaved backs of the creatures. It was watched after six days after harm few PCNA positive cells were recognized within the basal layer of the floor

of the wound and this can be due to moo rate of cell multiplication for recuperating because it cleared out without treatment.

Whereas, in group III with an initiated ulcer treated with silver nitrate for 14 days appeared to be more advancement within the wound mending than group II, which spoken to by pitifully positive atomic recoloring reactivity for PCNA of the basal and suprabasal cells of the surface epithelium. This may be due to the antimicrobial impact of silver which upgrade mending through invigorating expansion of the basal and suprabasal cells of the surface epithelium.

In group IV with an actuated ulcer treated with nano silver appeared with better enhancement in recuperating than group III, where the basal and suprabasal cells of the surface epithelium displayed with direct to solid positive atomic recoloring reactivity for PCNA.

Our findings were backed and in understood with those done by **Ghannam *et al***,[20] when having 40 grown-up female pale skinned adult rats with an initiated surgical wound were nano silver dressing was utilized after wound acceptance and other group gotten intradermal infusion of 1x 106 mesenchymal stem cells determined from bone marrow after wound acceptance. AgNPs dressing group appeared noteworthy increment within the cruel epidermal thickness, collagen optical thickness, and the positive PCNA immunoreactivity compared to the mesenchymal stem cells group.

Moreover, the thickness of PCNA expression and the number of positive cells within the labial mucosa expanded on the 14th day in nano silver group where the mending was completed. These discoveries coincided with **Liu *et al,***[21] who found noteworthy multiplication of the cells within the dorsal excisional wound of rodent begun from the 3rd day in AgNPs group, and illustrated the proliferative impact of AgNPs dressing which was supported after 10 days from injuring,

In group V with actuated ulcer treated with zinc oxide after 14 days appeared the basal and suprabasal cells of the surface epithelium after 14 days displayed with modestly positive atomic recoloring reactivity for PCNA, whereas in group VI which was treated with nano zinc showed the basal and suprabasal cells of the surface epithelium uncovered direct to emphatically positive atomic recoloring reactivity for PCNA and this finding may be due to the increment in the rate of cell expansion driving to extend the rate of wound healing which is made strides by utilizing nano zinc more than the group that utilized zinc oxide.

Zinc-oxide (ZnO) could be a well-known restorative for different skin conditions such as dermatitis or diaper hasty, and has been utilized in wound care for decades, indeed in spite of the fact that it is routinely debated. Moreover, antimicrobial properties have been detailed in vitro as well as in vivo ponders for ZnO impact [22].

Julian-Dario *et al*, [23] investigate the impact of zinc Subordinates on human fibroblasts and keratinocytes. This think about showed that a few Zn subordinates have a noteworthy proliferative effect on human fibroblasts and keratinocytes in an upsetting, nutrient-deficient environment.

CONCLUSIONS

From the results of the present investigation, the following conclusion could be reached:

- Infections may delay and deteriorate wound healing, so antimicrobial agents should be used.
- Nanomaterial has the best effect due to its size, improves penetration to wound.
- Nano silver improves healing faster than the silver nitrate, zinc oxide and nano zinc.

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