Initial Tissue Response after Partial Glossectomy by 3 Watt, 6 Watt CO_2 Laser and Scalpel

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Abstract

Objective: To compare advantages and disadvantages of CO_2 laser and scalpel in wound healing after an oral soft tissue surgical procedure.

Place and Duration of Study: Study was carried out at animal laboratory of Post Graduate Medical Institute, Lahore from January 2005 to March 2005.

Subjects and Methods: Study was conducted on 30 Sprague – dawley Albino rats, which were divided into 3 equal groups of 10. Partial glossectomy was done with 3 watts CO_2 laser in first group (A), with 6 watts CO_2 laser in second group (B) and with scalpel in third group (C). Wound healing was observed and compared grossly and histologically, 24 hours after surgery.

Results: Significant differences were seen in gross wound appearance in A and B groups as compared to C group. Histological formation of zones, thickness of blood and carbonization were highly significant in A and B groups as compared to group C.

Conclusion: Six watts CO_2 laser wound was much better as compared to scalpel in terms of bleeding duration of surgery, wound appearance, and initial inflammatory response.

Key words: CO_2 laser, Scalpel, Wound healing, Partial glossectomy, inflammation, zones of laser wound.

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Introduction

In recent years, CO_2 laser has become an alternative mode of treatment for the excision of various soft tissue lesions in oral cavity. In oral surgery a number of clinical advantages are reported by CO_2 laser as compared to conventional surgery by scalpel.[1,2]

Large areas in oral cavity can be excised or vaporized with CO_2 laser. It has coagulation and welding effect on blood vessels upto 0.5 mm of caliber which produces bloodless surgical field with good visualization.[3] these properties lead to better cosmetic and functional results[4] especially when delicate dissection of highly vascular areas such as tongue is involved. There is minimal hazard of malignant cell seeding even in deep seated oral cavity tumors due to sealing of lymphatics and blood vessels. These clinical advantages of CO_2 laser surgery leads to less post-operative complications and rapid recovery.[5]

Many studies comparing different wattages of CO_2 laser with those of scalpel on healing, has shown conflicting data.[6] A number of studies have reported that wound produced by CO_2 laser manifest more prominent initial inflammatory reaction than those produced by scalpel[7-9] while other studies reported delayed and less inflammatory reaction.[10,11]

Keeping in mind the existing controversies about laser the present study was planned. The aim was to compare and see the advantages and disadvantages of using CO_2 laser and scalpel in wound healing 24 hours after surgery.

Subjects and Methods

This study was conducted at Animal Laboratory of Postgraduate Medical Institute, Lahore. Thirty Albino

Sprague – dawley rats of about nine weeks age, weighing 250 to 350 grams, were included in the study.[12,13] Animals were divided into 3 equal groups (A, B, C) of 10 each. In Group A Partial glossectomy was performed with 3 watts CO_2 laser, Group B with 6 watts CO_2 laser and Group C with the scalpel. Animals were kept

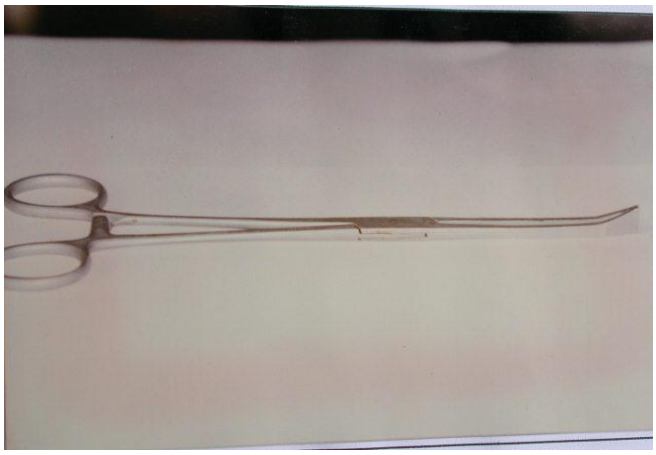

Fig. 1:

at room temperature (22 ± 2°C), under natural conditions of humidity and light.[14] The rats were allowed one week of acclimatization to the laboratory conditions before being used in the experiment.[15] Partial glossectomy was planned for each group with a specially designed forceps of non reflected surface having two blades. Each blade was 10 mm × 5 mm at right angle (Fig. 1).

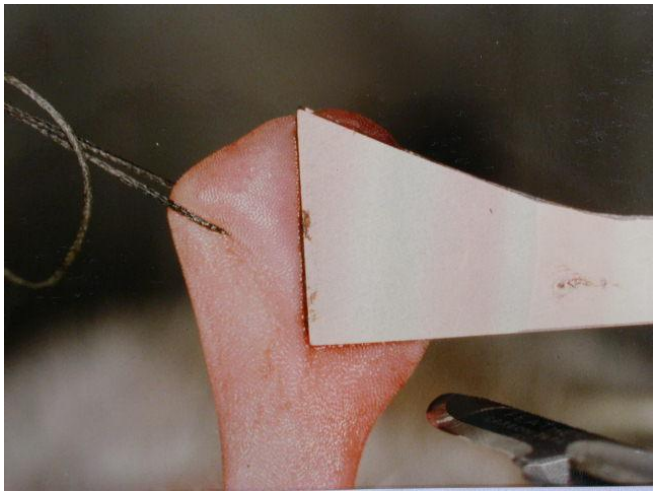

Fig. 2:

Standardized surgical conditions were maintained before undergoing surgical procedure. General anesthesia was induced with pentobarbital 4 mg per Kg body weight intraperitoneally.[15] Two rubber elastic bands were used to open the jaws by placing them in the upper and lower incisors.

The steel nails were placed on the surgical board to hold the bands. Extra oral skin was prepared with pyodine scrub and oral cavity was washed with sterile water. Saliva was sucked prior to surgery and tongue was retracted by placing 3/0 silk suture on animal's left half of tongue.

The tongue was kept gently stretched during surgery for accurate cutting. Right half of the tongue was held dorsoventrally by standard forceps so that 10 mm length was parallel to mid line of tongue while 5 mm side was perpendicular to mid line towards lateral margin (Fig. 2).

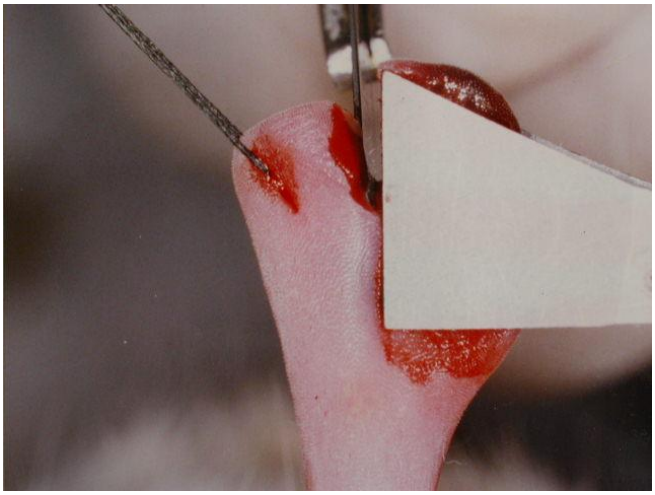

Fig. 3:

After application of standard forceps, partial glossectomy of group C was performed by scalpel blade No. 15. Right half of the tongue was excised all along the margin of standard forceps blades. The direction of excision was anteroposteriorly first, along 10 mm length and then from lateral margin to the mid line (Fig. 3).

No pressure or gauze was applied to stop bleeding. The wound was left exposed in the mouth and no sutures were inserted in order to produce standard test situation.[16,17,41]

Generally 3 watt is considered as minimal and 6 watt as maximum output power of waveguide laser for oral clinical application in practice.[18,19] Group A and B

were performed with 3 Watts and 6 Watts CO_2 laser respectively. LASERSAT – CO_2 Waveguide Laser by Satelec (France) was used which generates a wavelength of 10.6 µm in infrared spectrum.

The LASERSAT CO_2 laser had specifications of focal length 38 mm with beam diameter of 300 µm. The laser rays reached the tissue surface in a range of angles nearer to 90 degree; to spare the surrounding tissue.[20]

Right half of the tongue was excised along the standard forceps and left without suturing as described for scalpel. Continuous suction was done to prevent inhalation of saliva and the smoke produced during laser surgery (Fig. 4).

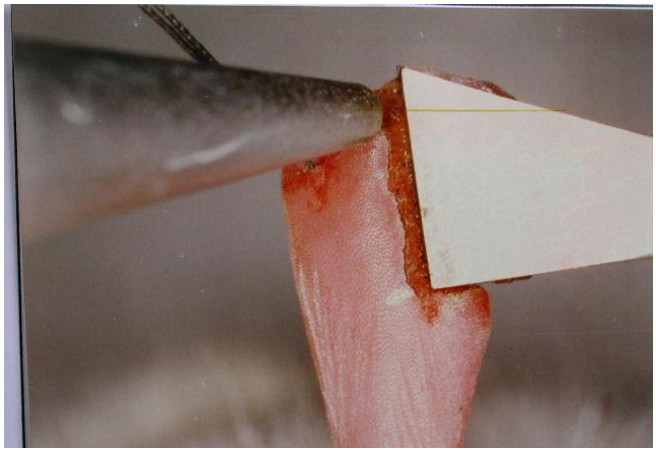

Fig. 4:

Gross Morphological Observations

The gross morphological observations were recorded for the assessment of wound healing after partial glossectomy.[16] The immediate observations like duration of surgery and bleeding were recorded. To evaluate the initial wound healing changes during first 24 hours after surgery, the wound base, wound edge appearance, contraction and colour of wound were recorded. For wound colour comparison, the left half of tongue mucosa was taken as control.[8,16]

Duration of surgery was noted by stop watch. Duration of bleeding was also recorded by another stop watch from the start of bleeding till it stopped. Colour, shape and surface of the wound base were grossly observed by a magnifying glass (3X). The wound was measured in millimeters by a caliper as per following measurements (Fig. 5).

The measurements were compared post-surgically for all 3 groups. Animals were killed by an over dose of general anesthesia after the measurements were recorded, and immediately fixed in 10% formalin. To maintain the tissues in correct relationship the tongue was preserved with mandible.[16] After at least 48 hours, the tongues were dissected out.

X = Length from point A_1 to A_2
Y = Length from point A_1 to B
Z = Length from point A_1 to C

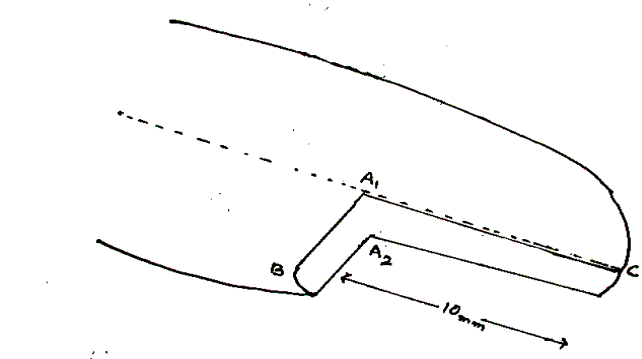

Fig. 5:

Histological Observations

Tissue processing was done in automatic processor.[21] Staining with haematoxylin and eosin was done as it define necrotic tissue most clearly and suitable to get all sort of measurements.[9,22] Van Geison Staining technique was done to assess the collagen fibers.[23] Histological observations were recorded under light microscope at 10 × and 40 × objectives.. The observations like formation of zones, carbonization, edema, surface granulation tissue formation, muscle necrosis and inflammatory cells in detail were recorded.[16]

Statistical Analysis

Mean, SD were calculated and Student "t" test, were used for comparing two means. Comparison of paired observation was carried out by Paired t test. The observation in the groups was compared by Chi square test and subgroups by Fisher's exact test in different kind of data analysis.

Results

The three groups were studied and compared after partial glossectomy macroscopically (gross) as well as histologically in the following manner.

Duration of Surgical Procedure

The mean ± SD duration of surgery in group A was 142.4 ± 8.6 seconds which was more as compared to group B and C and was statistically very highly significant. In group B surgical procedure took significantly ($P < 0.001$) longer time 92 ± 7.1 seconds as compared to group C 26.7 ± 3.9 seconds (Table 1).

Table 1: Comparison of Duration of Surgery in 3 Groups.

Group	No of Animals	Duration of Range(Sec.)	Surgery (Sec.) Mean ± SD
3 Watts CO_2 Laser (A)	10	130 – 166	142.4 ± 8.6
6 Watts CO_2 Laser (B)	10	74 – 113	92.0 ± 7.1
Scalpel (C)	10	20 – 38	26.7 ± 3.9

A Vs B $P < 0.001$, B Vs C $P < 0.001$, A Vs C $P < 0.001$

Table 2: Comparison of Duration of Bleeding in 3 Groups.

Group	No of Animals	Duration of Bleeding (Sec.)	
		Range	Mean ± SD
3 Watts CO_2 Laser (A)	10	65 – 88	76.9 ± 5.8
6 Watts CO_2 Laser (B)	10	50 – 84	68.0 ± 6.9
Scalpel (C)	10	210 – 268	236.7 ± 14.7

A Vs B $P < 0.01$, B Vs C $P < 0.001$, A Vs C $P < 0.001$

Duration of Bleeding

The mean ± SD duration of bleeding was significantly shorter in group B, 68.0 ± 6.9 sec. as compared to group A and C. Scalpel was however found to cause bleeding for a longer time 236 ± 14.7 seconds as compared to group A and B (Table 2).

Gross Wound Base Appearance

After 24 hours, group A wounds appeared more yellow than B and C groups. Elevated shape of the wounds appeared after 24 hours in all three groups, while irregular surface was found in all groups, but 50% of group B wounds appeared with smooth surface. Red colour of wound edge was present in all three groups, but 50% of the group A and B were of reddish white colour. The wound edges remained inverted in group A and B, but group C wound base became flat (Table 3).

Wound Measurements

After 24 hours each group showed highly significant increase in X, Y and Z measurements and was significantly more in group A (Table 4).

Histological Observations

Formation of Zones

The zones in the middle of the wound (0.699 ± 0.240 mm), on dorsal (0.550 ± 0.203) and on ventral surface (0.402 ± 0.177 mm) remained same after 24 hours and the difference was significant ($P<0.02$) only between middle of the wound and ventral surface. However range was reduced.

Carbonization

The mean ± SD µm thickness of carbonization in the middle of the wound was more than dorsal and ventral surfaces in the laser groups. The differences among the groups were statistically significant (Table 5).

Table 3a: Comparison of Wound Base and Wound Edge in 3 Groups.

Groups	Colour					Shape			Surface	
	Black	Black Red	Yellow	Yellow Red	Red	Elevated	Flat	Crater	Irregular	Smooth
A			6	4		10		10	10	
B			3	7		10		10	5	5
C			1	5	4	10			10	

Table 3b: Wound Edge

Groups	Colour				Shape		
	Black	White	Red White	Red	Inverted	Flat	Evertd
A			5	5	10		
B			5	5	10		
C				10*		10***	

*Significant
**Highly Significant
***Very Highly Significant

Table 4: Comparison of Wound Measurements in 3 Groups.

Group	Immediate (MM)		On Second Day (MM)		Difference
	Range	Mean + SD	Range	Mean + SD	
$X = A_1-A_2$					
A	4.3 – 5.2	4.5 ± 0.29	5.8 – 6.7	6.1 ± 0.29	P < 0.01
B	4.9 – 5.6	5.0 ± 0.21	5.8 – 6.5	6.0 ± 0.24	P < 0.01
C	5.0 – 5.5	5.18 ± 0.13	6.1 – 6.7	6.3 ± 0.26	P < 0.01
$Y = A_1-B$					
A	3.9 – 5.0	4.1 ± 0.27	5.0 – 5.8	5.5 ± 0.25	P < 0.01
B	4.5 – 5.1	4.6 ± 0.3	5.0 – 5.9	5.6 ± 0.26	P < 0.01
C	4.3 – 4.6	4.49 ± 0.17	5.0 – 5.2	5.1 ± 0.19	P < 0.01
$Z = A_1-C$					
A	7.8 – 8.1	7.9 ± 0.28	8.4 – 9.2	8.81 ± 0.26	P < 0.01
B	8.0 – 8.5	8.3 ± 0.20	8.5 – 9.1	8.82± 0.20	P < 0.01
C	8.0 – 8.8	8.5 ± 0.24	8.7 – 9.5	9.21 ± 0.28	P < 0.01

Table 5a: Comparison of Surface Carbonization in A and B Groups.

Surface	Group A Thickness (µm)		Group B Thickness (µm)		Difference in Groups
	Range	Mean ± SD	Range	Mean ± SD	
Dorsal	15 – 20	18.8 ± 2.9	10 – 20	13.2 ± 6.1	P < 0.01
Ventral	5 – 17	11.9 ± 3.2	0 – 15	7.5 ± 4.9	P < 0.01
Middle	20 – 26	22.9 ± 2.6	0 – 20	10.5 ± 6.7	P < 0.01

Table 5b: *Comparison of Surface Granulation*

Group	24 hours Mean ± SD (µm)	Difference in Groups	P value
A	129 ± 9.9	B Vs C	P < 0.001
B	148 ± 8.7	A Vs B	P < 0.01
C	219 ± 12	A Vs C	P < 0.001

Surface Granulation Tissue

Mean ± SD (µm) thickness of granulation at wound surface in group C (219 ± 12.0) was more than A (129 ± 9.9) and group B (148 ± 8.7) and the rise was statistically significant (Table 6).

Epithelization

Mean ± SD (µm) length of wound epithelization after 24 hours in group C at ventral surface (209 ±

Table 7: Comparison of Length of Healing Epithelium.

Group	Length of Epithelium Dorsal Surface (µm)		Length of Epithelium Ventral Surface (µm)		Difference
	Range	Mean ± SD	Range	Mean ± SD	
A	0	0	0	0	0
B	70 – 200	117 + 29	100 – 250	170 + 40	P < 0.01
C	200 – 400	271 + 59	130 – 350	209 + 65	P < 0.02

Table 8: Comparison of Cellular Response.

Cells	Groups with Cell Count Mean ± SD (0.0625 um^2)			Difference		
	A	B	C			
	Mean ± SD	Mean ± SD	Mean ± SD	A Vs B	B Vs C	C Vs A
Neutrophils	89 ± 14.9	80.5 ± 19.7	74.9 ± 13.5	NS	NS	NS
Lymphocytes	13.5 ± 3.9	13.8 ± 2.4	14.9 ± 2.2	NS	NS	NS
Fibroblasts	15.8 ± 5.0	9.7 ± 2.9	26.0 ± 3.9	P < 0.01	P < 0.001	P < 0.001
Macrophages	----	----	----	----	----	----
Plasma Cells	----	----	----	----	----	----
Mast Cells	0.03 ± 0.04	0.06 ± 0.05	1.0 ± 0.9	NS	NS	NS

65) was significantly less than dorsal surface (271 ± 59). Group B showed reversed pattern of epithelization whereas ventral surface (170 ± 40) length was more than dorsal surface (117 ± 29) and this rise was highly significant. Group A showed no sign of epithelization (Table 7).

Hyperemia

Moderate hyperemia after 24 hours, was noticed in all three groups and in group A it was more than B and C but this rise was not significant.

Edema

Edema was insignificantly more in group A and C than group B after 24 hours.

Muscle Necrosis

The necrosis after surgery was more in group A than B and difference was highly significant and this difference became highly significant (P < 0.005) after 24 hours.

Neovascularisation

Active neovascularisation after 24 hours was found in group C which was significant as compared to group A. It was more than group B but statistically not significant.

Inflammatory cells Neutrophils

Mean ± SD count of Neutrophils, after 24 hours, group A (89 ± 14.9) count was more than group B (80.5 ± 19.7) and C (74.9 ± 13.5), but the difference was not significant (Table 8).

Lymphocytes

Mean ± SD count of lymphocytes, after 24 hours, in group C (14.9 ± 2.2) count was more than group B (13.8 ± 2.4) and A (13.5 ± 3.9), but this difference was not significant (Table 8).

Fibroblasts

The mean ± SD count after 24 hours in group C (26.0 ± 3.9) was more than group A (15.8 ± 5.0) and B (9.9

± 3.1) and the difference was highly significant ($P < 0.001$), group A also had significantly ($P < 0.01$) more count than group B (Table 8).

Macrophages / Plasma Cells

The mean SD count of macrophages and plasma cells after 24 hours was not considerable in any group (Table 8).

Mast Cells

The mean SD count / 0.0625 um^2 of mast cells in group C (1.0 ± 0.9) was more than group A (0.03 ± 0.04) and group B (0.06 ± 0.5), but was not significant (Table 8).

Discussion

Since the development of the CO_2 laser as a surgical knife in 1970, many studies have been conducted to compare wound healing at different watts of CO_2 laser with the scalpel, but these studies have certain deficiencies like these studies were conducted on very limited animal models, gross morphological observations of the wound were not being taken. Wound measurements for the assessment of the wound expansion, contraction during healing were not emphasized earlier.[6] Many histological observations like cellular response, surface coverings, blood, carbonization, granulation tissue formation and thickness of the healing epithelium etc, were not emphasized. The limited spectrum of research and controversies in the previous studies let us to perform this study. The 3 watts CO_2 laser as minimum power and 6 watts CO_2 laser as maximum power (a routine clinical practice) have never been compared with scalpel to see the soft tissue response.

The weight of each animal was reduced 24 hours after surgery. This reduction in group A and B was not significant. In scalpel group the weight was significantly ($P < 0.05$) reduced. This reduction in weight in scalpel group was due to less intake, more postoperative pain and excessive bleeding duration as compared to laser groups where sealing of nerve ending and vessels up to 0.5 mm, added comfort to the animals.[24,25]

The colour distribution in the scalpel wound was more towards yellowish red to red as compared to laser groups whereas it was yellow mainly in group A. The yellowish hue was due to more denatured collagen on the surface of the lasered area and less neovascularisation. The wound shape was elevated (convex) in three groups and surface was irregular due to edema.[10,11,16] In group B 50% animal showed smooth surface that may be due to less inflammatory response. The wound edges in laser groups had reddish white colour distribution, and inverted shape due to denatured proteins,[6,26] while scalpel had flat and red colour wound edges. The increase in the size of the wound after 24 hours was due to the hydration which expands intercellular space, promoting inflammatory cell movement,[25] protein extravasation[28] and release of muscle spasm.[6] Less expansion in group B was noticed as compared to group A and C. This reduced expansion was due to covering of wound surface with fibrinous coagulated coagulum which prevent the wound from external environments[29] and less thermal conductivity during surgery.[6]

Walsh et al[18] described the first phase of healing in laser as the loss of carbonized material from the wound surface. Histologically surface carbonization in group A was more as compared to group B and difference was highly significant ($P < 0.01$). The carbonization was more in middle, and dorsal surface of the wound (due to more energy impact) as compared to ventral surface in both laser groups. The difference was highly significant ($P < 0.01$) in group A and was significant in group B. This shows the slow healing response in group A due to longer thermal effect, which causes retardation of phagocytic activity of leukocytes.[28]. The scalpel wound surface was covered with fibrino purulent membrane having a fibrin layer underneath and inflammatory reaction extending to lingual muscles.

Mean ± SD (µm) length of healing epithelium was measured (Table 7). No sign of epithelial regeneration was noticed in group A and same was noticed by Kardos et al[26] by using 4 watts CO_2 laser. Re-epithelization was seen to be on its way more on dorsal and less on ventral surface in group B and C, the difference was significant ($P < 0.02$) in group C but was more significant ($P < 0.01$) in group B, the percentage of epithelization was raised in group C as compared to group B. Fisher and Frame[31] used 10 to 20 watts power in oral cavity and found the same result, however, Luomanen and Veritan[32] used average 6 watts power of CO_2 on tongue and found early migration of epithelium in scalpel group and same was observed in our study but Pogrel et al[27] reported faster re-epithelization in skin incisions by laser due to more hyaluronidase activity. Kardos et al[26] mentioned the start of epithelization in 3 watts CO_2 laser after 24 hours but his treatment was limited to epithelial surface which do not

require more time for ablation. Hell and Lawrence[35] analyzed that DNA synthesis occurs within one day in cut wound while in burn wounds it was delayed. This reflects that 6 watts laser has least thermal damage (due to less cutting time) and it appeared nearer to scalpel.

Mean ± SD (μm) formation of surface granulation tissue was more in group C as compared to group B and A (Table 6) and the difference was highly significant ($P < 0.001$). This can be supported by the presence of endothelial proliferation at the edges of scalpel wound as reported by Margic[34] and Morosolli et al.[42] This showed the active wound healing in scalpel as compared to laser groups. In group A less granulation tissue was seen due to heat effect that retards the capillary proliferation and fibroblast activity.

Mean ± SD (0.0625 μm^2) cellular count of group C was more as compared to group A and B except neutrophils (Table 8). The difference was not statistically significant amongst groups. Neutrophils are primary inflammatory cells and appear in excisional wounds after 24 hours.[35,37] Group A had more neutrophils count as compared to group B and C. More inflammation was noted by Madden et al,[7] Pogrel et al[27] in scalpel group but this pattern was not noted by Luomanen et al,[8] Johnson et al.[37] Increase in cutting duration time of laser raised the inflammatory response.[38] The rat tissues contains higher percentage of fibroblasts than other cells.[39] Beginning of fibroblasts reaction in wound after one day was observed by Mondain et al.[40] Fibroblast count was less but dominated in group C than group A and B and the difference was highly significant ($P < 0.001$). Group B had more fibroblasts count than group A and was statistically significant ($P < 0.01$). Mast cell count was more in group C but degranulated mast cells were seen in laser groups that were also observed by Basu et al[6] and Pinhero et al.[14] The under granulated mast cells were found more in group B than group A but the difference was not statistically significant.

Conclusion

6 Watts CO_2 laser wound initial healing response was much better as compared to scalpel in terms of bleeding duration, surgery duration, wound appearance surface coverings and inflammatory cells response. The 3 Watts CO_2 laser wound in spite of less Wattage had not good healing response due to deeper diffusion of heat by more cutting duration.

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