

4.4 EXPERIMENTAL RESULTS

Results of the microscopic evaluation included observation on macrophages, epithelialization, angiogenesis, fibroplasia, collagen content. Detail of the results are found below.

4.4.1 NUMBER OF MACROPHAGES

According to the data obtained from the microscopic evaluation, the inflammation status of the experimental wound was determined by observing the approximation of number of macrophages present in the wound (Table 4.6). The number of macrophages was scored according to the scale based on high magnification microscopic observation at 400X. The scale values, 0=None; 1=few; 2=Scant 3=Moderate and 4=Abundant (Table 4.5).

According to the results obtained (Figure 4.5), the number of macrophages increased from Day 1 to Day 5 of treatments. There was no significant difference ($p < 0.05$) found on Day 1 of treatments among all the treatment groups. On Day 1 of treatments, the average scales of macrophages present in wounds of Group IN, GE and NE (Intrasite, Gelam, Nenas) were 2.33, reflecting scant presence of macrophages. Values for macrophages found in Group NO (No dressing) and Group SA (Saline) were lesser.

On Day 5 of treatments, the number of macrophages present in wounds was increased in all treatment groups. The highest scale value of macrophages found in Group IN (Intrasite) and Group GE (Gelam) were 3.67 (almost abundant). In comparison, the scale value of macrophages in Group NE (Nenas) was the least. On Day 10 of treatments, macrophages in the wound of all the treatment groups were

slightly decreased. The decrease continued to Day 15 in all treatment groups, except for Group NO (No dressing). On day 15 of treatments, the scale number of the honey treatments (Group GE and Group NE) was the lowest in comparison with other groups. The trend observed was that the number of macrophages increased in the early stage of wound healing process and decreased after Day 10 of treatments in all treatment groups, except Group NO (No dressing).

Table 4.6: Number of macrophages found in the wounds in the five treatments groups.

Group Name		Day 1	Day 5	Day 10	Day 15
NO	No Dressing	1.16±0.17 ^{a,w}	3.33±0.21 ^{b,w}	2.50±0.22 ^{a,w}	2.83±0.16 ^{b,w}
SA	Saline	1.33±0.21 ^{a,w}	3.33±0.21 ^{b,w}	3.17±0.40 ^{b,x}	2.00±0.26 ^{a,w,x}
IN	Intrasite	2.33±0.42 ^{ab,w}	3.67±0.21 ^{a,w}	2.67±0.21 ^{b,w,x}	1.00±0.52 ^{b,xy}
GE	Gelam	2.33±0.33 ^{a,w}	3.67±0.21 ^{a,w}	3.00±0.26 ^{a,x}	0.33±0.21 ^{b,y}
NE	Nenas	2.33±0.33 ^{a,w}	3.00±0.37 ^{a,w}	2.83±0.31 ^{a,x}	0.67±0.21 ^{a,y}

All values were expressed as mean ± SEM; a value of $p < 0.05$ was considered significant. Means with different superscripts (a, b within each row) were significantly different ($p < 0.05$) due to days of treatments. Means with different superscripts (w, x, y within each column) were significantly different ($p < 0.05$) due to types of treatments.

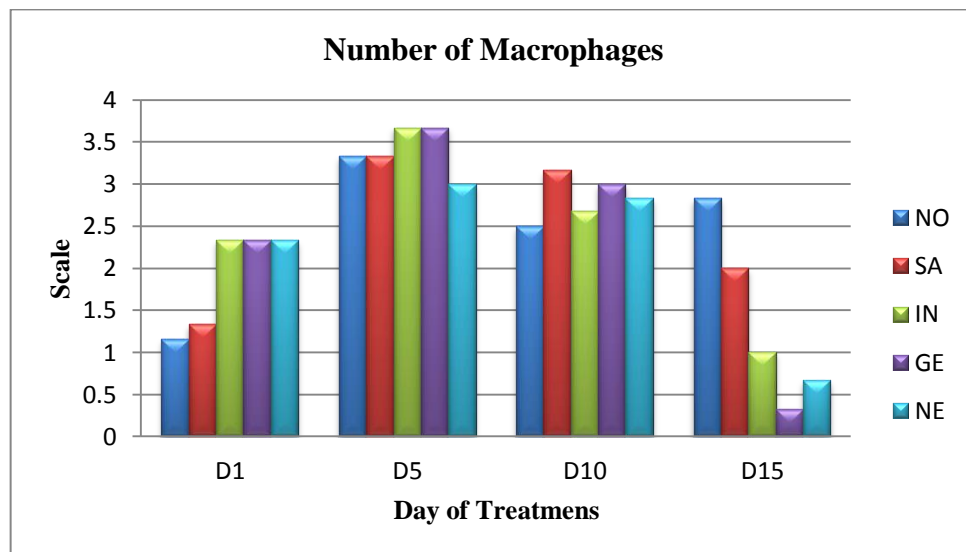


Figure 4.5: Number of macrophages in the treatment groups on different day of treatments. NO: No dressing; SA: Saline; IN: Intrasite; GE: Gelam honey and NE: Nenas honey.

4.4.2 EPITHELIALIZATION

The epithelialization status was scored according to the scale: 0= None, 1=Partial (thin), 2= Partial (thick); 3= Complete (thin); and 4= Mature (Thick). There was a gradual increase in the rate of epithelialization in all treatment groups (Figure 4.6). On Day 1 of treatments, there was only little epithelialization found in all treatment groups. The highest rate of epithelialization was found in Group IN (Intrasite). In comparison, epithelization found on Day 5 of treatments was greater compared to Day 1 of treatments. Most of the experimental wound showed partial epitheliazation with the wound bed not fully covered by newly formed epithelial cells. Epithelialization found in Group SA (Saline) and Group IN (Intrasite) seemed to have occurred only slightly in comparison with other groups.

Results on Day 10 of treatments showed that epithelialization continued to increase in all treatments groups. Epithelialization in Group IN (Intrasite), Group GE (Gelum) and Group NE (Nenas) were significantly greater compared to Group NO (No treatment). This showed the epithelialization of wounds in these groups were completed but at immature stage. The new formed epithelial cell layer was thin. On Day 15 of treatments, all treatments groups except Group NO (No dressing) and Group SA (Saline) showed completed epithelization and the starting of maturation.

Table 4.7: Epithelialization occurred in the wounds in the five treatment groups.

Group Name		Day 1	Day 5	Day 10	Day 15
NO	No Dressing	0.00±0.00 ^{a,w}	1.33±0.21 ^{b,w}	1.50±0.22 ^{b,w}	1.50±0.22 ^{b,w}
SA	Saline	0.33±0.21 ^{a,w}	1.67±0.17 ^{b,w}	1.67±0.21 ^{b,w,x}	1.50±0.22 ^{b,w}
IN	Intrasite	0.67±0.21 ^{a,w}	1.67±0.17 ^{a,w}	2.50±0.22 ^{b,x}	3.50±0.34 ^{c,x}
GE	Gelum	0.33±0.21 ^{a,w}	1.50±0.22 ^{b,w}	2.50±0.22 ^{b,x}	3.67±0.21 ^{b,x}
NE	Nenas	0.33±0.21 ^{a,w}	1.33±0.21 ^{b,w}	2.17±0.17 ^{b,w,x}	3.50±0.34 ^{b,x}

All values were expressed as mean ± SEM; a value of p<0.05 was considered significant. Means with different superscripts (a, b within each row) were significantly different (p<0.05) due to days of treatments. Means with different superscripts (w, x, within each column) were significantly different (p<0.05) due to types of treatments.

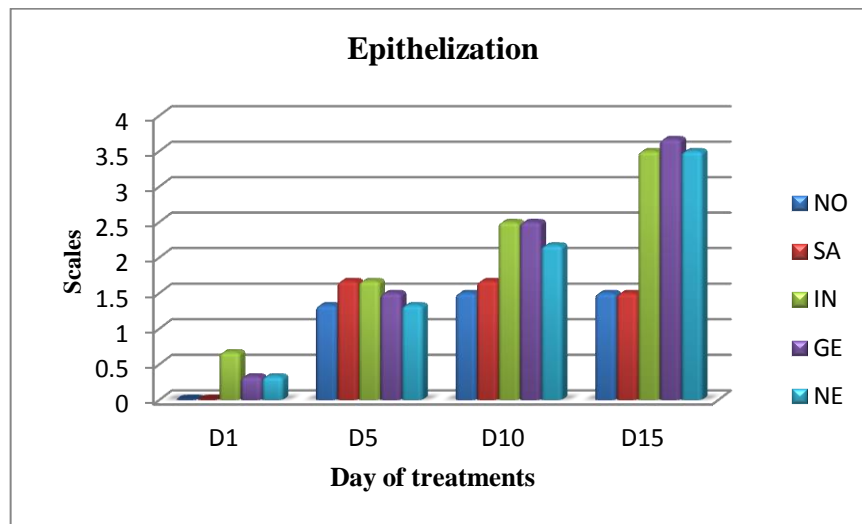


Figure 4.6: Epithelialization of the wounds in the treatments groups on different days of treatments. NO: No dressing; SA: Saline; IN: Intrasite; GE: Gelam honey and NE: Nenas honey.

4.4. 3 ANGIOGENESIS

The blood vessels in the wounds were counted and graded accordingly. Angiogenesis was scored according to the scale on Table 4.5: 0=None, 1=<5 vessels/HPF (High Power Field); 2= 6-10 vessels/ HPF; 3>10 vessels/HPF, 4=>20 vessels/HPF. The process of angiogenesis in the wound healing showed an increase in the beginning but decreasing in late phase of wound healing process in all the treatment groups (Figure 4.7).

There was little or no blood vessels found within wound areas on Day 1 of treatments in all treatment groups. On Day 5 of treatments, there was a gradual increase in the number of blood vessels found in the wounds of all treatment groups. The number of blood vessels was significantly ($p<0.05$) highest in Group IN (Intrasite), Group GE (Gelam) and Group NE (Nenas) compared to Group NO (No dressing) and Group SA (Saline).

On Day 10 of treatments, blood vessels found in wound beds of Group NO (No dressing) and Group SA (Saline) had increased compared to Day 5 of treatments.

However, blood vessel in the wound beds of Group IN(Intrasite), Group GE (Gelang) and Group NE (Nenas) were not as many as before.

On Day 15 of treatments, the number of blood vessels in Group NO (No dressing) was still increasing. Number of blood vessels in Group SA (Saline) started to reduce but it was still greater than other treatment groups. The range of blood vessels in Group IN (Intrasite), Group GE (Gelang) and Group NE (Nenas) were significantly greater than Group NO (No dressing) and Group SA (Saline).

Table 4.8: Angiogenesis found in wound of all treatments groups.

Group Name		Day 1	Day 5	Day 10	Day 15
NO	No Dressing	0.00±0.00 ^{a,w}	1.50±0.22 ^{b,w}	2.33±0.21 ^{c,w}	2.83±0.17 ^{c,w}
SA	Saline	0.16±0.17 ^{a,w}	2.50±0.22 ^{b,wy}	3.33±0.21 ^{b,w}	2.00±0.22 ^{c,w}
IN	Intrasite	0.83±0.40 ^{a,w}	2.83±0.31 ^{b,xy}	2.67±0.21 ^{b,w}	1.67±0.21 ^{ab,w}
GE	Gelang	0.67±0.33 ^{a,w}	3.67±0.21 ^{b,x}	3.17±0.31 ^{b,w}	1.33±0.21 ^{a,w}
NE	Nenas	0.50±0.22 ^{w,a}	3.50±0.22 ^{b,xy}	2.67±0.21 ^{b,w}	1.67±0.21 ^{c,w}

All values were expressed as mean ± SEM; a value of $p < 0.05$ was considered significant. Means with different superscripts (a, b, c within each row) were significantly different ($p < 0.05$) due to days of treatments. Means with different superscripts (w, x, within each column) were significantly different ($p < 0.05$) due to types of treatments.

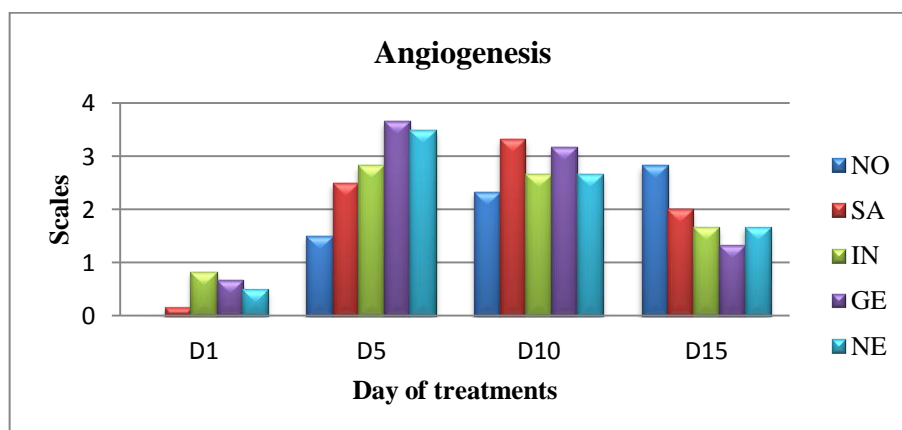


Figure 4.7: Angiogenesis process in the wounds of all treatments groups on different days of treatments. NO: No dressing; SA: Saline; IN: Intrasite; GE: Gelang honey and NE: Nenas honey.

4.4.4 FIBROPLASIA

The level of fibroplasias was graded based on the scale in Table 4.5: 0=None; 1=Few, 2=Moderate; 3= More; 4=Predominantly.

The general trend of fibroplasia was an increase from Day 1 to Day 5 of treatments and started to decrease from Day 10 to Day 15 of treatments (Figure 4.8). More fibroblast in the wound area were found in Group GE (Gelum), followed by Group IN (Intrasite) on Day 1 of treatments. The amount of fibroblast showed a drastic increase from Day 1 to Day 5 of treatments in all groups, except Group NO (No dressing). Greatest amount of fibroblast was found in Group NE (Nenas) on Day 5 of treatments. On Day 10 of treatments, the greatest amount of fibroblast was found in Group GE (Gelum). On Day 15 of treatments, the amount of fibroblast was found to decrease in all treatment groups, except Group NO (No dressing).

Table 4.9: Process of fibroplasia in the wound area according to the group of treatments.

Group Name		Day 1	Day 5	Day 10	Day 15
NO	No Dressing	1.00±0.00 ^{a,w}	1.33±0.21 ^{a,w}	1.17±0.17 ^{a,w}	2.67±0.21 ^{b,w}
SA	Saline	1.33±0.21 ^{a,w}	2.33±0.43 ^{a,w}	1.83±0.31 ^{a,wy}	2.50±0.22 ^{a,w}
IN	Intrasite	1.67±0.21 ^{ab,w}	2.67±0.33 ^{a,w}	2.67±0.21 ^{a,xy}	1.67±0.21 ^{b,x}
GE	Gelum	1.83±0.17 ^{a,w}	2.67±0.33 ^{ab,w}	3.17±0.31 ^{b,x}	1.83±0.17 ^{a,w}
NE	Nenas	1.16±0.31 ^{a,w}	3.33±0.21 ^{b,w}	2.83±0.18 ^{g,b,xy}	1.50±0.22 ^{a,x}

All values were expressed as mean ± SEM; a value of $p < 0.05$ was considered significant. Means with different superscripts (a, b, c within each row) were significantly different ($p < 0.05$) due to days of treatments. Means with different superscripts (w, x, within each column) were significantly different ($p < 0.05$) due to types of treatments

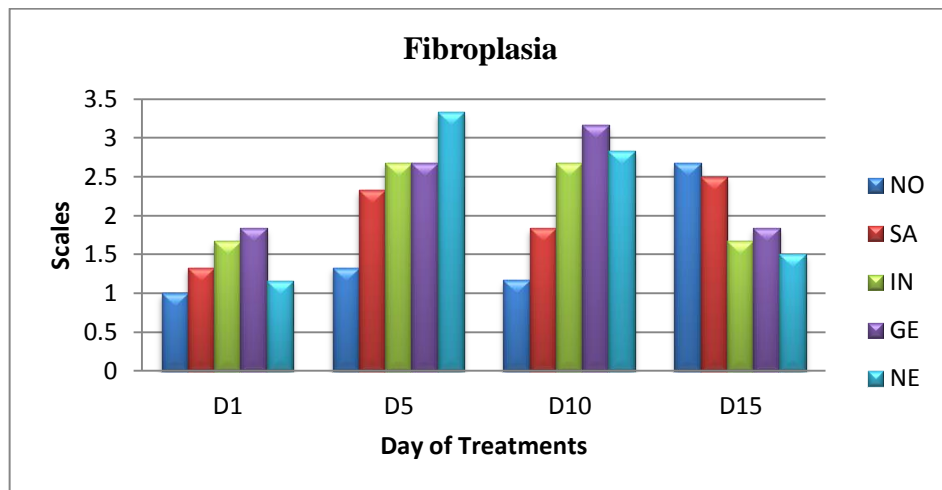


Figure 4.8: Fibroplasia process in the wounds of all treatments groups on different days of treatments. NO: No dressing; SA: Saline; IN: Intrasite; GE: Gelam honey and NE: Nenas honey.

4.4.5 COLLAGEN CONTENT

The level of collagen content was graded based on the scale in TABLE 4.5: 0=None; 1= Little, 2=Scant; 3= Moderate; 4=Abundant.

The level of collagen content increased from Day 5 to Day 15 of treatments in all treatment groups (Figure 4.9). No collagen existed on Day 1 of treatments in all the groups. On Day 5 of treatments, the collagen level of Group NO (No dressing) and Group SA (Saline) were significantly lower compared to the others. The collagen level in Group IN (Intrasite) was the greatest among all groups of treatments.

On Day 10 of treatments, an increase was found in the collagen level of all group of treatments especially in wounds treated with topical dressings (Group IN, Group GE and Group NE). The collagen level kept increasing till the end of the wound healing process. Thus, on Day 15 of treatments, the collagen level was the highest for all group of treatments. Wound in Group IN (Intrasite) showed the highest amount of collagen level.

Table 4.10: Collagen content in wounds according to the treatment groups.

Group Name		Day 1	Day 5	Day 10	Day 15
NO	No Dressing	0.00±0.00 ^{a,w}	0.33±0.21 ^{a,w}	1.67±0.21 ^{a,w}	1.83±0.21 ^{b,w}
SA	Saline	0.00±0.00 ^{a,w}	1.83±0.17 ^{a,w}	2.67±0.21 ^{a,wy}	2.67±0.21 ^{a,wy}
IN	Intrasite	0.00±0.00 ^{ab,w}	2.50±0.22 ^{a,w}	3.83±0.17 ^{a,xy}	3.83±0.16 ^{b,x}
GE	Gelam	0.00±0.00 ^{a,w}	2.33±0.21 ^{ab,w}	3.67±0.21 ^{b,x}	3.5±0.22 ^{a,xy}
NE	Nenas	0.00±0.00 ^{a,w}	1.33±0.21 ^{b,w}	3.50±0.21 ^{b,xy}	3.00±0.26 ^{a,x}

All values were expressed as mean \pm SEM; A value of $p < 0.05$ was considered significant. Mean with different superscripts (a, b,c within each row) were significantly different ($p < 0.05$) due to days of treatments. Mean with different superscripts (w, x, within each column) were significantly different ($p < 0.05$) due to types of treatments.

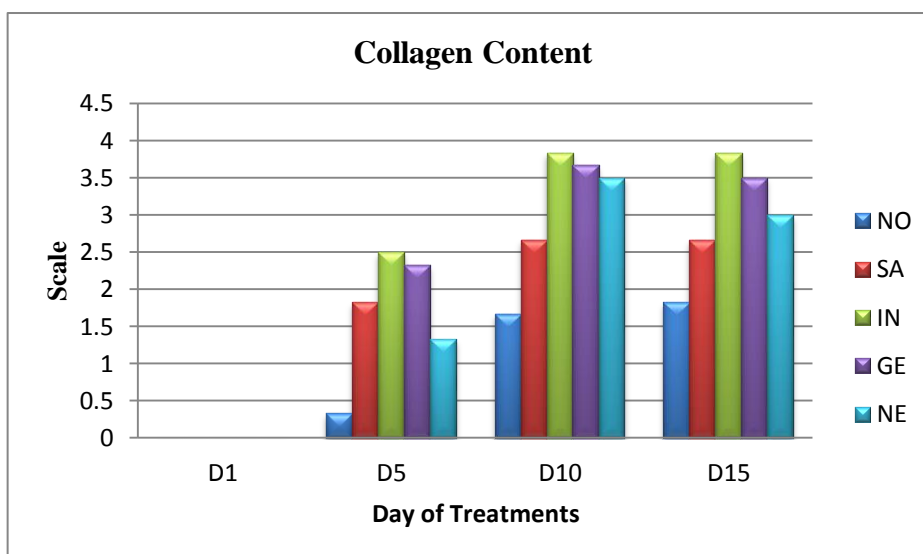


Figure 4.9: Collagen content in the wounds of all treatments groups on different days of treatments. NO: No dressing; SA: Saline; IN: Intrasite; GE: Gelam honey and NE: Nenas honey.