manufactures. In brief, the ELISA plate was pre coated with anti rat TNF-α monoclonal antibodies overnight at 4°C and blocked with 3% bovine serum albumin (BSA). Plate was incubated for 2 h with different dilutions of standard TNF-α and serum samples. Anti rat TNF-α biotin conjugated monoclonal detection antibodies were added in each well and incubated for 2 h. Plate was again incubated after adding avidin –HRP conjugate for 90 min. The colour was developed by TMB and the reaction was stopped with 2N H_2SO_4. The reading was taken at 450-560 nm dual wavelengths. The concentrations in control and *P. acerifolium* treated animals were estimated by standard curve of TNF-α.

Effect of *P. acerifolium* extract on lipopolysaccharide (LPS) induced TNF-α production

Rats were divided into two groups of six animals each. Group A included *P. acerifolium* extract fed rats as described above. Group-B animals served as control. Lipopolysaccharide (LPS) 1 mg/kg body weight in 100 µl normal saline (vehicle) was injected ip. After 48h, blood was collected by retro orbital route. Collected blood was centrifuged and the serum was used for the determination of TNF-α induction by sandwich ELISA.

Statistical analysis

Results were expressed as mean ± SEM and analysed using sigmastat trial version. The statistical differences, between groups in terms of the mean of wound healing, were calculated using student's t- test. *P* values < 0.05 were considered statistically significant.

RESULTS

Effect of *P. acerifolium* extract on the rate of healing

Wound inflicted on group-2 and group-3 was found to be epithelized faster in 14 and 11 days respectively as compared to control animals where the wound was healed in 20 days. Days required for healing in group-2 and group-3 were significant ($P<0.001$) in respect to group-1. The results indicated that *P. acerifolium* flower extract promoted wound repair (Fig. 1). In group-3 animals the healing was significant in respect to group-2 animals indicating the *in vivo* potential of *P. acerifolium* flower extract.

Effect of *P. acerifolium* on TNF-α production during healing

The results indicated that TNF-α level increased during 12 to 48 h. TNF-α was augmented in *P. acerifolium* fed rats as compared to control rats. The concentration of TNF-α induction was maximum after 24 h of wound formation and reduced after 48 h in control and topically applied, unfed animals while it sustained in *P. acerifolium* fed animals (Fig. 2)

Effect of *P. acerifolium* on LPS Induced TNF-α production

TNF-α production in serum was found to be significantly ($P<0.001$) enhanced in the *P. acerifolium* fed animals after 2h of LPS stimulation (from 600 pg/ml in *P. acerifolium* fed animals).

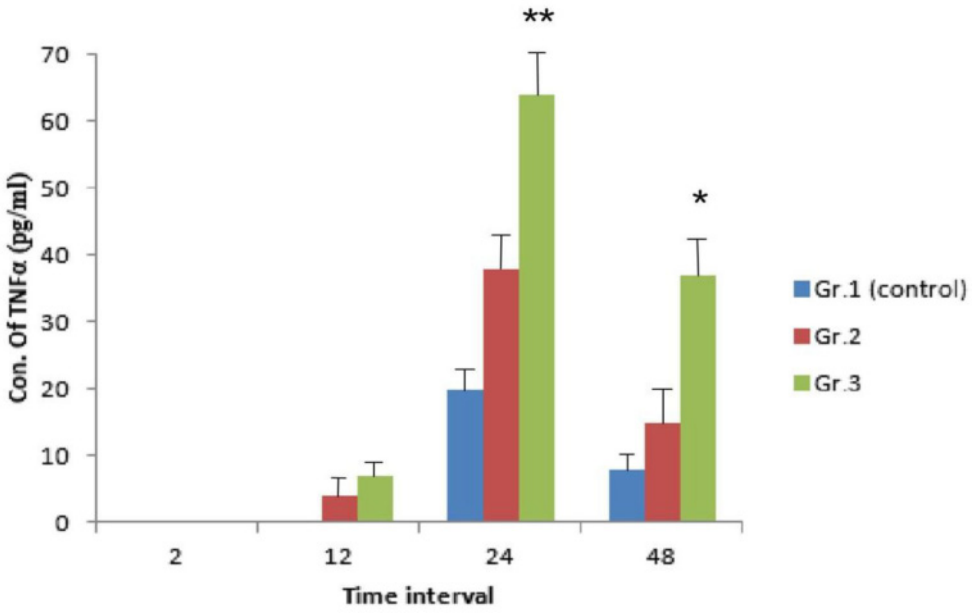
DISCUSSION

The results showed that topical application of *P. acerifolium* in petroleum jelly, with or without oral feed of *P. acerifolium*, accelerated wound healing and TNF-α production. However the rats fed with *P. acerifolium* extract along with topical application of the extract (group-3 animals) exhibited more rapid wound healing process as compared to group-2, indicating that *P. acerifolium* may have some ingredients for regulatory mechanism due to which there was an acceleration in wound repair. All stages of wound repair are controlled by a wide variety of different growth factors and cytokines [17, 18]. Beneficial effect of many of these growth factors e.g. platelet derived growth factors (PDGFs), fibroblasts growth factors (FGFs) and granulocytes macrophages colony stimulating factors (GM-CSF) on the healing process have been demonstrated [19-22].TNF-α, a macrophage derived cytokine, is also known to play a major role in the inflammatory phase of wound healing by enhancing angiogenesis [15]. Hubner et al. [23] also revealed that during the early phase of wound repair, TNF-α was predominantly expressed in polymorphonuclear leukocytes suggesting a normal function of these cells in the initiation of wound healing.

In the present study during wound healing TNF-α was detected after 12 h of wound infliction in *P. acerifolium* fed animals but remained undetectable in unfed animals. After 24 h, TNF-α level increased and reached maximum in *P. acerifolium* fed animals. The level of TNF-α declined after 24 h of wounding in unfed animals while remained static up to 48 h in *P. acerifolium* extract fed animals. It is thus apparent that *P. acerifolium* extract, during the first stage of healing, increased the TNF-α production from polymorphonuclear leucocytes and macrophages. Hubner *et al.* [23] also observed a strong and early induction of TNF-α and IL-1α and β after cutaneous injury and highest level of these cytokines were seen as early as 12-24 h after wounding. This supports the present finding that TNF-α increased up to 24 h of wounding and later down regulated after 48 h. In wound repair, expression of proinflammatory cytokines; IL-1α, IL-1β, IL-6 and TNF-α was shown to be strongly up regulated during the inflammatory phase of healing [24, 25].

To support the view that up regulation of TNF-α was due to the *P. acerifolium* extract feeding, induction of TNF-α by LPS mitogen was also studied and it was found that after 2 h of post LPS administration there was a significant rise in TNF-α production in *P. acerifolium* fed rats as compared to control rats and the TNF-α level returned to its basal level with in 24 h.

Figure 2: Effect of P. acerifolium on TNF- production after different time interval of wound formation. During the wound healing process at 24 h, TNF- production was significant. **P<0.01 (Gr.3 vs Gr.1); *P<0.05 (Gr.3 Vs Gr.2). At 48 h, concentration of TNF-α was significant. *P<0.05 (Gr.3 vs Gr.1) and *P<0.05 (Gr.3 vs Gr.2).



However, there are reports [26,27] that TNF-α inhibits collagen formation and hydroxyproline production which are essential for the final part of proliferative phase in wound healing, but the low TNF-α level after 48 h did not interfere with the collagen formation and hydroxyproline production.Antioxidant property of *P. acerifolium* and presence of flavonoids which scavenges the free radicals helps in the healing of wounds [12-14].

CONCLUSION

From the above study it was concluded that the *P. acerifolium* has a good wound healing potential. The accelerated healing process and induction of TNF-α by *P. acerifolium* extract may be the mechanisms involved in wound healing processes.

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