

Effect of andrographolide on the pathological events during the surgical open wound healing process

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Abstract

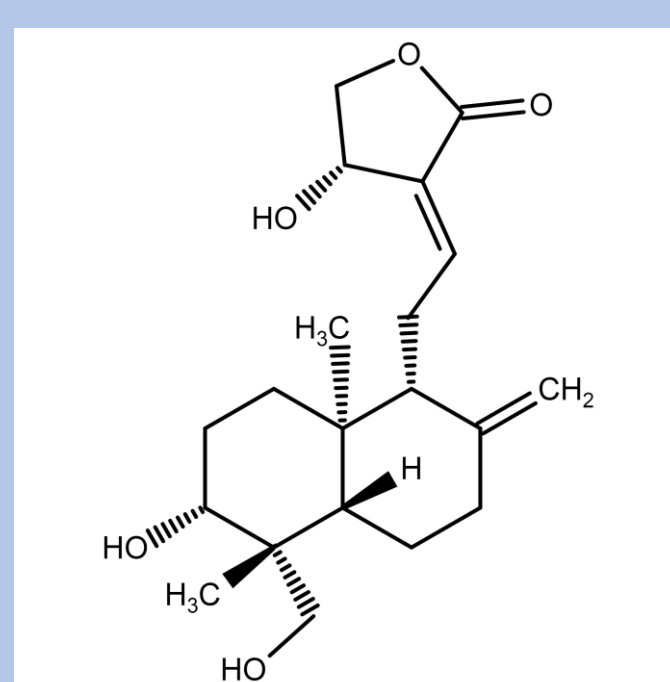
Wound healing is an important physiological process and several natural and synthetic drugs are being tested to speed up the process to avoid infection and other undesirable pathological events. Although andrographolide has been reported to be an excellent bioactive compound that can influence multiple pathways in the cells, its wound healing property has not been widely appreciated. In this study, we wanted to exhibit the improvement in the pathological events concerning wound healing process by injecting andro in a surgical wound model in rats. The right paw of female SD rats was operated and after 5 days, pathological events in the operated right paw was observed by staining the sections using hematoxylin & eosin dyes to elucidate the dermal and epidermal changes, while Mason's trichrome staining was performed on the sections to show the granulation layer formation and provide supporting evidence for angiogenesis and ECM deposition. Pathological score was given to the sections according to the extent of the changes observed and the results suggested that epidermal changes were scored almost similar in both saline and andro injected rats (2-3), while angiogenesis and inflammation were moderately improved in favor of wound healing in the andro injected rats (2.5 & 3.7 respectively) compared to saline-injected rats (3 & 4 respectively). The results clearly suggested that, though the improvement due to andro injection was moderate, these observations might create an awareness regarding the wound healing property of andro. Further studies are required to optimize the dosage of andro to influence the molecular pathways in order to show a better wound healing activity.

Background

Andrographis paniculata is one of the popularly known medicinal herbs with numerous bioactive compounds. The plant is widely known as "King of bitter" and this is mainly because of its high terpenoid content



Andrographis paniculata
Image source: <https://www.123rf.com>



Andrographolide (Andro)

It is the major constituent with more than 1% of the whole plant material. Andro and structurally similar terpenoids were explored for many biological activities

In the current study, we aim report the effect of andro on specific pathological events of the wound healing process in a surgical wound model rat hind paw.

Methods

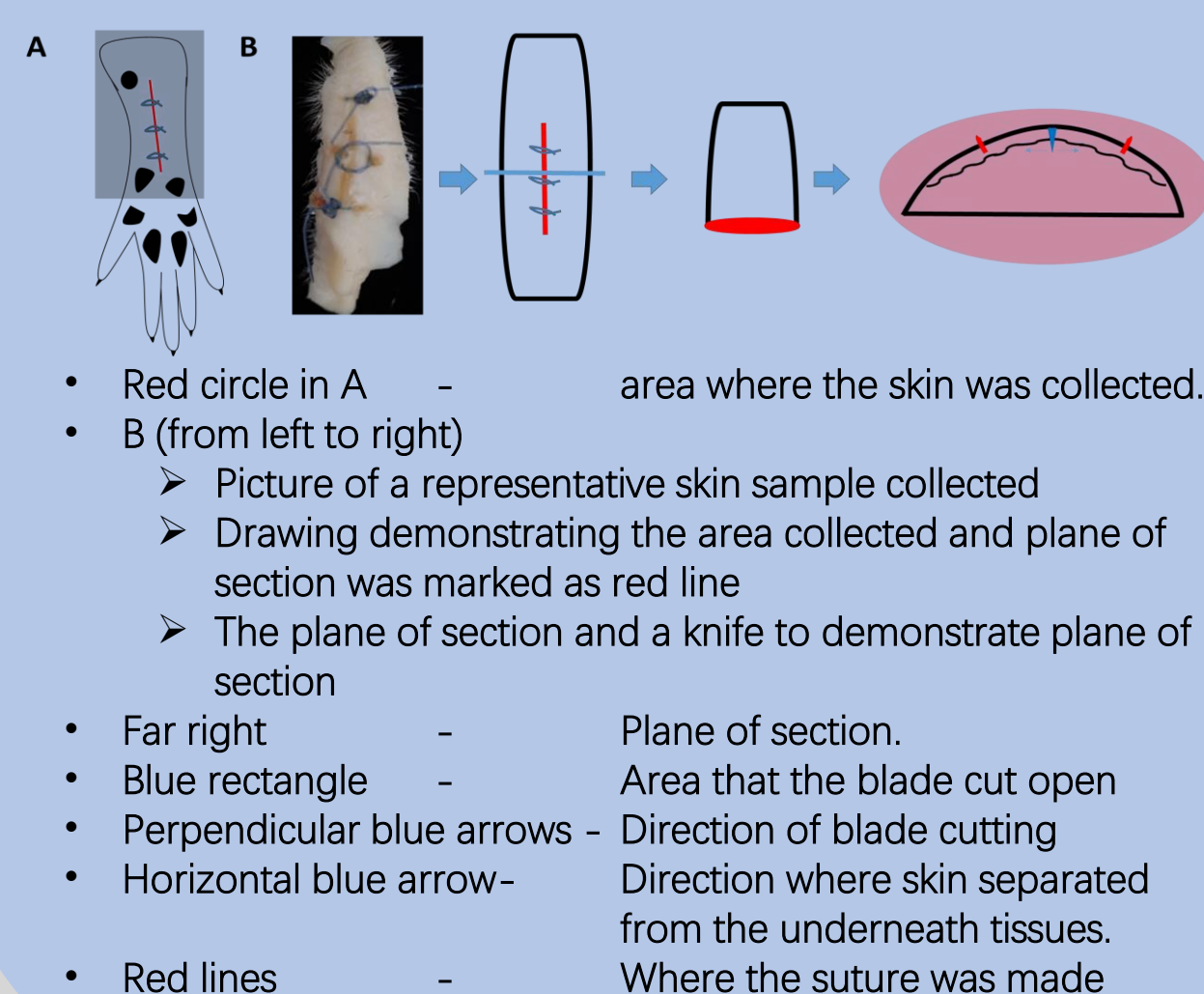
Surgical procedure

A longitudinal incision of 1 cm length using surgical blade No. 11, was made on the medial plantar surface of the right hind paw.

The incision began 0.5 cm distal from the end of the heel to the first set of footpads after which the skin, fascia, and plantaris muscle were all elevated using forceps.

The epidermis and dermis were separated by scissors in the area directly underneath the wound and then the skin was closed with 5-0 nylon sutures.

After surgery, the animals were allowed to recover from the anesthesia in their cages.



Pathological studies

Animals were sacrificed by bloodletting
 Paw skin was collected from both the operated and non-operated feet
 Fixed by 4% paraformaldehyde in 1x PBS overnight
 Treated with graded alcohol to dehydrate, followed by embedding in a wax block
 Stained with hematoxylin-eosin (HE) and masons trichrome stain (MT)

Results

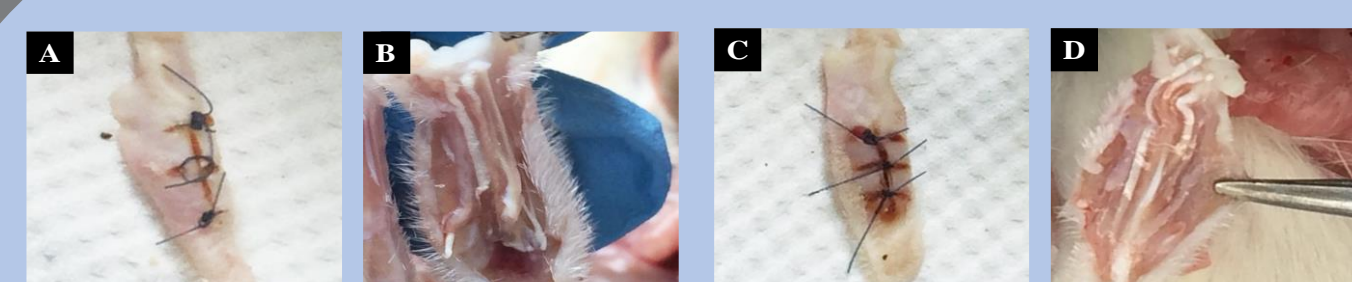


Figure 1: Gross anatomy of the incisional surgical wound in the right foot collected on day 4 after surgery. Saline injected (A & B); Andro injected (C & D).

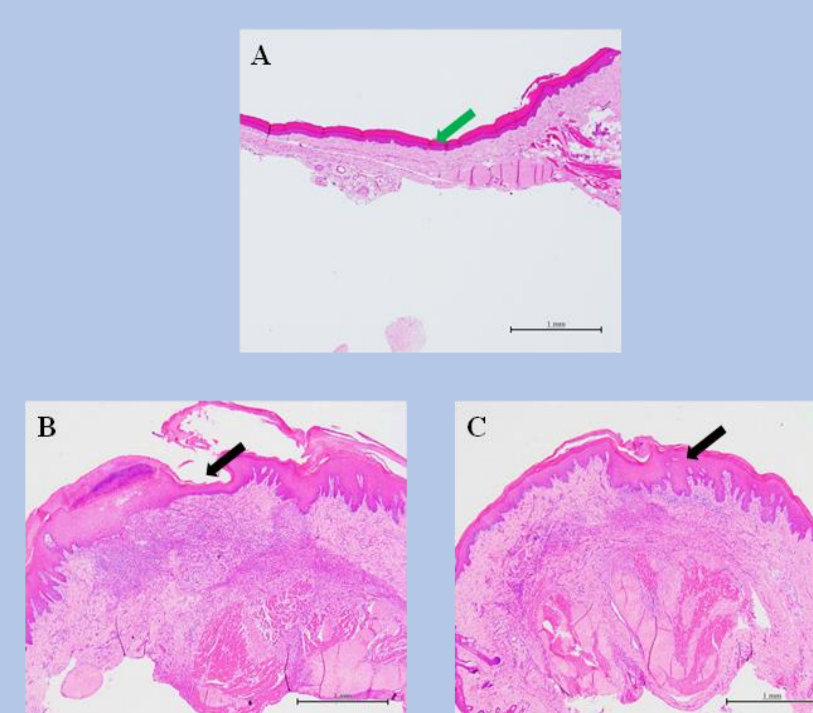


Figure 2: H & E staining of foot tissue sections of saline and andro injected animals showing changes in the epidermal region. Control without surgery (A); Saline injected after surgery (B); Andro injected after surgery (C). The images clearly show re-epithelialization (Black arrow) in andro and saline-injected animals. The normal epithelium is indicated with a green arrow in the un-operated paw.

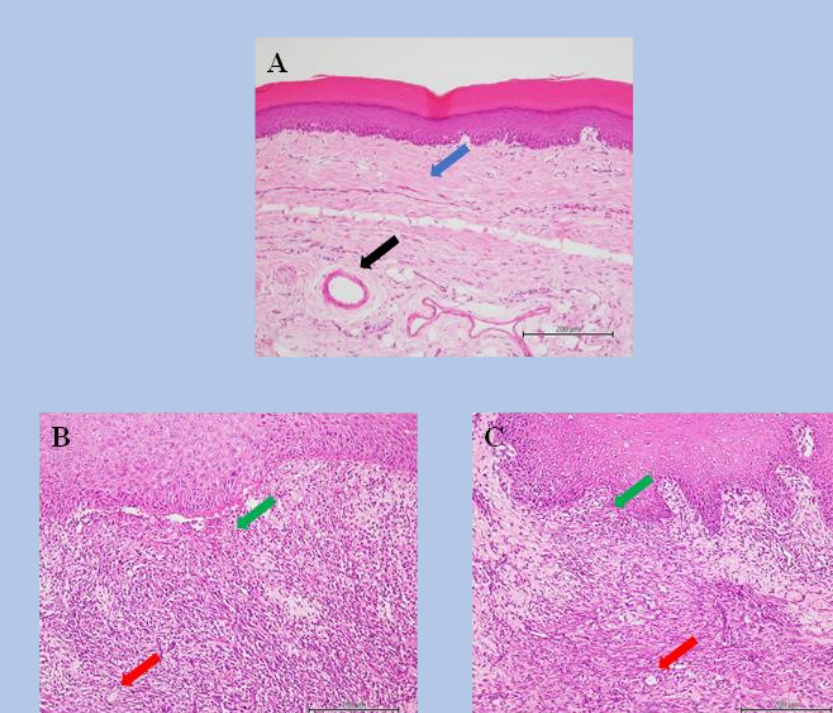


Figure 3: H & E staining of foot tissue sections of saline and andro injected animals showing changes in dermis region. Control without surgery (A); Saline injected after surgery (B); Andro injected after surgery (C). Figure 3A depicts minimal infiltration of inflammatory cells (Blue arrow) & intact blood vessels (Black arrow) in un-operated paw. Figure 3B & C clearly show the infiltrated inflammatory cells (Green arrow) in saline (Grade: 4) and andro (Grade: 4) injected animals. Newly formed blood vessels (Red arrow) in saline (Grade: 3) and andro groups (Grade: 3).

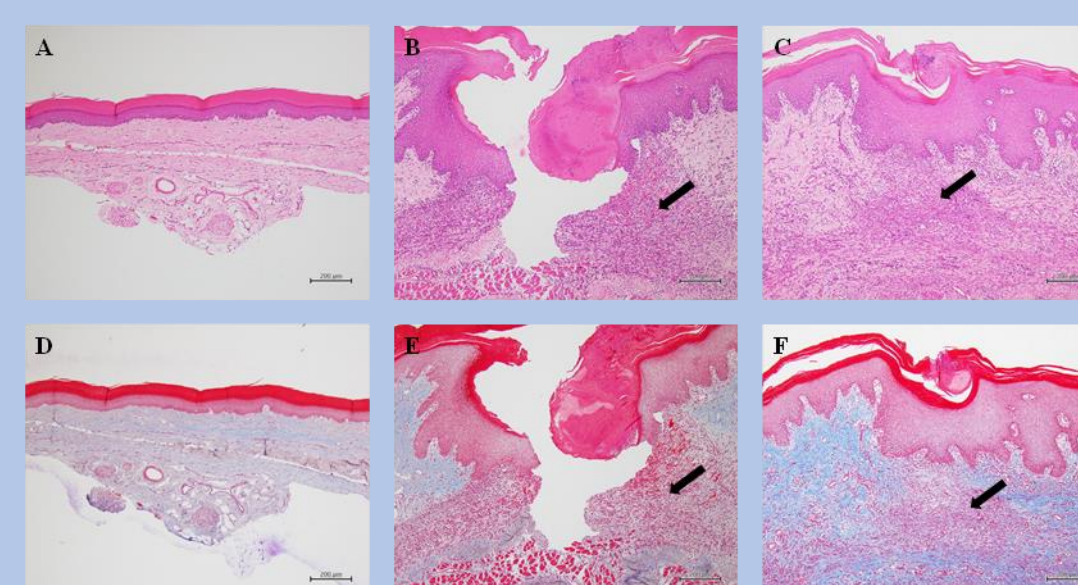


Figure 4: H & E (A, B & C) and Mason's trichrome (D, E & F) staining of foot tissue sections from saline and andro injected animals showing granulation in the dermis region. Control without surgery (A & D); Saline injected after surgery (B & E); Andro injected after surgery (C & F). Granulation layer (Black arrow) was clearly shown in saline (Figure 4B & E; Grade: 2) and andro (Figure 4C & F; Grade: 2) treated groups respectively.

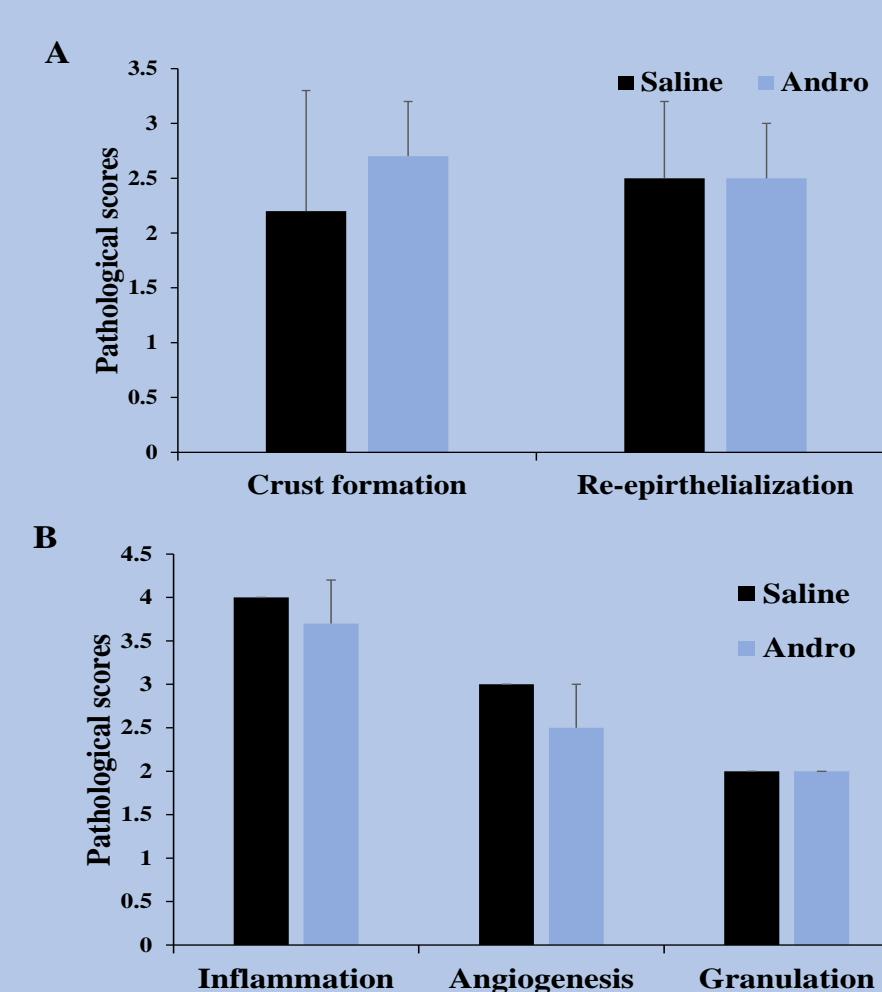
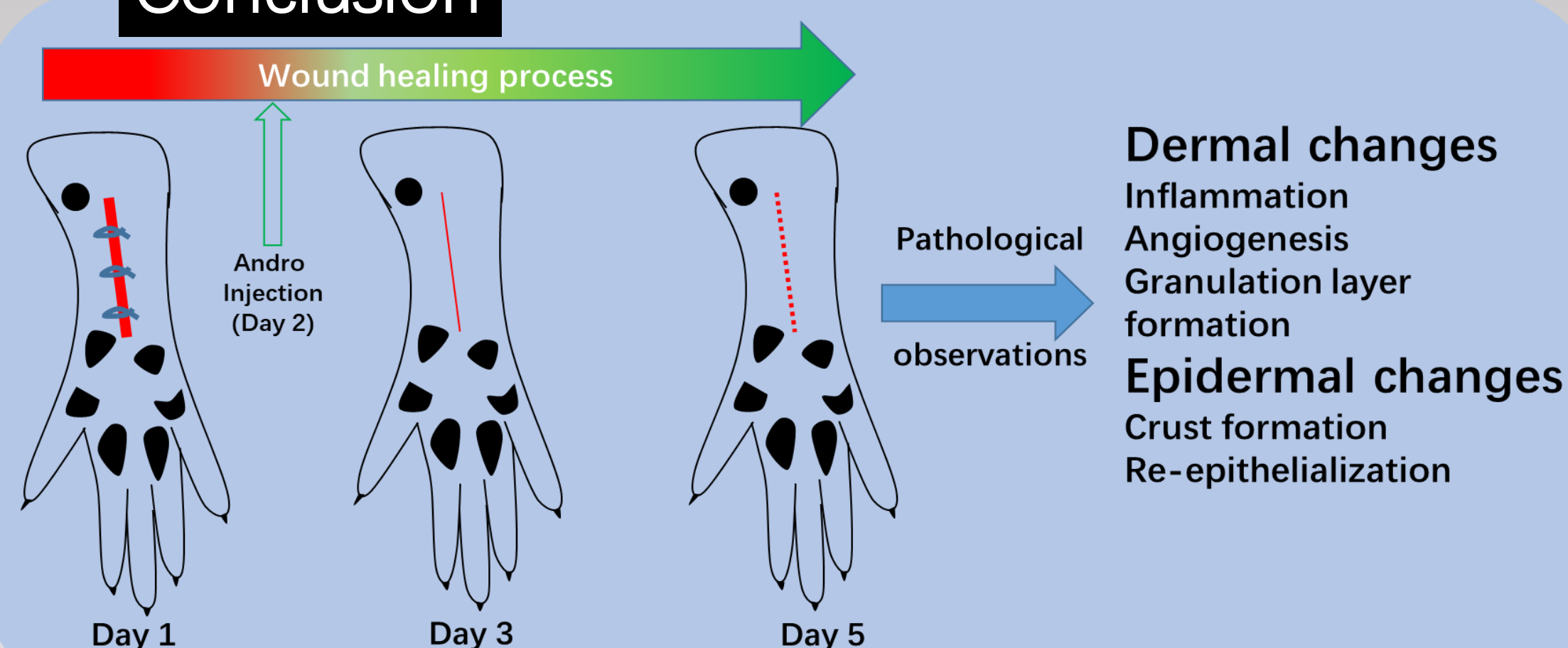


Figure 5: Pathological score of control, saline-injected and andro injected rats

Conclusion



Summary

- The pathological events pertaining to wound healing were observed in the study and at the epidermal region, crust formation and re-epithelialization were prominently illustrated in saline and andro injected animals.
- Similarly, dermal architecture was also observed to have similar changes between the two groups, but the extent of angiogenesis and inflammation showed slightly better improvement in the andro injected animals' tissue.
- Andro has moderately increased blood vessel formation than the saline-injected animals. On the other hand, mild and less significant reduction in inflammation due to andro injection, which also was in favour of the wound healing process which was slightly higher in the saline group.

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