CHAPTER IV

RESULTS

This chapter is composed of seven parts which are served for the examination the effects of Aloe vera on changes of microcirculation and of TNF-α and IL-6 levels.

I. Effects of aloe- treated burn wound on healing area

Changes of healing area were evaluated as percentage in the burn wound-rats, the NSS-treated burn wound- rats and the aloe- treated burn wound- rats. Mean values and standard deviation of means were shown in Table 4.1 and Figure 4.1.

The results indicated that percentage of healing area in the NSS-treated burn wound-rats and the aloe-treated burn wound-rats were of no significant differences from the burn wound-rats within both 3 and 7 days. However, on day 14 the percentage of healing area in the NSS-treated burn wound-rats and the aloe-treated burn wound-rats were significantly higher than the untreated burn wound-rats. However, the percentage of healing area in the aloe-treated burn wound-rats was significantly higher than the NSS-treated burn wound-rats. These data represented that on day 14, aloe-treated burn wound healed almost completely and the healing time was shorter compared to NSS-treated burn and untreated burn.

II. Effects of aloe-treated burn wound-rats on tissue perfusion

The Laser Doppler Flowmetry was used to measure the tissue perfusion.

The results of skin blood flow in the control rats, the burn wound- rats, the NSS-treated burn wound- rats and the aloe- treated burn wound- rats.

Mean values and standard deviation of means were shown in Table 4.2 and Figure 4.2.The results indicated that the tissue perfusion of burn wound-rats was significantly lower than that of controls in all three monitored time points. Aloe- treated burn wound- rats had increased tissue perfusion on day 14. As these results Aloe vera was able to increase skin blood flow to burn wound area.

III. Effects of aloe-treated burn wound on arteriolar diameters (40-70 μm)

The diameters of arterioles (40-70 μm) were determined on day 3,7 and 14. Means and standard deviation of means were shown in Table 4.3 and Figure 4.3. The results indicated that on day 3, there were no significantl differences among the groups of the control rats, the burn wound-rats, the NSS-treated burn wound-rats and the aloe-treated burn wound-rats. On day 7, the arteriolar diameters in the NSS-treated burn wound-rats and the aloe-treated burn wound rats were significantly decreased compared to the control rats. On day 14, the arteriolar diameters in aloe-treated wound-rats was significantly increase compare to the burn wound-rats and the NSS-treated burn wound-rats.

IV. Effects of aloe-treated burn wound on arteriolar diameter (15-40 μm)

The diameters of arterioles (15-40 μm) were determined the means and the standard deviations of means were shown in Table 4.4 and Figure 4.4.

The results of these arteriolar diameter indicated that on day 3, there were significantly increase in diameters in the burn wound-rats and the NSS-treated burn wound-rats compared to the control rats. On day 7, the arteriolar diameters in the burn wound-rats, the NSS-treated burn wound rats and the aloe-treated burn wound-rats was reduced compared to the control rats. On day 14, the arteriolar diameters in the burn wound-rats and the NSS-treated burn wound-rats were largely reduced with significant difference as compared to the control rats. However, there were significantly increases in diameters in the aloe-treated burn wound-rats compared to the control rats and the NSS-treated burn wound rats.

V. Effects of aloe-treated burn wound on leukocyte adhesion

The leukocyte which was defined as the adherent cell to endothelium of postcapillary venule if that cell remained stationary for ≥ 30 seconds was determined and expressed as the percentage per 100 μm length of the postcapillary venules. The means and the standard deviation of means were shown in Table and Figure 4.5.The percentage of leukocyte adhesion were not different among groups of the burn wound rats, the NSS-treated burn wound-rats and the aloe-treated burn wound-rats on day 3 and 7. However, on day 14 leukocyte adhesion was significantly decreased in the aloe-treated burn wound-rats compared to the burn wound-rats. Aloe vera could reduce the percentage of leukocyte adhesion to endothelium of postcapillary venule. The intravital microscopic demonstration of leukocyte adhesion of 3,7,14 days postburn were shown in Figures 4.8, 4.9 and 4.10, respectively.

VI. Effects of aloe-treated burn wound on TNF-α and IL-6 levels

By using ELISA technique, the means and the standard deviations of

Means were shown in Tables 4.6, 4.7 and Figures 4.6 , 4.7.

The results indicated that TNF-α and IL-6 values of burn wound-rats, NSS-treated burn wound-rats and aloe-treated burn wound rats were significantly higher than those of control rats on days 3 and 7. Aloe vera had an effect to reduce the increased TNF-α and IL-6 whichin 14 days.

VII. Effects of aloe-treated burn wound on histopathologic findings

The second degree burn or partial thickness burn involves the epidermis (top layer of skin) as well as part of the dermal layer (Figure 4.11).

On day 7, the burn wound areas after treatment with Aloe vera were covered with necrotic tissue debris and red blood cells. In addition, the NSS-treated burn wound area was also covered by acute inflammatory exudate intermixed with necrotic tissue (Figure 4.12).

Epithelialization was fully developed on day 14 in the aloe-treated burn wound. Newly formed squamous epithelium showed prominent mitotic figures. A thick epithelial layer containing numerous rows of epithelial cells including skin appendages can be observed and vascularization in papillary dermis was normal in pattern (Figure 4.13).

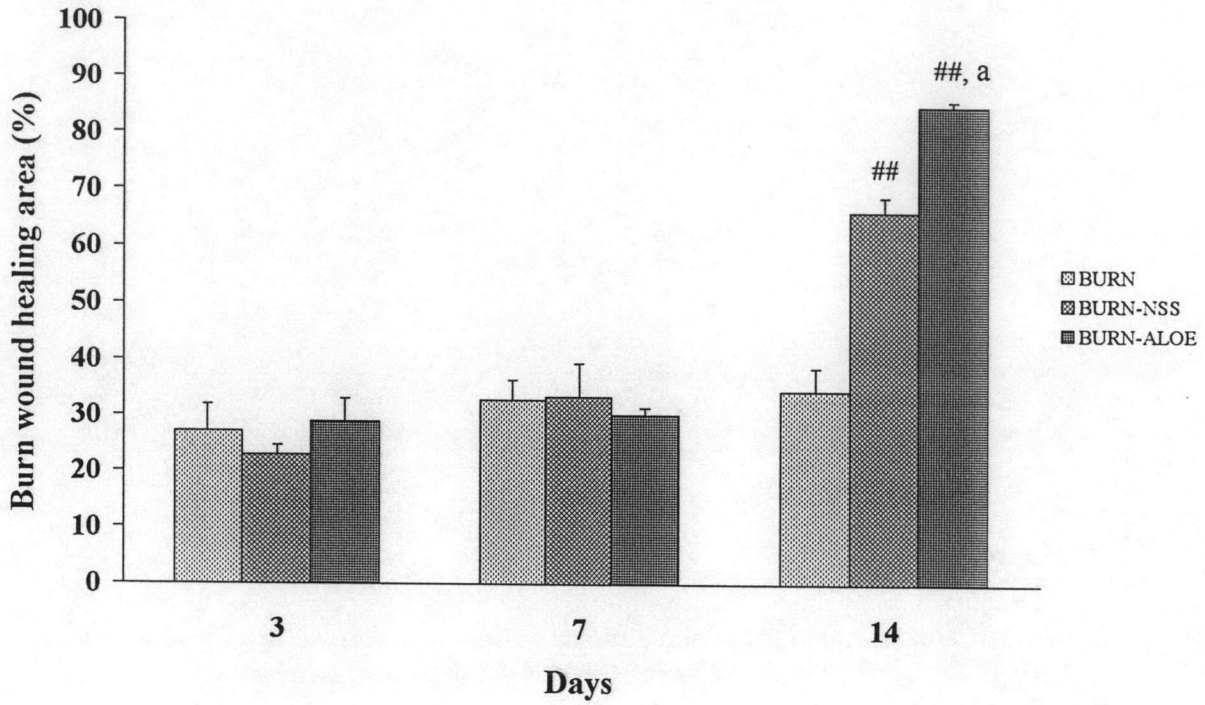
Table 4.1 *Means ± SD of burn wound healing area of burn wound-rats (BURN), NSS-treated burn wound-rats (BURN-NSS) and aloe-treated burn wound-rats (BURN-ALOE)*

Duration (Days)	Burn wound area (μm^2)			Burn wound healing area (%)		
	BURN	BURN-NSS	BURN-ALOE	BURN	BURN-NSS	BURN-ALOE
0	326,993	333,848	316,735	-	-	-
3	238,279	257,563	225,293	27.13±4.78	ns 22.85±1.81	Ns, nss 28.87±4.09
7	220,131	226,048	221,302	32.68±3.55	ns 32.29±5.93	Ns, nss 30.13±1.26
14	212,831	113,842	48,872	34.31±4.19	## 65.90±2.61	##,a 84.57±0.94

$$\% \text{ Healing area} = \frac{\text{Burn area on day 0} - \text{Burn area on day n}}{\text{Burn area on day 0}} \times 100$$

##	Significant difference compared to BURN ($p < 0.01$)
a	Significant difference compared to BURN-NSS ($p < 0.01$)
ns	No Significant difference compared to BURN
nss	No Significant difference compared to BURN-NSS

Figure 4.1 Bar graph showing the mean ± SD of burn wound healing area



BURN; burn wound-rats

BURN-NSS; NSS-treated burn wound -rats

BURN-ALOE; aloe-treated burn wound-rats

Significant difference compared to BURN ($p < 0.01$)

a Significant difference compared to BURN-NSS ($p < 0.01$)

Table 4.2 *Means ± SD of average of tissue perfusion of burn wound rats (BURN), NSS-treated burn wound-rats (BURN-NSS) and aloe-treated burn wound-rat (BURN-ALOE) (100 means is normal tissue perfusion in the control group)*

Duration (Days)	Average of tissue perfusion (%)		
	BURN	BURN-NSS	BURN-ALOE
* 0	83.86±0.64	ns 78.10±1.84	ns, nss 70.10±0.76
3	58.68±1.14	ns 55.78±2.00	#, a 81.19±1.11
7	67.77±1.00	ns 79.66±0.90	#, nss 88.45±1.87
14	73.69±2.35	# 110.50±5.54	##,nss 163.12±7.95

$$\% \text{ tissue perfusion} = \frac{\bar{X}\text{exp} \times 100}{\bar{X}\text{ control}}$$

\# Significant difference compared to BURN ($p < 0.05$)

\#\# Significant difference compared to BURN ($p < 0.01$)

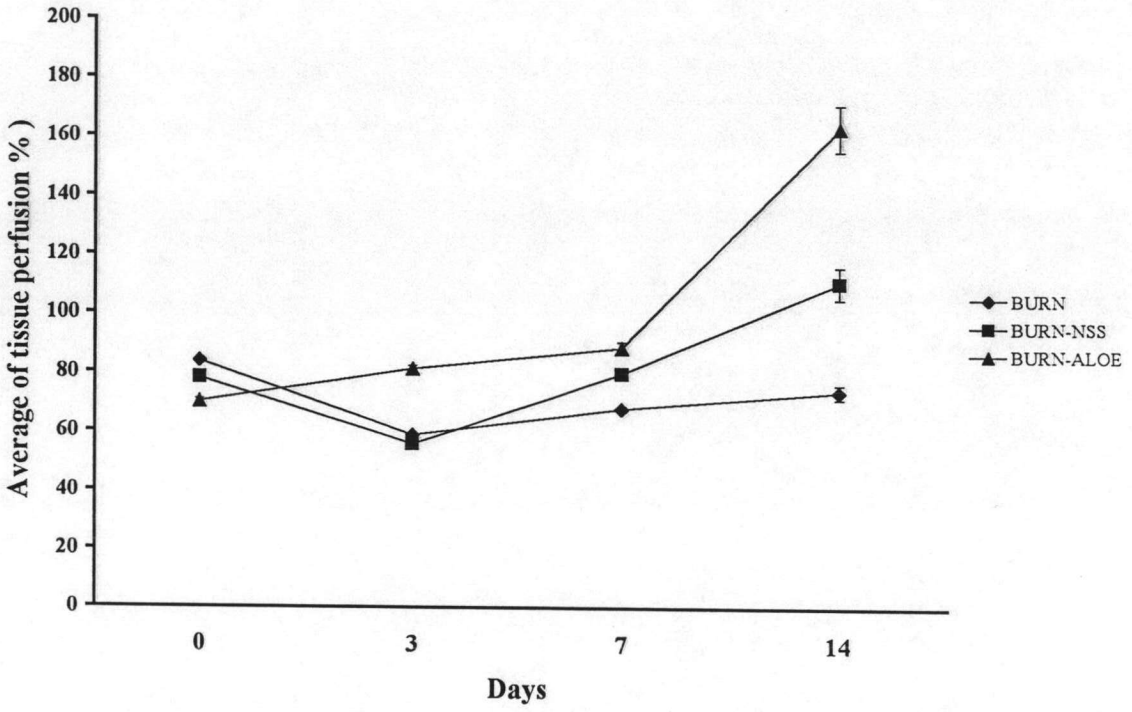
a Significant difference compared to BURN-NSS ($p < 0.01$)

ns No Significant difference compared to BURN

nss No Significant difference compared to BURN-NSS

* The value was monitored immediately postburn

Figure 4.2 *The percentage of average tissue perfusion*



BURN; burn wound-rats

BURN-NSS; NSS-treated burn wound -rats

BURN-ALOE; aloe-treated burn wound-rats

Table 4.3 *Means ± SD of arteriolar diameter (40-70 μm) of control rats (CON), burn wound-rats (BURN), NSS-treated burn wound-rats (BURN-NSS) and aloe-treated burn wound-rats (BURN-ALOE)*

Duration (Days)	Arteriolar diameter (μm)			
	CON	BURN	BURN-NSS	BURN-ALOE
3	45.80±10.30	NS 51.79±5.75	NS, ns 53.03±2.15	NS, ns, nss 44.07±8.13
7	50.55±9.88	* 34.79±20.37	*, ns 28.50±6.51	*, ns, nss 28.73±4.68
14	48.96±6.99	* 37.78±6.23	*, ns 35.89±2.14	NS, #, b 48.37±7.79

* Significant difference compared to CON ($p < 0.05$)

\# Significant difference compared to BURN ($p < 0.05$)

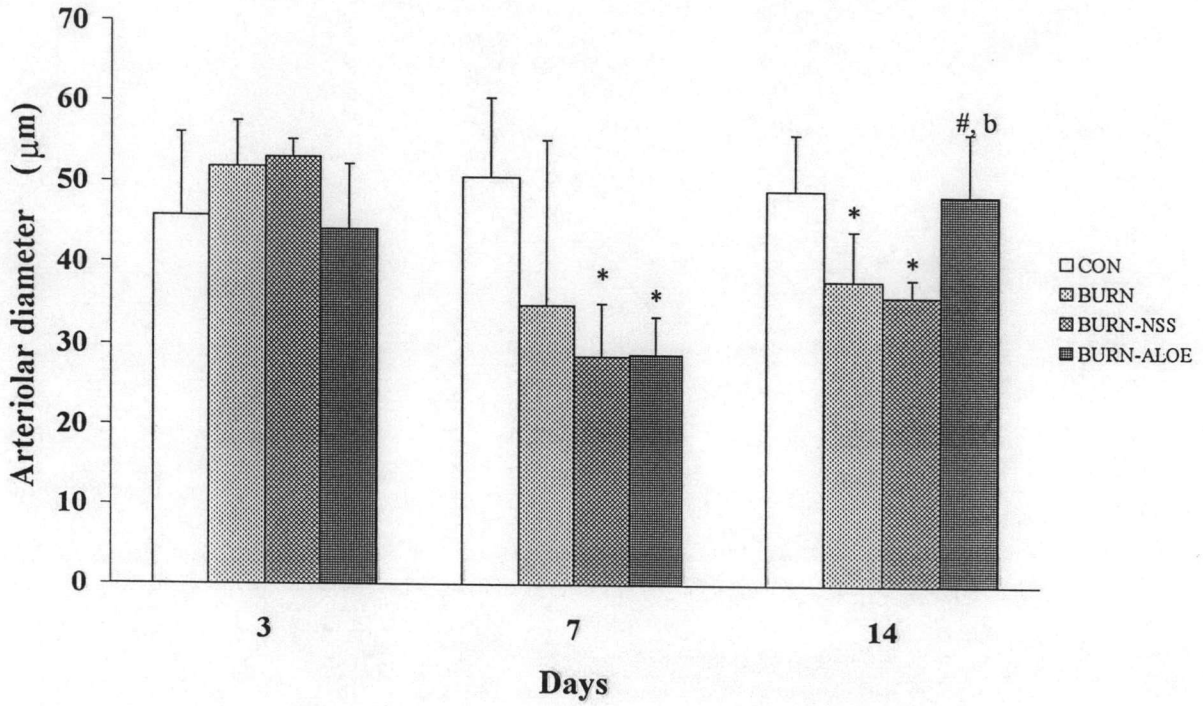
b Significant difference compared to BURN-NSS ($p < 0.05$)

NS No significant difference compared to CON

ns No Significant difference compared to BURN

nss No Significant difference compared to BURN-NSS

Figure 4.3 *Bar graph showing the mean ± SD of arteriolar diameter (μm)*



CON; control rats

BURN; burn wound-rats

BURN-NSS; NSS-treated burn wound -rats

BURN-ALOE; aloe-treated burn wound-rats

* Significant difference compared to CON ($p < 0.05$)

\# Significant difference compared to BURN ($p < 0.05$)

b Significant difference compared to BURN-NSS ($p < 0.05$)

Table 4.4 *Means ± SD of arteriolar diameter (15-40 μm) of control rats (CON),burn wound- rats (BURN), NSS-treated burn wound-rats (BURN-NSS) and aloe- treated burn wound-rats (BURN-ALOE)*

Duration (Days)	Arteriolar diameter (μm)			
	CON	BURN	BURN-NSS	BURN-ALOE
3	32.40±4.76	* 38.38±0.66	*,ns 37.84±0.49	NS, ##, nss 29.06±3.59
7	39.64±6.15	* 32.33±0.46	**, ## 23.12±4.13	**,ns, nss 30.16±2.16
14	34.98±6.84	** 24.11±2.04	*,ns 27.31±3.43	NS, ##, b 34.16±3.82

* Significant difference compared to CON ($p< 0.05$)

** Significant difference compared to CON ($p< 0.01$)

\#\# Significant difference compared to BURN ($p< 0.01$)

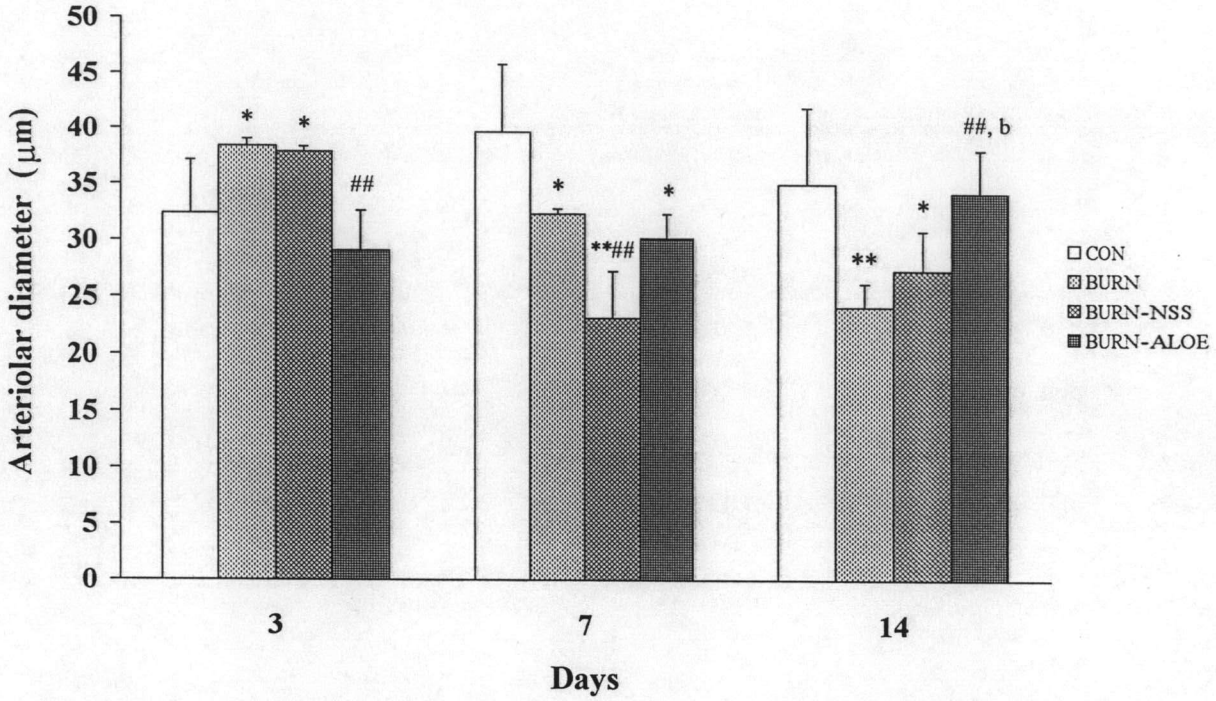
b Significant difference compared to BURN-NSS ($p< 0.05$)

NS No significant difference compared to CON

ns No Significant difference compared to BURN

nss No Significant difference compared to BURN-NSS

Figure 4.4 *Bar graph showing the mean ± SD of arteriolar diameter (μm)*



CON; control rats

BURN; burn wound-rats

BURN-NSS; NSS-treated burn wound -rats

BURN-ALOE; aloe-treated burn wound-rats

* Significant difference compared to CON ($p < 0.05$)

** Significant difference compared to CON ($p < 0.01$)

\#\# Significant difference compared to BURN ($p < 0.01$)

b Significant difference compared to BURN ($p < 0.05$)

Table 4.5 *Means ± SD of leukocyte adhesion (percentage/100 μm) on postcapillary venules of control rats (CON), burn wound-rats (BURN), NSS-treated burn wound-rats (BURN-NSS) and aloe-treated burn wound-rats (BURN-ALOE)*

Duration (Days)	Leukocyte adhesion (percentage/100μm)			
	CON	BURN	BURN-NSS	BURN-ALOE
3	3.84±1.14	26.33±4.80 **	22.98±2.97 **, ns	23.60±9.02 **, ns, nss
7	2.31±1.23	24.77±10.31 **	19.95±0.55 **, ns	19.15±5.03 **, ns, nss
14	4.74±2.40	22.12±1.75 **	18.77±3.81 **, ns	15.40±2.75 **, #, nss

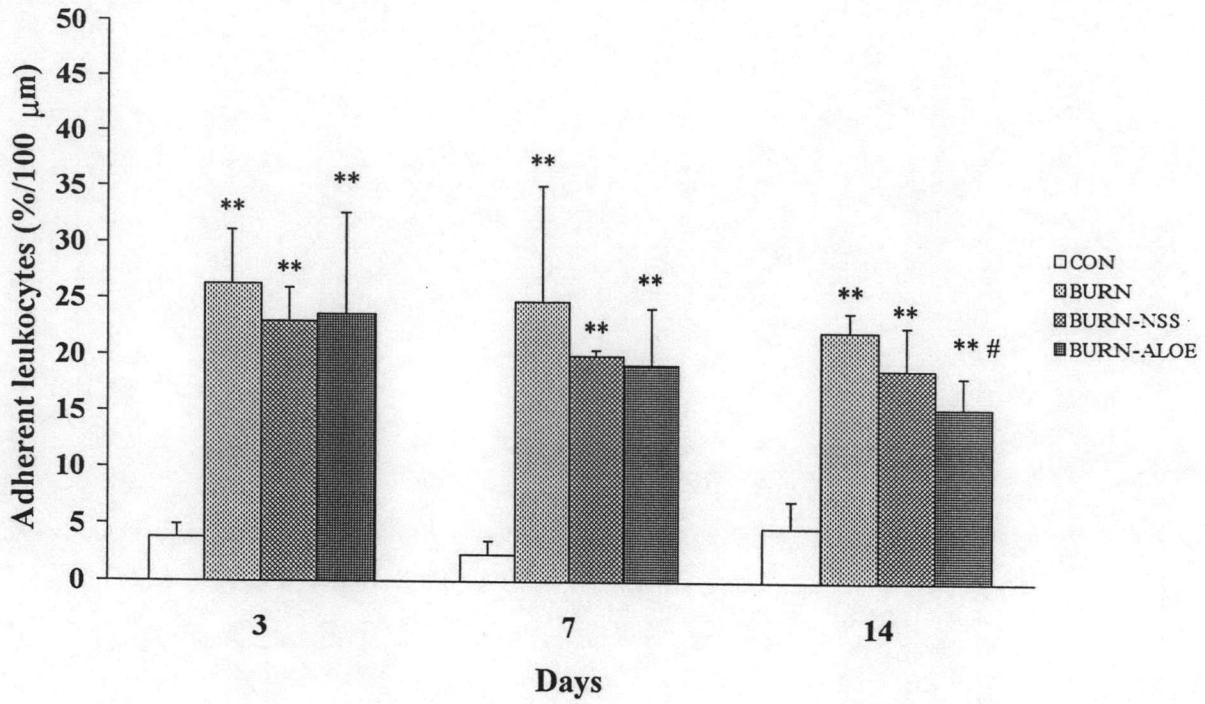
** Significant difference compared to CON ($p < 0.01$)

\# Significant difference compared to BURN ($p < 0.05$)

ns No Significant difference compared to BURN

nss No Significant difference compared to BURN-NSS

Figure 4.5 *Bar graph showing the mean ± SD of leukocyte adhesion*



CON; control rats

BURN; burn wound-rats

BURN-NSS; NSS-treated burn wound -rats

BURN-ALOE; aloe-treated burn wound-rats

** Significant difference compared to CON ($p < 0.01$)

\# Significant difference compared to BURN ($p < 0.05$)

Table 4.6 *Means ± SD of TNF-α levels of (CON), burn wound- rats (BURN), NSS-treated burn wound-rats (BURN-NSS) and aloe treated burn wound-rats (BURN-ALOE)*

Duration (Days)	OD 450 nm ($\times 10^{-2}$)				TNF-α levels (pg/ml)			
	CON	BURN	BURN-NSS	BURN-ALOE	CON	BURN	BURN-NSS	BURN-ALOE
3	8.2±0.8	13.9±1.9	11.7±0.4	11.3±0.6	82.0±8.0	139.0±19.0 **	117.0±4.0 **,#	113.0±6.0 **,##, nss
7	8.5±0.2	3.8±1.0	13.6±1.2	12.8±2.7	85.0±2.0	138.0±10.0 **	136.0±12.0 **,ns	128.0±27.0 **, ns, nss
14	8.2±0.7	11.7±2.1	10.5±2.2	9.0±0.2	82.0±7 .0	117.0±21.0 **	105.0±22.0 NS, ns	90.0±2.0 NS,#, nss

** Significant difference compared to CON ($p < 0.01$)

\# Significant difference compared to BURN ($p < 0.05$)

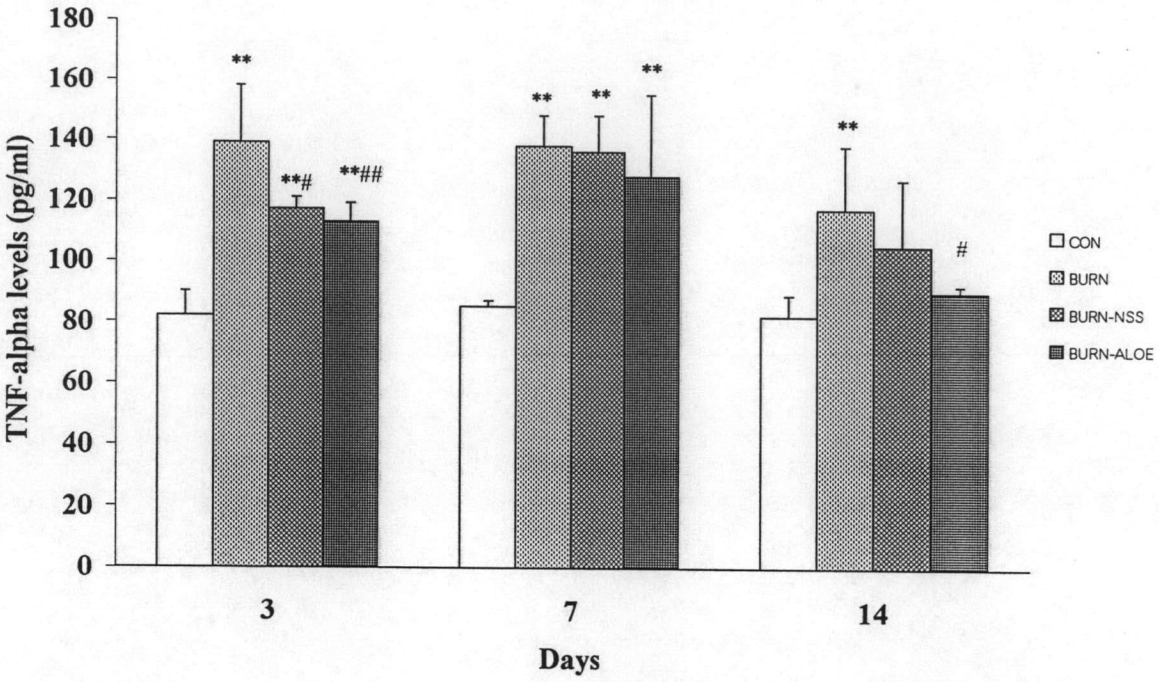
\#\# Significant difference compared to BURN ($p < 0.01$)

NS No significant difference compared to CON

Ns No Significant difference compared to BURN

nss No Significant difference compared to BURN-NSS

Figure 4.6 *Bar graph showing the mean ± SD of TNF-α levels*



CON; control rats

BURN; burn wound-rats

BURN-NSS; NSS-treated burn wound -rats

BURN-ALOE; aloe-treated burn wound-rats

* * Significant difference compared to CON ($p < 0.01$)

\# Significant difference compared to BURN ($p < 0.05$)

\# \# Significant difference compared to BURN ($p < 0.01$)

Table 4.7 *Means ± SD of IL-6 levels of (CON), burn wound- rats (BURN), NSS-treated burn wound-rats (BURN-NSS) and aloe treated burn wound-rats (BURN-ALOE)*

Duration (Days)	OD 450 nm ($\times 10^{-2}$)				IL-6 levels (pg/ml)			
	CON	BURN	BURN-NSS	BURN-ALOE	CON	BURN	BURN-NSS	BURN-ALOE
						**	**,ns	*, #, nss
3	6.5±0.3	10.2±1.1	9.0±0.4	8.4±1.8	62.1±2.9	97.4±10.5	85.9± 3.8	80.2±17.2
						**	NS,##	NS,##,nss
7	6.5±0.3	9.3±0.5	7.0±.06	7.0±0.7	62.1±2.9	88.8±4.8	66.9±5.7	66.9±6.7
						**	NS,##	NS,##,nss
14	6.5±0.3	8.9±1.2	6.8±0.5	6.4±0.5	62.1±2.9	85.0±11.5	64.9±4.8	61.1±4.8

* Significant difference compared to CON ($p < 0.05$)

** Significant difference compared to CON ($p < 0.01$)

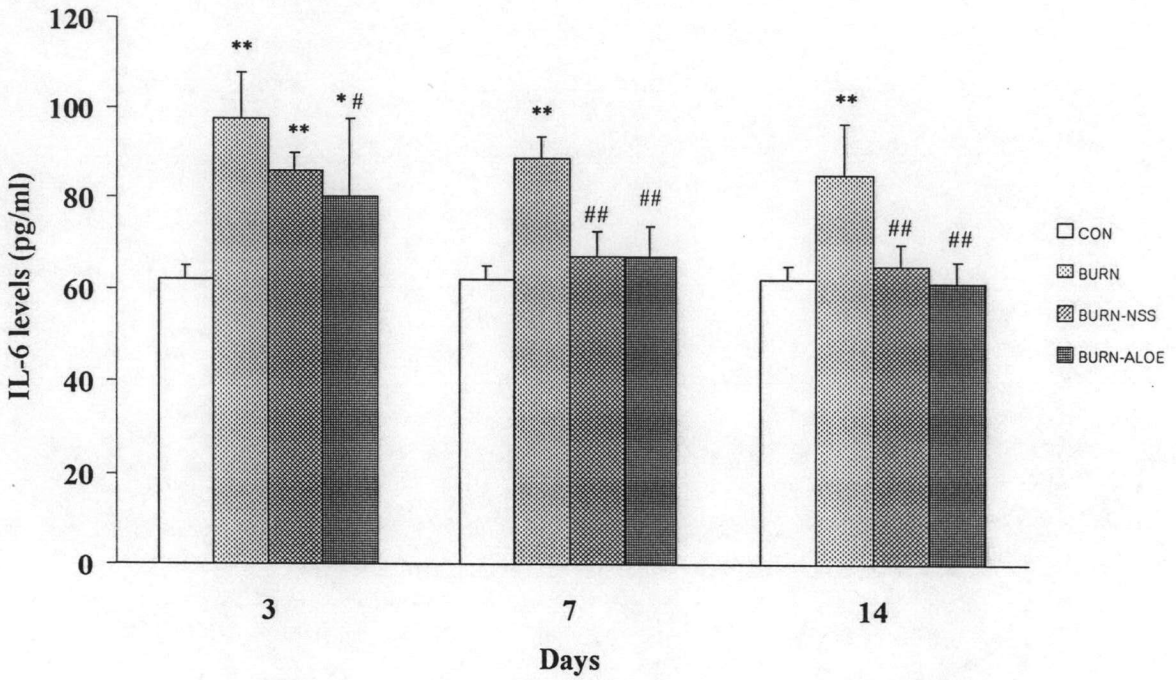
\# Significant difference compared to BURN ($p < 0.05$)

\#\# Significant difference compared to BURN ($p < 0.01$)

NS No significant difference compared to CON

ns No Significant difference compared to BURN

Figure 4.7 *Bar graph showing the mean ± SD of IL-6 levels*



CON; control rats

BURN; burn wound-rats

BURN-NSS; NSS-treated burn wound -rats

BURN-ALOE; aloe-treated burn wound-rats

* Significant difference compared to CON ($p < 0.05$)

** Significant difference compared to CON ($p < 0.01$)

\# Significant different compared to BURN ($p < 0.05$)

\#\# Significant different compared to BURN ($p < 0.01$)

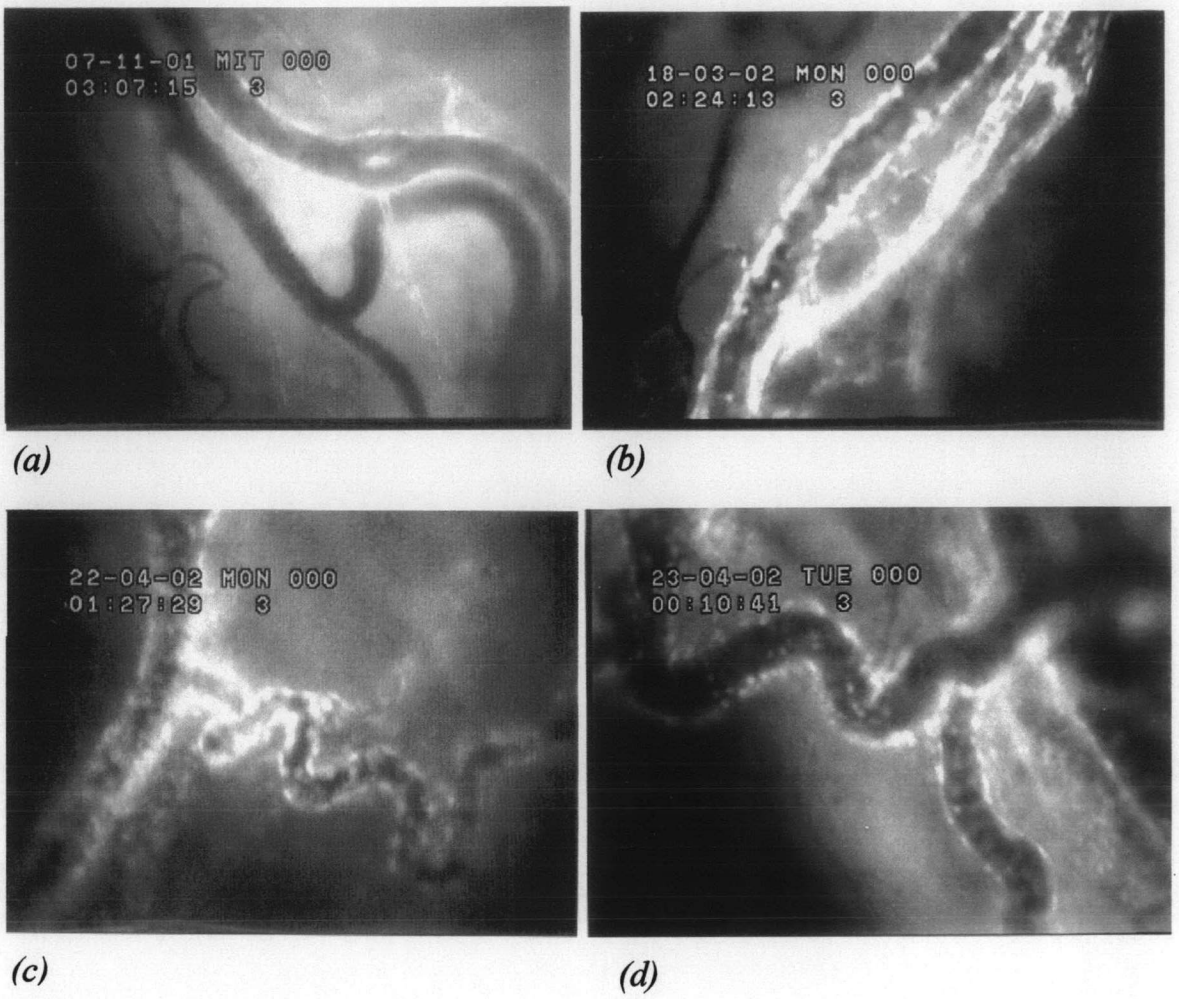


Figure 4.8 *Intravital microscopic demonstration of leukocyte adhesion on the postcapillary venule (PV) 3 days postburn of a) CON, b) BURN, c) BURN-NSS, d) BURN-ALOE. White dots represent leukocytes stained by intravenously- injected of the fluorescein marker, acridine orange.*

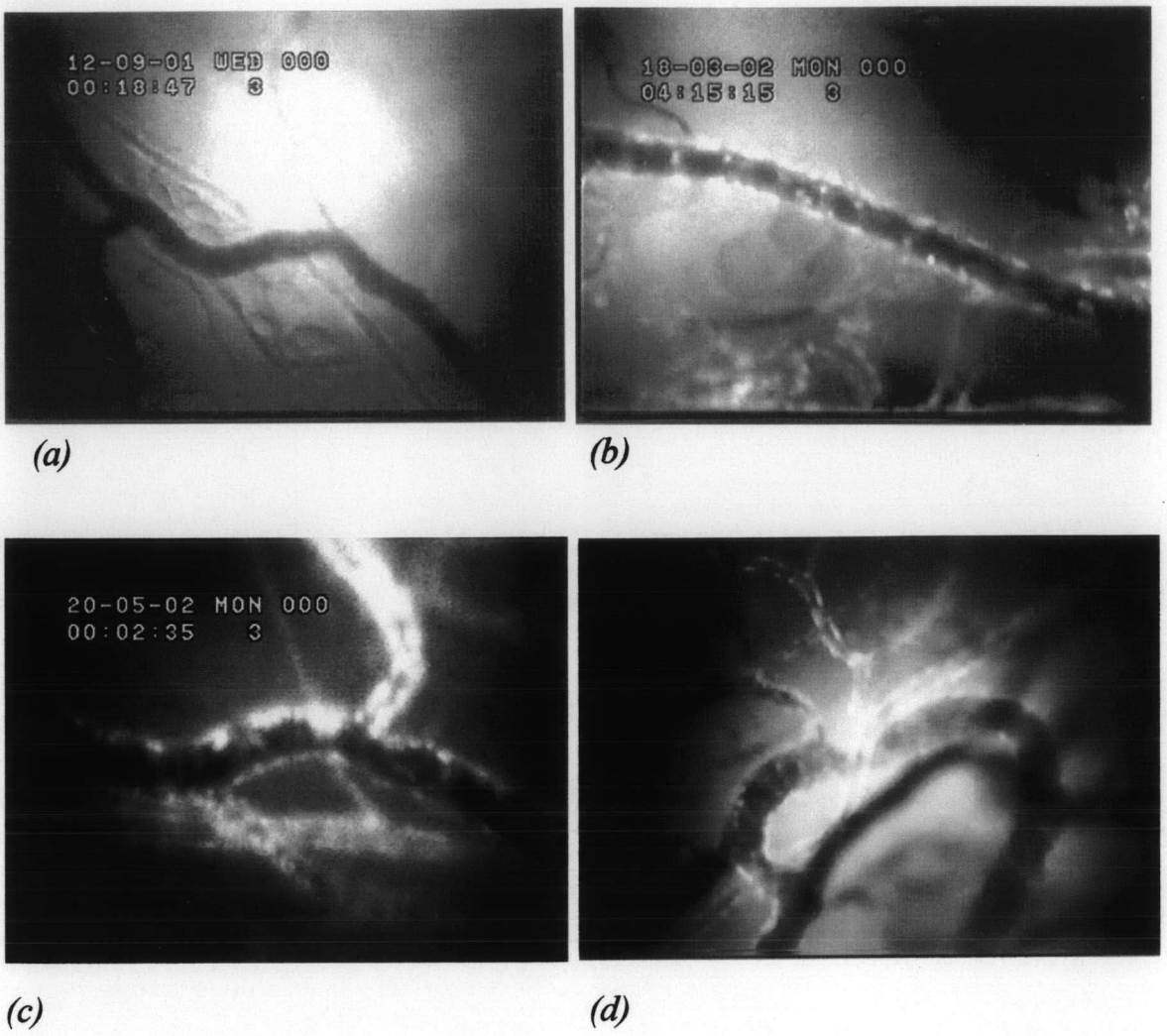


Figure 4.9 *Intravital microscopic demonstration of leukocyte adhesion on the postcapillary venule (PV) 7 days postburn of a) CON, b) BURN, c) BURN-NSS, d) BURN-ALOE. White dots represent leukocytes stained by intravenously- injected of the fluorescein marker, acridine orange.*

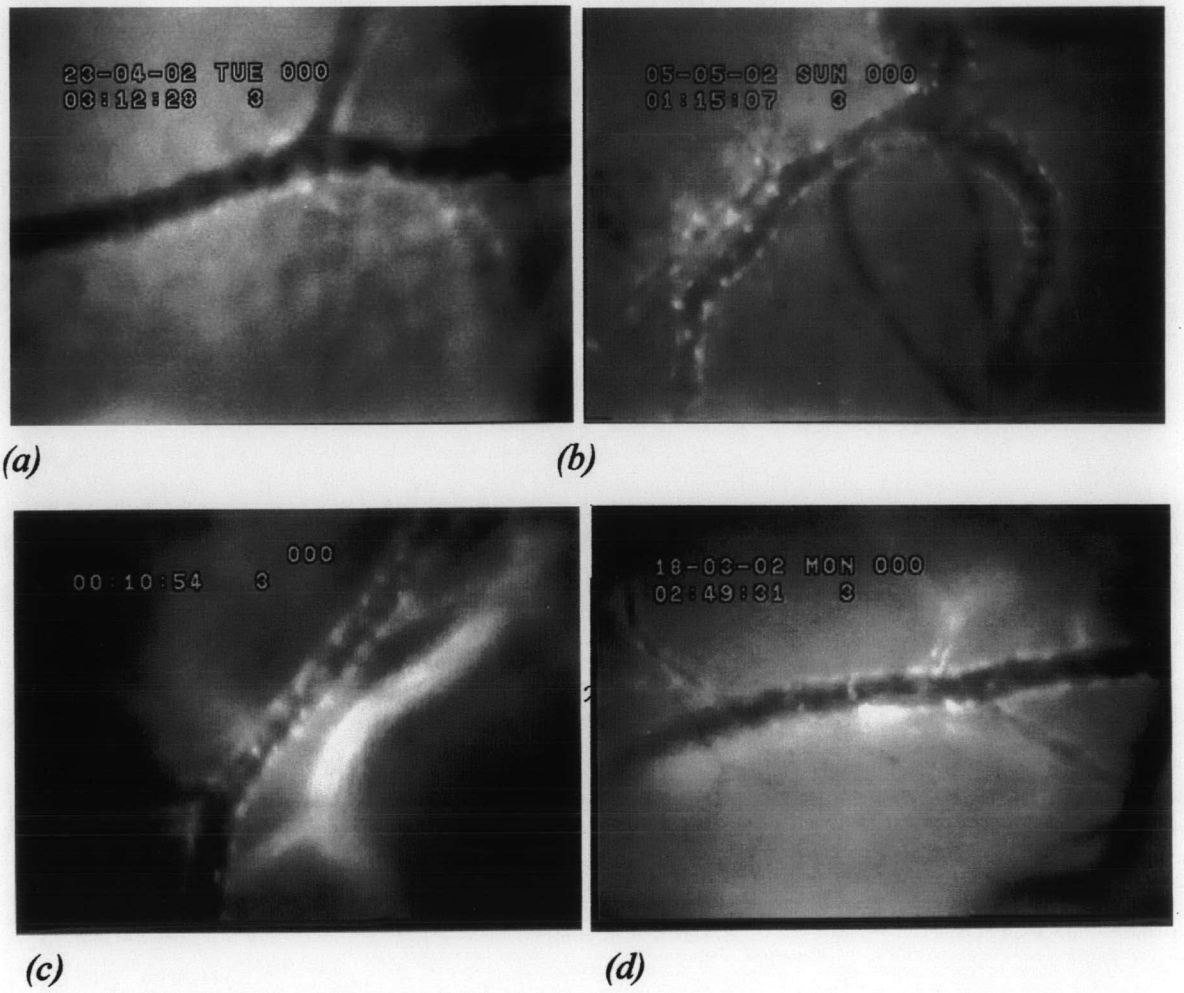


Figure 4.10 *Intravital microscopic demonstration of leukocyte adhesion on the postcapillary venule (PV) 14 days postburn of a) CON, b) BURN, c) BURN-NSS, d) BURN-ALOE. White dots represent leukocytes stained by intravenously- injected of the fluorescein marker, acridine orange.*

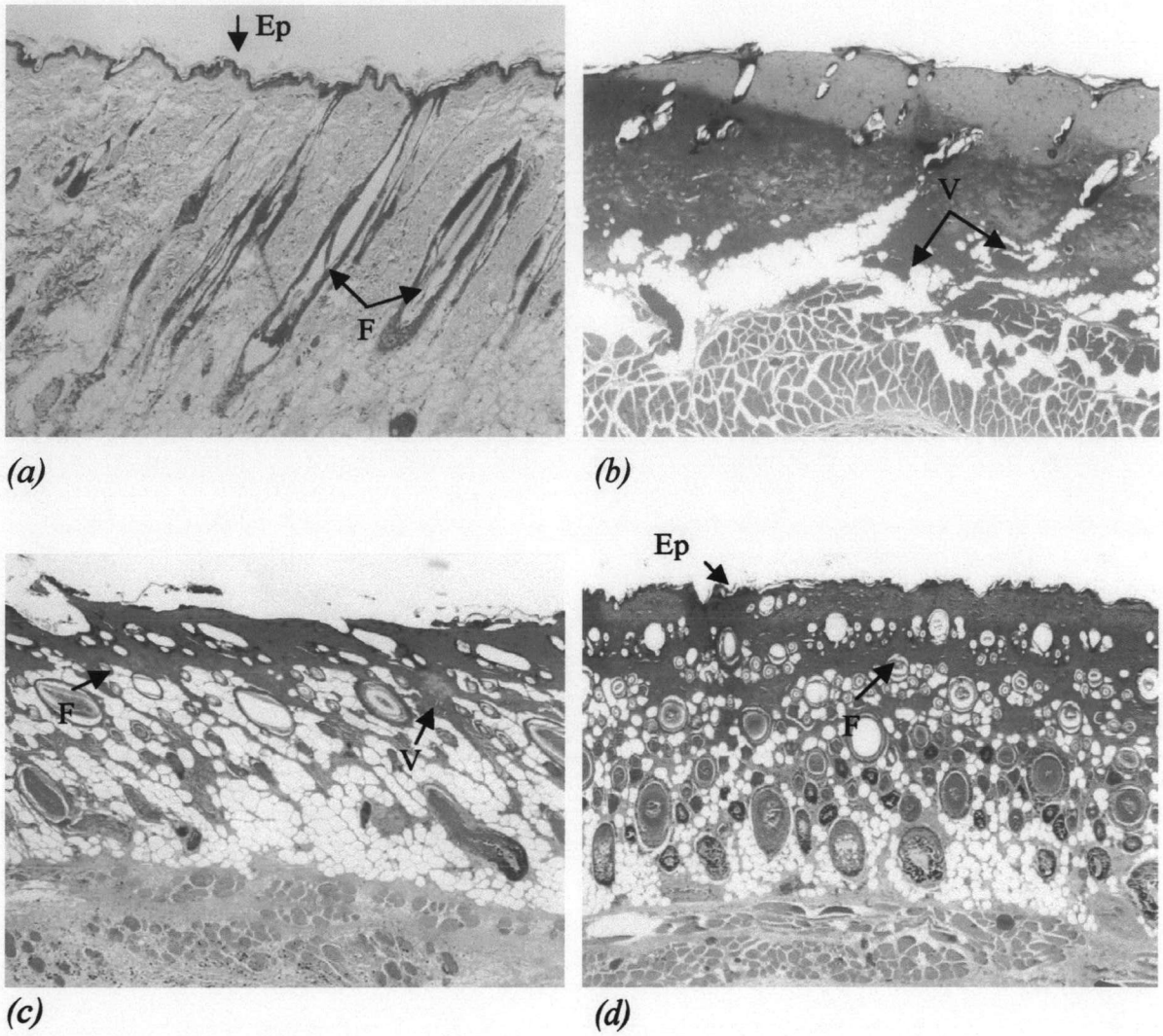


Figure 4.11 *Haematoxylin - eosin stain. Histological change of skin section 3 days postburn of a) CON, b) BURN, c) BURN NSS, d) BURN-ALOE; Ep=epidermis, V= blood vessel, F= hair follicle (Original magnitude × 40)*

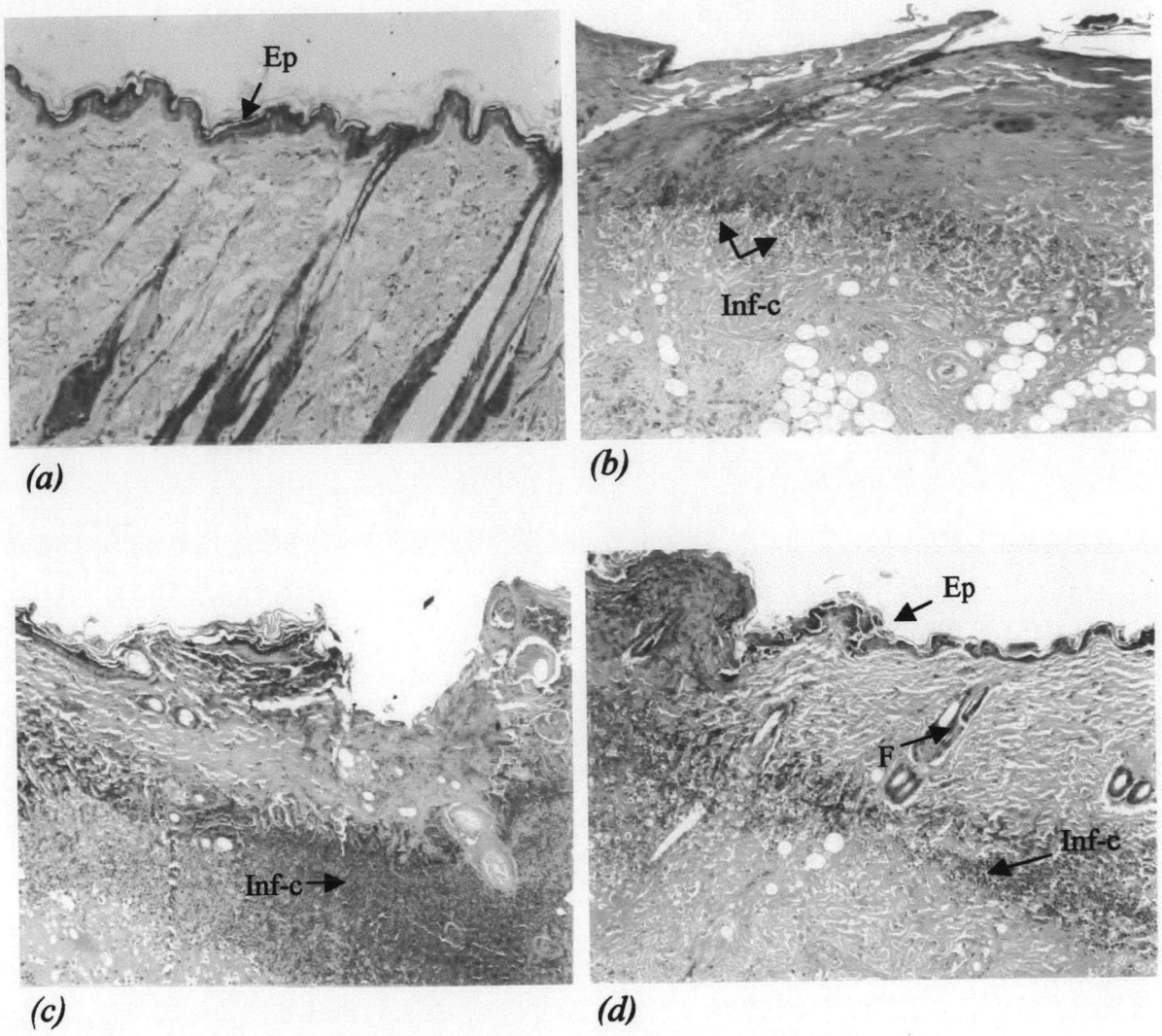


Figure 4.12 *Haematoxylin - eosin stain. Histological change of skin Section 7 days postburn of a) CON, b) BURN, c) BURN-NSS, d) BURN-ALOE; Ep=epidermis, V= blood vessel, F= hair follicle, Inf-c=inflammatory cell (Original magnitude × 100)*

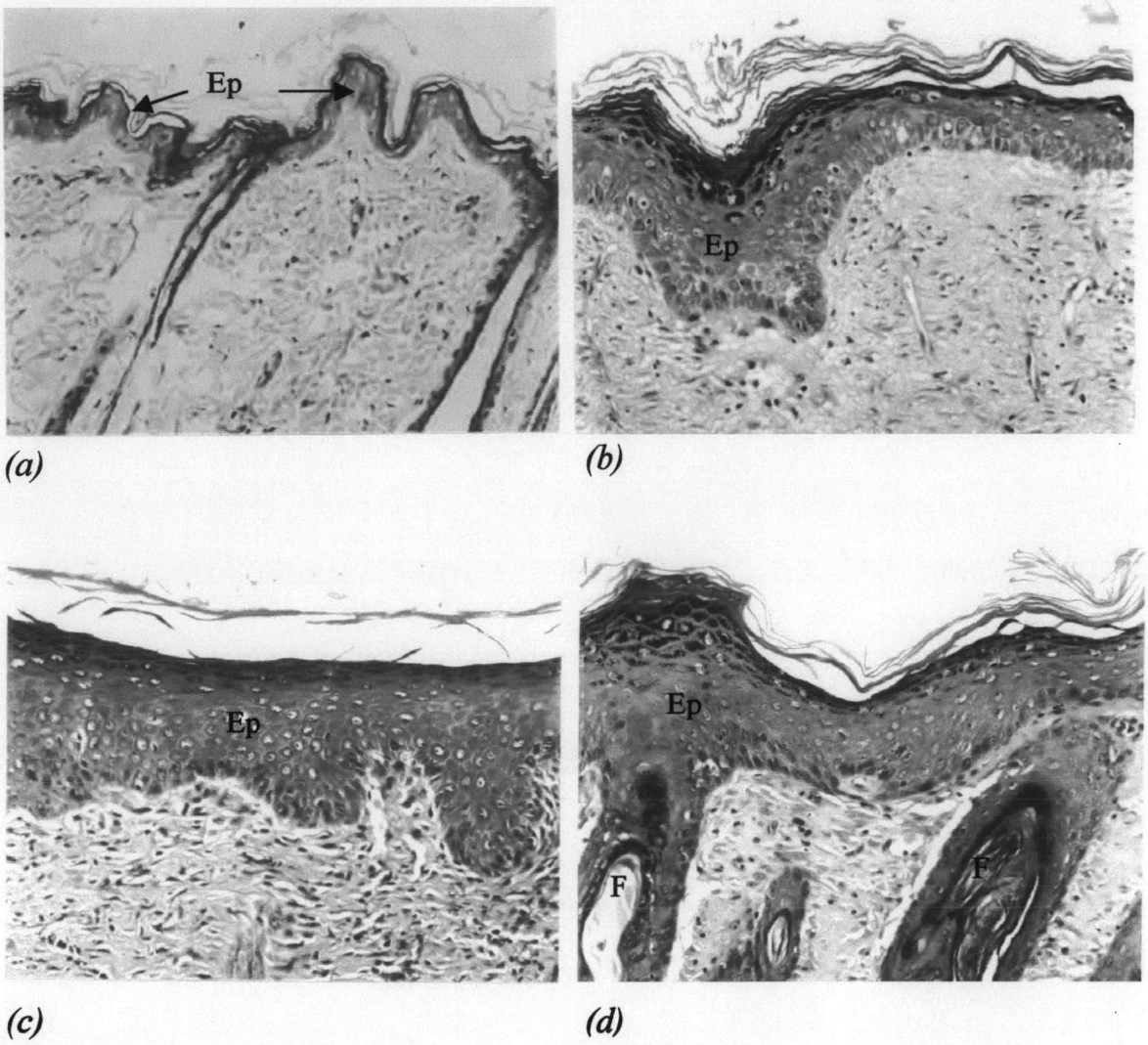


Figure 4.13 *Haematoxylin - eosin stain. Histological change of skin section 14 days postburn of a) CON, b) BURN, c) BURN-NSS, d) BURN-ALOE ; Ep=epidermis, F= hair follicle (Original magnitude × 200)*