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## RESEARCH ARTICLE

### Effect of diode laser depigmentation on gingival tissue of dogs.

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#### Abstract

**Introduction:** Intraoral soft tissue esthetics has become a significant aspect of dentistry and clinicians are faced with achieving acceptable gingival esthetics as well as addressing biologic and functional problems.

**Aim of study:** was to evaluate the effect of diode laser irradiation on gingival depigmentation.

**Materials and methods:** 3 watts continuous mode diode laser was used to remove gingival hyperpigmentation of dogs. Specimens were divided according to the follow up periods of (1, 2, 4 and 8 weeks) where the experimental specimens were contralateral to the control ones. Specimens were evaluated pre- and post-treatment histologically using H&E, histochemically using Masson Fontana and ultrastructurally by transmission electron microscope.

**Results:** histological, histochemical and ultrastructural evaluation revealed a statistically significant decrease in melanin content in the follow up periods compared to the baseline. Meanwhile, the recurrence of melanin was observed more in groups of 4 and 8 weeks after laser irradiation yet did not reach the baseline.

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#### Introduction:-

A smile expresses a feeling of joy, success, sensuality, affection and can reflect self-confidence and kindness. The harmony of a smile is not only determined by the shape, position and color of the teeth, but also by the gingival tissues. Gingival health and appearance became of a great concern since they are essential components of an attractive smile. Melanin pigmentation of the gingiva occurs in all ethnicities. Therefore, an increasing number of persons are found seeking treatment for this condition (Lagdive et al., 2009).

Melanin, a brown pigment, is the most common natural pigment contributing to endogenous pigmentation of gingiva and is produced by melanocytes in the basal and suprabasal cell layer of the gingival epithelium. The gingiva is the most frequently pigmented tissue of the oral cavity (Rosa et al., 2007).

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Gingival hyperpigmentation is increased pigmentation beyond the normally expected degree of the oral mucosa. Several physiologic and pathologic factors can cause hyperpigmentation (Dummett and Gupta, 1964). However, the most common cause is physiologic or ethnic hyperpigmentation which is genetically determined and is clinically manifested as variable amounts of diffuse or multifocal melanin pigmentation in different ethnic groups (Hegde et al., 2013).

Numerous intrinsic and extrinsic factors, including body distribution, ethnicity/gender differences, variable hormone-responsiveness, genetic defects, hair cycle- dependent changes, age, UV-R, climate/season, toxin, pollutants, chemical exposure and infestations, are responsible for a whole range of responses in melanosome structure and distribution (Costin and Hearing, 2007).

Depigmentation is the treatment of removal of melanin hyperpigmentation. Various methods have been used for this procedure including gingivectomy, electrosurgery, cryosurgery, chemically using phenol and alcohol and abrasion with diamond bur (Atsawasuwan et al., 2000).

Laser has been used in dentistry since the beginning of the 1980s. Recent research has centered on using diode laser for oral surgery of the tongue and gingiva and to remove infected epithelium in chronic periodontitis. Diode laser has offered some advantages over the others, such as easy gingival reshaping, reduced need of local anesthesia and dry operative field due to excellent hemostasis associated with significant decrease in pain and inflammatory postoperative score. Minimal scarring and satisfactory clinical outcome on the long term have also been achieved. Moreover, there is evidence in the recent literature of successful depigmentation using diode laser (Hedge et al., 2013).

### **Aim of the study:-**

The aim of the present study was to evaluate the effect of diode laser irradiation on gingival depigmentation, histologically, histochemically and ultrastructurally.

### **Materials and methods:-**

Twenty-eight adult male dogs were evenly divided into 4 main groups; groups (I, II, III and IV) correspondent to post-operative follow-up periods of (1, 2, 4 and 8 weeks) respectively. Specimens of each group were divided into 2 groups; control (A) and experimental (B), where specimens of group B (right side) were selected from contralateral area to group A (left side).

The melanin pigmented gingiva at the region of the canines was ablated by diode laser irradiation with a flexible, fiber-optic delivery system (320 microns diameter) in a contact technique, air cooling handpiece with 3 W continuous mode under standard protective measures. The procedure was performed on pigmented areas until blister formation occurred. Laser ablation started from the mucogingival junction towards the free gingival margin including the inter-dental papilla. The laser beam was guided in brush stroke movements until the entire pigmented spot had disappeared without causing any bleeding which was beneficial for clear visualization (Agarwal et al., 2014). This procedure was repeated until the desired depth of tissue removal was achieved. Remnants of the ablated tissue were removed using sterile gauze damped with saline so as to allow clear visibility of the field. Gingival specimens were examined by light microscope using Hematoxyline and Eosin stain and Masson Fontana special stain and by electron microscope.

### **Results:-**

Histologically, before laser irradiation, the gingiva of dogs showed extensive physiologic pigmentation of a brown color. The specimens showed that most of the melanin is located in the basal and suprabasal layers. After 1, 2, 4 and 8 weeks of the laser irradiation, the specimens were free of the the brown coloration. Complete re-epithelialization was seen after 1 week (subgroup IB) of the application. Some cytoplasmic vacuoles were seen in the granular layer after 2 weeks (subgroup IIB) of the irradiation. Also the granular cells were of faintly stained and less dense cytoplasm. A remarkable thickening in the keratin layer of subgroup IIIB (4 weeks after irradiation) was observed. Normal epithelial cells were seen after 8 weeks (subgroup IVB) of laser irradiation. Lamina propria was normal with some inflammatory cell infiltration (Fig. 1).

Using Masson Fontana, the specimens before laser irradiation showed positive reaction throughout the entire epithelial layers. After 1 and 2 weeks (subgroups IB and IIB), there was a remarkable decrease in the melanin concentration. But at 4 and 8 weeks (subgroups IIIB and IVB), there was a little increase in the melanin concentration compared to subgroups IB and IIB but yet didn't reach the baseline (Fig. 2).

Our histological and histochemical results were confirmed with the histomorphometry where subgroups IB and IIB were highly statistically significant with our histological results as the melanin concentration in the basal layer was significantly decreased while subgroups IIIB and IVB statistically significant where melanin concentration in basal layer was decreased but yet more than subgroups IB and IIB (Fig. 3).

Electron microscopic examination of the gingiva of the control subgroup showed the basal cells of their two types; the serrated basal cells which are a single layer of cuboidal or high cuboidal cells with protoplasmic processes projecting from their basal surface towards the connective tissue. They contain some melanin granules in their cytoplasm around the nucleus. The other population of the basal cells which are the non-serrated could also be seen. They give rise to a population of cells amplified for cell division. Considerable amount of melanosomes of variable sizes and electron density were seen inside their cytoplasm (Fig. 4a). Melanocytes with many melanin granules were scattered between lower prickly layer. Some melanocytes had a large rounded nucleus and others with elongated or ellipsoid nucleus containing a nucleolus. The nuclear membrane was smooth with regular outline which may however exhibit a single small cleft. The cytoplasm was relatively clear or electron lucent surrounding the nucleus. Numerous electron dense melanin granules (melanosomes) of different size and shape correspondent to their maturative stage were seen throughout the cytoplasm (Fig. 4b). Relatively less melanin granules were seen between upper prickly cell layer. The granular cell layer consisted of flatter, wider and larger cells than those of the prickly layer. Their nuclei showed signs of degeneration and pyknosis. A cytoplasmic vacuole surrounded the nucleus and some melanosomes were detected in it (Fig. 4c). The keratin layer presented some melanosomes and pyknotic nuclei. Gingival specimens of subgroup IB showed that almost all basal cells were devoid of melanin granules meanwhile few of them presented some. The nuclear outline tended to be more irregular (Fig. 4d). Most of the prickly cells were devoid of melanin granules and normal desmosomal attachments were seen between them (Fig. 4e). The specimens of subgroup IIB showed the basal cells with variable sized electron dense melanin granules (Fig. 4f). Cells of the prickly layer were almost devoid of melanin granules similar to those of subgroup IB. However, some of them presented cytoplasmic vacuoles (Fig. 4g). Subgroup IIIB showed prominent melanin granules in some of their prickly cells (Fig. 4h). A remarkable thickening of the keratin layer was noticed in this subgroup (Fig. 4i). Abundant melanin granules were seen between adjacent keratinocytes of subgroup IVB (Fig. 4j).

### Discussion:-

Laser was used in the current study because its use is currently the most common technique in gingival depigmentation. Laser depigmentation is a superior treatment modality for a number of conditions when compared to traditional techniques in terms of precision cutting, hemostasis and lowering the risk of other complications commonly seen such as pain, edema and infection (Romeo et al., 2014).

Gingival depigmentation performed in this study was carried out by a 970 nm wave length diode laser and a 3 W irradiation power settings as it has near optimal absorption for melanin and hemoglobin (Lagdive et al., 2010). In the present study there was minimal side effects such as the very slight coagulation on the treated surface as laser has been recognized as one of the most effective, comfortable and reliable techniques for the gingival depigmentation (Shenawy et al., 2015).

In the present study, dogs have been chosen for the gingival depigmentation procedures rather than any other experimental animal due to the marked presence of melanin pigments in their gingiva. Dummett et al., 1964 declared the presence of melanin pigments in the stratum spinosum and the basal layers of the dog's attached gingiva through a heavy patchy distribution.

It is known that the nature of the pigmentation and the depth or the degree of its penetration into the tissue are decisive factors in the outcome of the laser treatment (Sharon et al., 2000). In the current study, the utilized laser wavelength was 970nm. Such wavelength is known to induce relatively deep tissue penetration (Atsawasuwan et al., 2000).

In the present study, the light microscopic examination of subgroup IB (1 week after laser irradiation) revealed the completion of re-epithelialization and normal maturation of the epithelium manifested by the presence of all layers

till the stratum corneum. It also showed the absence of the brown colored melanin pigmentation in the basal cell layer compared to the control subgroup (IA). The results of subgroup IIB (2 weeks after laser irradiation), showed that the basal and prickles cells presented almost normal histological features. The granular cells were of faintly stained cytoplasm. Also some cells showed a relatively large vacuolar spaces. A remarkable thickening in the keratin layer of subgroup IIIB (4 weeks after irradiation) was observed. Normal epithelial cells were seen in subgroup IVB.

These findings were supported by Yousuf et al., 2000 who evaluated the possibility of the removal of canine gingival melanin pigmentation in dogs with the semiconductor diode laser using a 3 W in a continuous mode. They also checked for the brown pigmentation after 3 weeks of the laser irradiation and they did not find any repigmentation when examined by H&E stain.

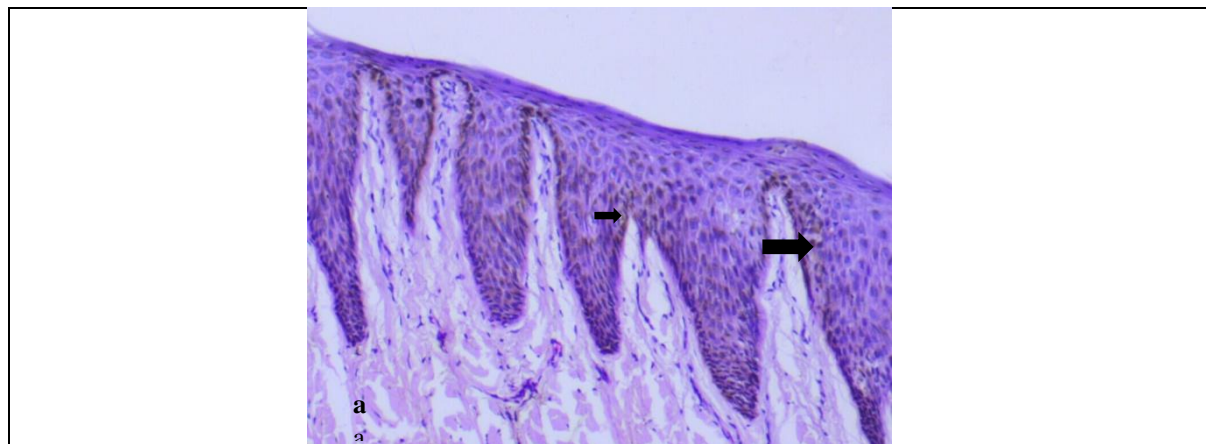
However, in our study using Masson Fontana stain reflected that there was rare amount of melanin in basal and suprabasal layers of the epithelium of the dog gingiva after 1 week (subgroup IB) of the diode laser application. Also after 2 weeks (subgroup IIB) of laser application, Masson Fontana stain revealed very small spots of melanin. And according to the statistical analysis of the histomorphometric results, these two groups showed a highly statistically significant decrease in melanin concentration in the basal and suprabasal layers of the gingival epithelium in comparison to the baseline treatment of each subgroup.

Masson Fontana results of subgroups IIIB and IVB (4 and 8 weeks postoperative respectively) revealed a slight to moderate appearance of melanin throughout the whole thickness of the epithelium. The statistical analysis of the histomorphometry revealed a statistically significant decrease in amount of melanin in relation to the baseline of each subgroup.

In the present study, the ultrastructural changes observed in the epithelial cells after laser irradiation were consistent with the histological and histochemical examinations.

Despite the different technique of erbium:YAG laser that was utilized by Attwa et al., 2015 in treatment of melasma of skin (acquired hyperpigmentation involving cheeks, nose, upper lip and forehead) in humans. Skin of the face was treated with 2 consecutive passes. This treatment was repeated monthly for a total of 6 months. Evaluation was done at baseline, before each session and at 1, 3 and 6 months after final treatment. Similarly, their electron microscopic results seemed similar to those of the current study. Their results showed that there was a decrease in the aggregation of melanin granules within keratinocytes and melanocytes of the epidermis.

In accordance to the results of this study, it may be concluded that the application of diode laser appears to be a safe and effective alternative method for gingival depigmentation procedure. However, the esthetic outcome may not last for long time. Therefore, further studies are recommended with different laser treatment parameters for a better understanding of the potential benefits of laser depigmentation.





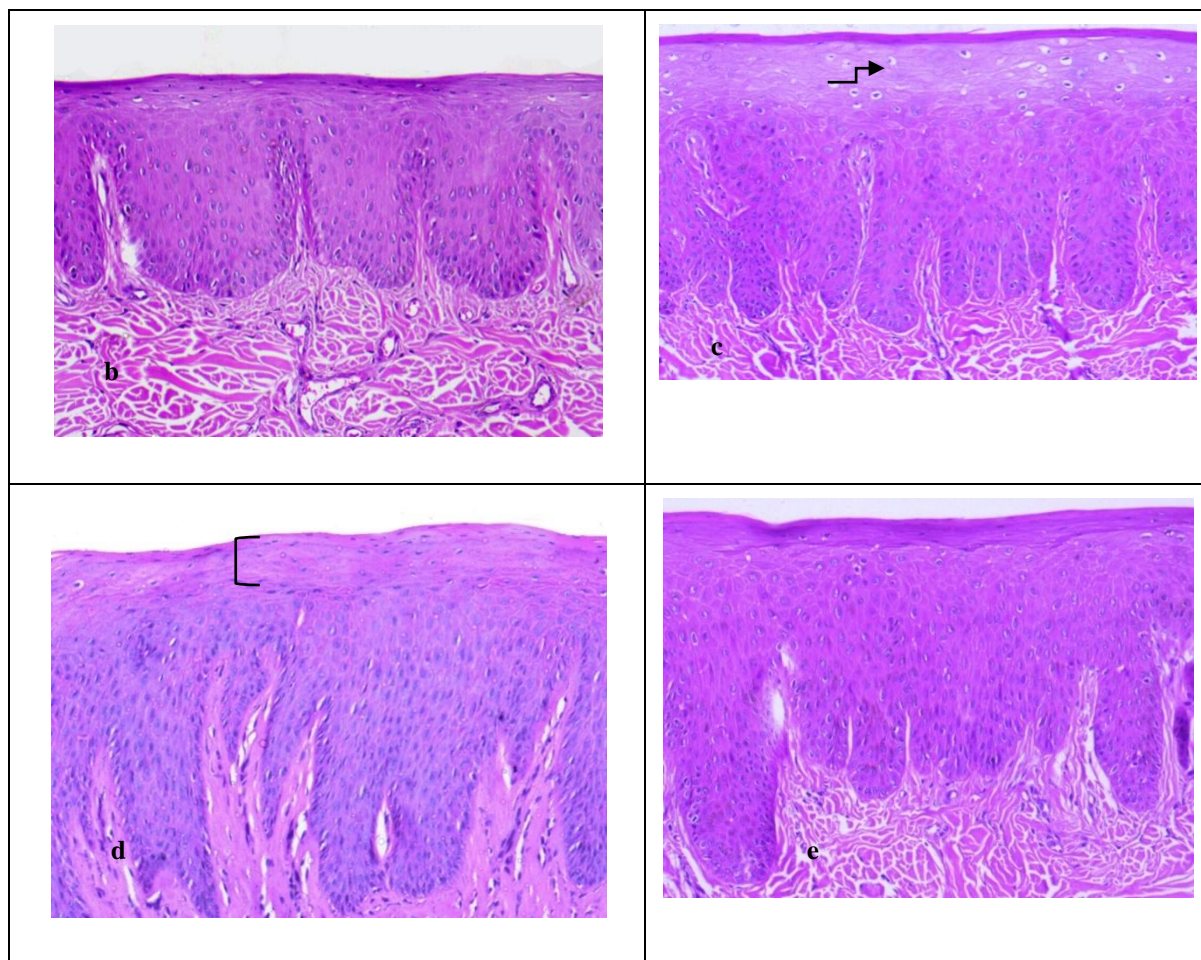
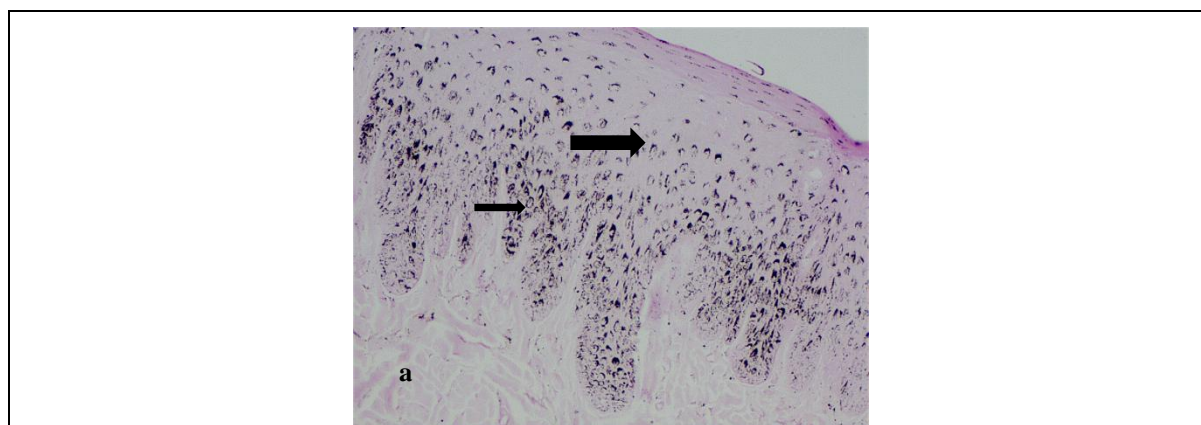


Fig. 1: Photomicrograph of the gingiva of the control subgroups showing: (a) the brown pigmentation in basal layer. (b) 1 week after irradiation no brown pigments. (c) 2 weeks after irradiation showing some vacuoles in granular layer. (d) 4 weeks after irradiation showing remarkable thickening of keratin layer. (e) 8 weeks after irradiation showing normal epithelial cells (H&E org. mag. X 200).



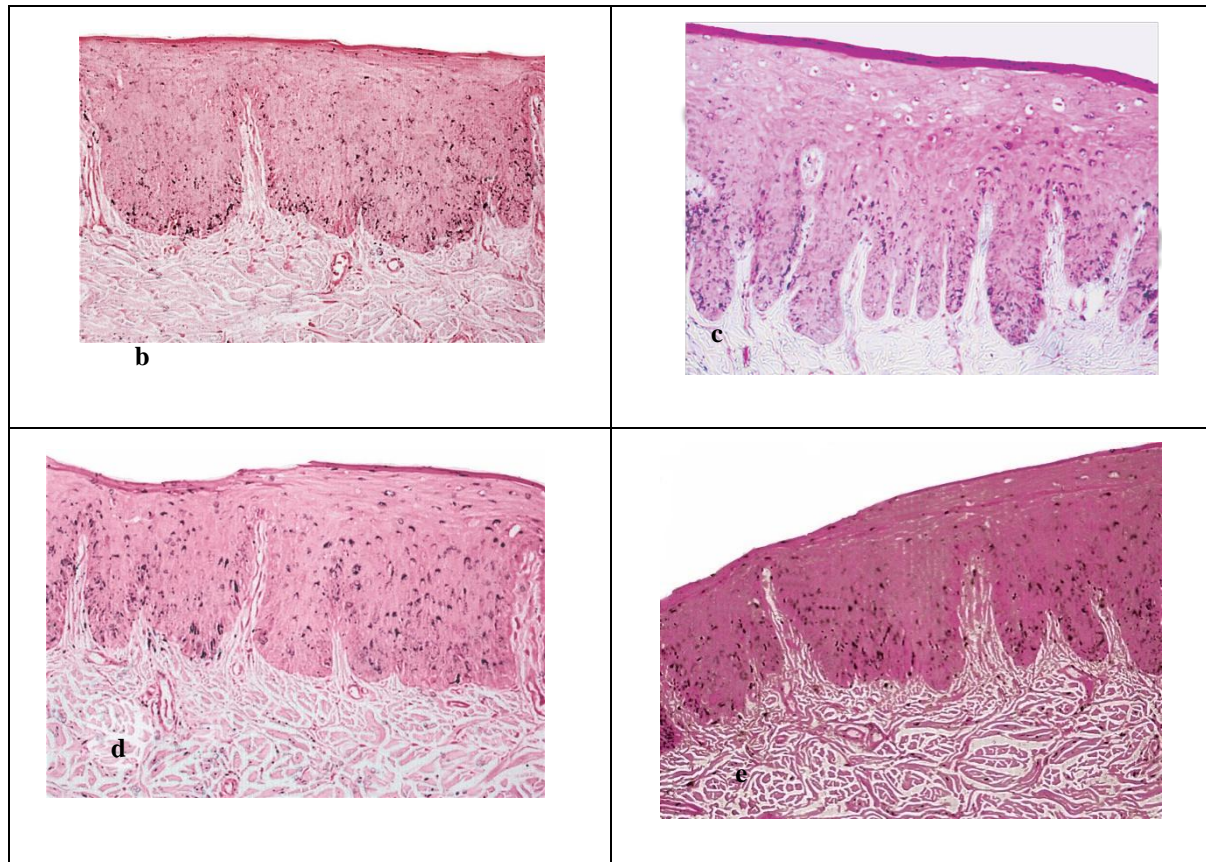


Fig. 2: Photomicrograph of the gingiva of the control subgroups showing: (a) melanin pigmentation in full thickness of epithelium (b) 1 week after irradiation scarce amount of melanin. (c) 2 weeks after irradiation showing few amount of melanin. (d) 4 weeks after irradiation showing scattered areas of melanin (e) 8 weeks after irradiation showing scattered areas of melanin (Masson Fontana org. mag. X 200).

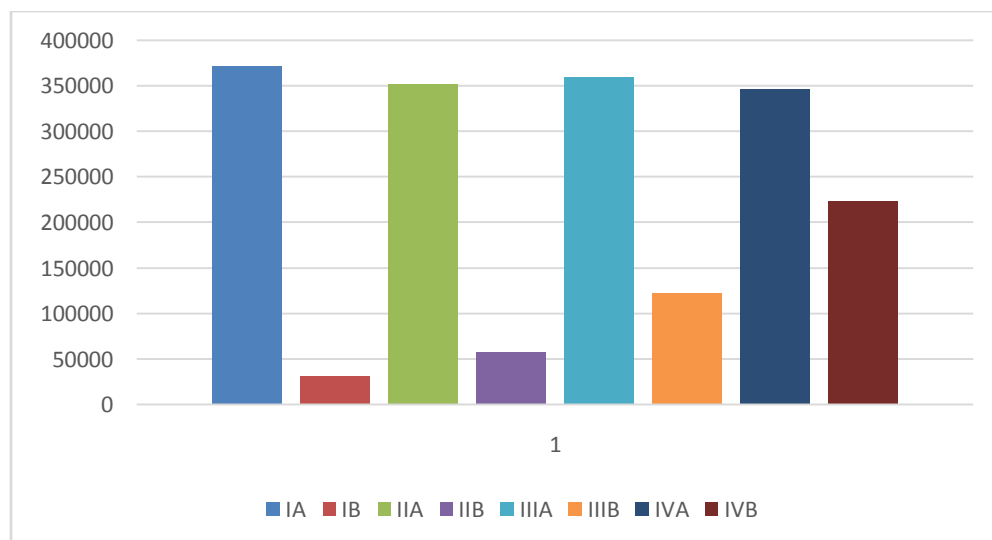
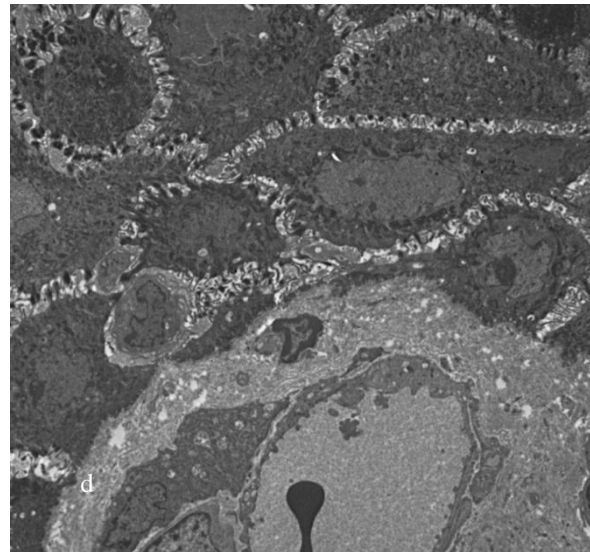
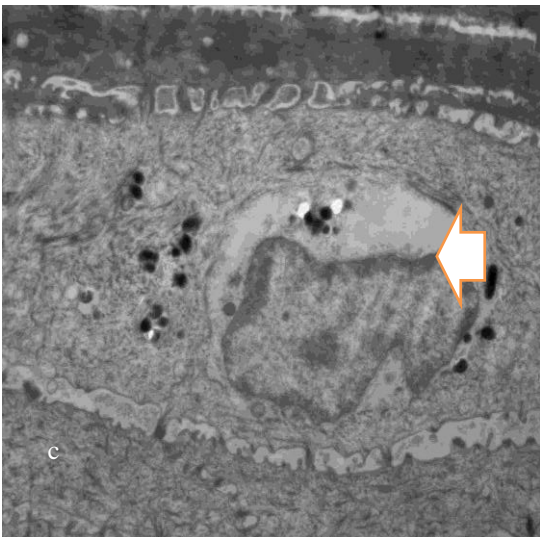
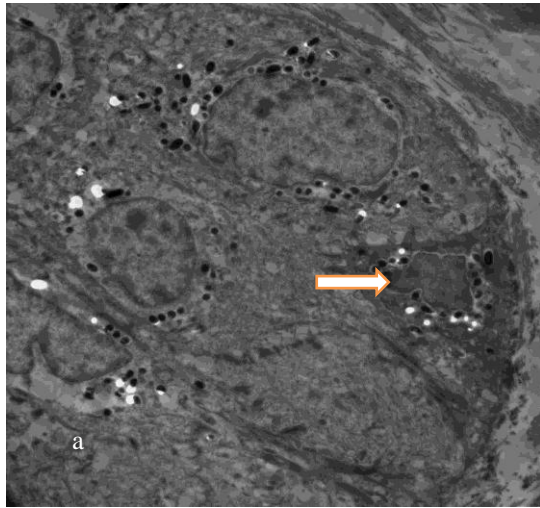
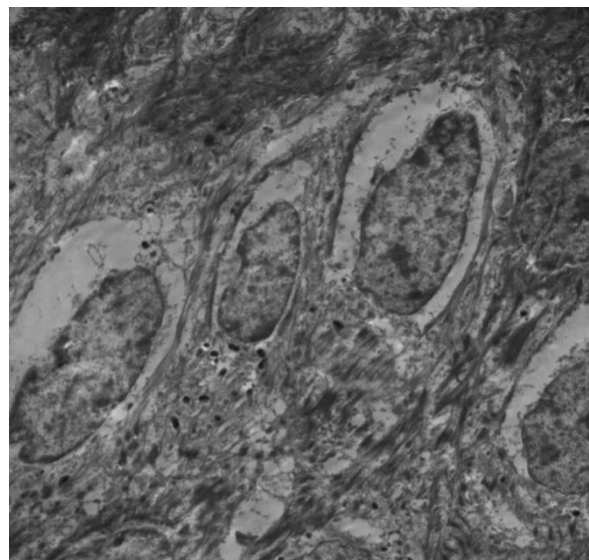
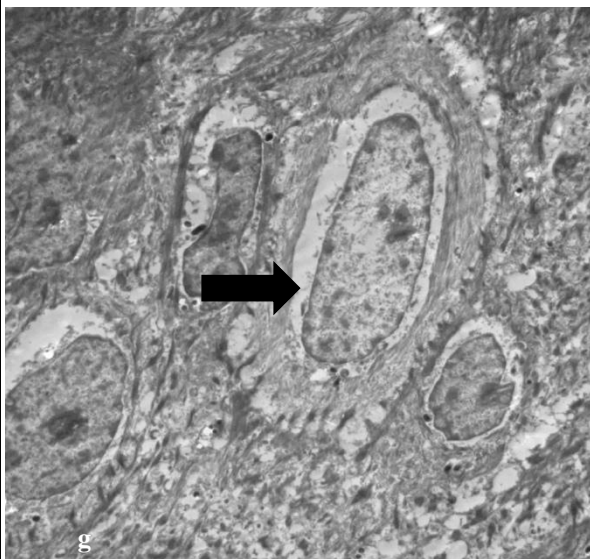
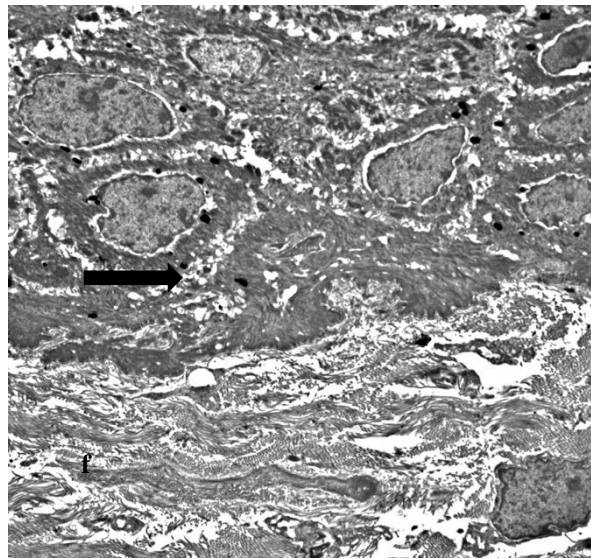
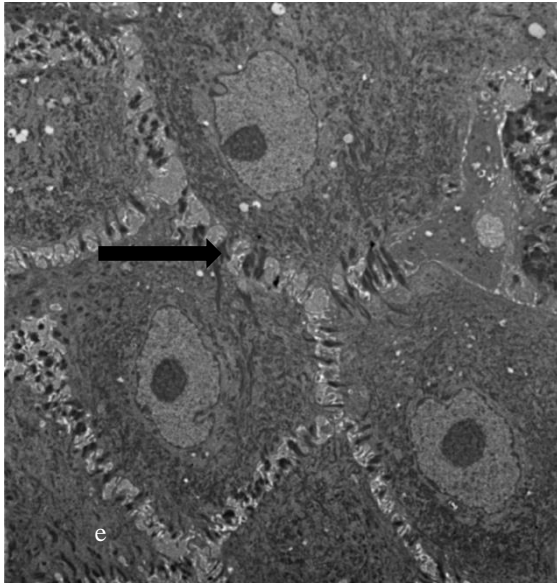


Fig.3: Bar chart showing melanin mean area percent expression in gingiva between all the subgroups.









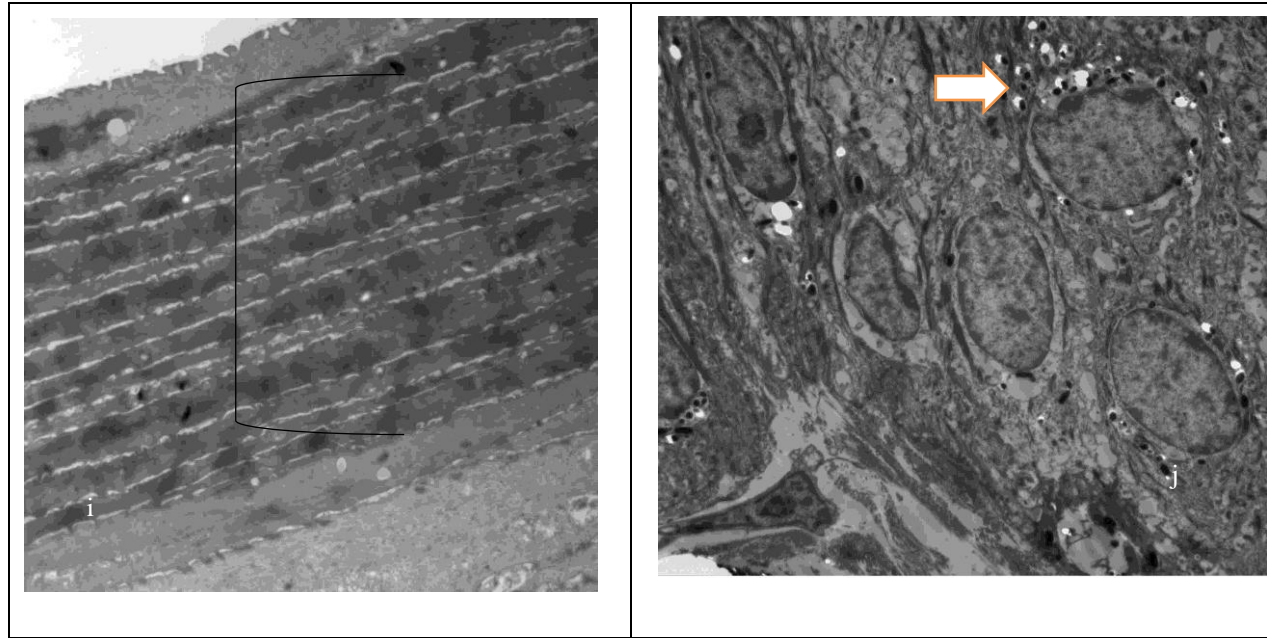


Fig. 4: An electron photomicrograph of the gingiva of the control subgroups showing: (a) the serrated type of basal cells with melanin pigmentation (b) showing the the melanocytes with variable sized melanosomes in control group. (c) granular cell with pyknotic nucleus and some melanosomes (d) basal cells of group IB devoid of melanosomes (e) prickle cells with desmosomal attachments in group IB (f) basal cells showing some melanosomes in group IIB. (g) prickle cells of group IIB with cytoplasmic vacuoles. (h)prickle cells of group IIIB with scanty amount of melanosomes and cytoplasmic vacuoles. (i) thickening of keratin layer in group IIIB. (j) basal cells of group IVB with few melanosomes.

### Conclusions:-

- Using diode laser of 970nm wave length and 3 watts power caused significant decrease in gingival pigmentation in all follow-up periods.
- Gingival pigmentation began to reappear on the 8th week after laser irradiation yet did not reach the baseline.

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