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Evaluation of Wound Healing and Burn Healing Activity of Ethanolic Fruit Extract of Nyctanthes Arbor-Tristis

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KEYWORDS
Excision wound, Burn wound, Nyctanthes Arbor-tristis, Wound healing, Burn healing, NAT, Ointment

ABSTRACT:
To study the wound healing potential of Nytanthes Arbor-tristis, the ethanolic fruit extract was used for evaluation of excision and burn in Wistar albino rats. Powdered crude material was extracted with 95% ethanol in soxhlet apparatus. Ointment containing 5% w/w ethanolic fruit extract of NAT was applied once daily to test groups of excision and burn wounds groups. The percentage of wound contraction and degree of healing were assessed at day 4, 8, 12 and 16, till complete closure of wounds. The extract ointment treated test group of excision and burn wound groups significantly increased wound healing and decreased epithelization time as compared to standard and control groups. Hence, 5%w/w fruit extract of NAT could be used for treatment of both types of wounds.

1. INTRODUCTION

Herbal plants are richest source of various bioactive components, which having various pharmacological activity. Now these days, peoples are turning back to use herbal medicines for treatment of a number of diseases (1).

Skin is the principle organ of the body which play major roles in a number of functions such as thermoregulation, protection from external environment, vitamin D synthesis, excretion and absorption of substances and prevention from direct microbial entrance into the body (2).

A skin wound is the disruption or loss of epithelial integrity and damage to underlying tissue due to any physical, chemical or mechanical stress to the skin(3).

Burns, surgery, trauma, and other external events as well as internal factors including problems associated with the local blood supply can result in wounds(4).

Healing of wound is a protective and biological processes in the body usually begins after Post-wounding days and completed within few months followed by different healing stages(5).

Nyctanthes Arbor-tristis (NAT) is an ornamental plant belonging to family Oleaceae. It is a terrestrial, woody long standing plant that gets larger up to 10 meters height and has highly scented flowers that bloom at night and fall off before daylight. This plant constitutes a number of therapeutic activity such as anticancer, antidiabetic, antihypertensive, hepatoprotective, antibacterial and antioxidant activity (6).

Till now, no study had been reported to show wound healing and burn healing activity of fruit extract of NAT. The current study was undertaken to evaluate the excision wound and burn wound healing activity of fruit extract of NAT using rat model.

2. MATERIAL AND METHODS

2.1. Collection and authentication of plant

The fruits of Nyctanthes arbor-tristis were collected from rural area of district Bhadohi, Uttar Pradesh, India, in October 2022. The plant was identified and

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authenticated at Botanical survey of India, Prayagraj, Uttar Pradesh, under the guidance of Dr. Arti Garg (Scientist- E and Head of office).

2.2. Selection of Animals

Healthy Wistar Albino rats (150-180g) of either sex (male or female) were selected for study. All animals were caged under standard condition at 25±2ºC of 12:12 hours day and night cycle with free access to water and standard food supply. The experiment was carried out according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) now becomes as Institutional Animal Ethics Committee (IAEC) with reference number (IAEC/008/03/23) guidelines.

2.3. Preparation of extract

A mechanical grinder was used to ground the dried fruit extract of Nyctanthes Arbor-tristis into coarse powder. 500g of powdered material was heated continuously in a Soxhlet apparatus and extracted with 95% ethanol. The solvent was heated to between 40 and 45ºC in a rotary evaporator before being concentrated over a water bath to form a semisolid substance (7).

2.4. Preliminary phytochemical screening

Ethanolic fruit extract of NAT were tested for presence of various components such as alkaloids, glycosides, sterols, carbohydrates, tannins and phenolic compounds (8),(9).

2.5. Thin layer chromatographic analysis

The slurry of silica gel-G was spreading out to a glass plate to make TLC plate and was dried for 30 minutes in the air, and then activated it by heating at 110°C in a hot air oven. This was done for the thin layer chromatographic examination of the ethanolic fruit extract of NAT. Using a capillary tube, the material was spotted from the 2 cm margin. The plate was put in a saturated TLC chamber with a mobile phase that included 8.1: 1.1: 0.8 ethyl acetate, methanol, and water. Anisaldehyde-H_2SO_4 spraying is used to identify phytoconstituents, which are then visible at 254 nm. Spot color and Rf values are contrasted with the standard drug (10), (11).

2.6. Preparation of ointment

The formulation of an ointment containing 5% w/w NAT extract followed the steps indicated by Demilew et al. (2018). In a beaker over a water bath, all the components (Table-1) were heated in a decreasing order of melting points while being constantly stirred. After cooling in a mortar, the mixture was continually triturated with a pestle to achieve homogeneity. After that, 10g of the extract was added to a small quantity of ointment base, and it was triturated. The leftover ointment base was added and continually mixed at the last stage of the procedure, then stored in a clean container for topical treatment during the experiment (12).

Ingredients	Quantity used
Wool fat	10g
Hard paraffin	10g
White soft paraffin	170g
Cetostearyl alcohol	10g

Table-1 Composition of ointment.

2.7. Acute dermal toxicity study

Acute dermal toxicity of formulation was assessed as per OECD guidelines number 402. The patches of 1cm^2 were marked on the back skin surface of the animal. The dorsal back regions of animals were wrapped with maximum concentration of 5%w/w formulation for 24 hours. After that the patches were exposed and observed the skin for any toxic dermal effects. A repeated observation after 72 hours was made to any physical changes (13).

2.8. Grouping and dosing of animals

A Total 30 healthy Wistar albino rats (weight about 150 to 180g) of either sex (male or female) was divided randomly into six groups (Table-2). Each group having 5 animals (n=5), acclimatized for 7 days in clean, sterile propylene cage with free access to standard food and water supply till the end of the experimental period.

	Animal Group	Received Treatment
For Excision wound		
Group- I	Standard Group	5% Povidone-iodine (Betadine)

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		ointment
Group- II	Control Group	Normal saline
Group- III	Test Group	5% ointment containing NAT fruit extract
For Burn wound		
Group- I	Standard Group	1% Silver sulfadiazine ointment
Group- II	Control Group	Normal saline
Group- III	Test Group	5% ointment containing NAT fruit extract

Total 30 animals were used. Where, n=5.

Table-2 Grouping and dosing of animals for wound healing assessment.

3. Induction of wound

3.1. Excision wound

Three groups of healthy animals were rendered unconscious by intraperitoneal administration of Ketamine Hcl 80mg/kg, and shaving cream was used to remove hair from the dorsal back area, which was then sterilized with 70% v/v ethanol. The rats' dorsal thoracic area is then exposed, and 400 mm2 circular sections are removed with a surgical blade and scissor. Following that, the animals were separated and kept in a clean, disinfected cage. Day zero is the day of wound induction (14).

3.2. Burn wound

The dorsal back hairs of the three groups of rats was removed by using shaving cream, after they had been given 80 mg/kg of Ketamine Hcl by intraperitoneal route to induce anesthesia. 70% v/v ethanol is used to sterilize the shaved area. The wound was inflicted with the use of a metal plate that was heated for 30 seconds and had a diameter of around 300mm^2 to produce second degree burn wound. Animals were housed separately in a clean and sterilized cage. Day 0 was accounts for wound induction day (15).

4. EVALUATION PARAMETER

4.1. Percentage of wound closure

After induction of excision wounds, the closure of wounds were observed by using transparent paper sheet and marker on days 4, 8, 12 and 16 and thereafter days till complete closure of wounds. A graph paper of 1mm^2 scale was used to measure the recorded wound closure. To calculate the percentage wound healing, recorded surface area of wound was used by taking initial wound size as 100% (16).

4.2. Degree of burn wound healing

After burn injury, the wound healing was assessed at day 4, 8, 12 and 16 thereafter until healing was completed. The color photographs of wounds were taken at respective healing days. The wounds area was measured using a caliper, transparent paper and marker followed by tracing wounds boundaries (17).

4.3. Epithelization period measurement

After wounding, the time period (day) required for complete healing of wound with falling stiffed outer layer leaving no traces of wound was considered as epithelization period. Epithelization of excision and burn wounds was measured by the total days required for complete wound healing (18).

4.4. Histopathological examination

Granulation tissue (0.50.5) from the center of the wound was removed and stored in a newly prepared 10% neutral formline buffer solution for 24 hours for histological evaluation of excision and burn skin wounds. Hematoxylin and eosin (HE) dyes were used to stain the tissue, which was then viewed using a light microscope with a 20x and 40x objective lens (19).

4.5. Statistical analysis

The data was expressed as mean ± standard error of mean (SEM). The statistical significance was assessed by using analysis of varience (ANOVA) two factor with replication followed by Tukey's multiple comparison tests for significance difference between groups and ($P < 0.05$) was considered as significant.

5. RESULT AND DISCUSSION

5.1. Physicochemical analysis

The powdered fruits of NAT were extracted by hot

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continuous soxhlet extraction method with 95% ethanol used as solvent. The extractive value of powdered drug was found 13.84% w/w.

Table-3 Physicochemical analysis of powdered fruit of NAT.

S.No.	Parameters	Result
1	Total ash	11.29 % w/w
2	Water soluble ash	3.81 % w/w
3	Acid insoluble ash	2.12 % w/w
4	Moisture content	7.14 % w/w
5	Extractive value	13.84% w/w

The powdered crude drug were subjected to various physicochemical analysis such as total ash, water soluble ash, acid insoluble ash and moisture content and results was significantly obtained as seen in (Table-3).

5.2. Preliminary phytochemical analysis

The preliminary phytochemical screening was done by treatment of solution of extract with different chemicals for the presence of phytoconstituents. The results confirms the presence of alkaloids, glycosides, tannins and phenolic compounds and carbohydrates, while absence of saponins and phytosterols.

5.3. Thin layer chromatographic analysis

The NAT extract was utilized for thin layer chromatographic analysis and six spots were visualized (Figure-1) and their RF values were recorded. The color of spots were found to yellowish brown (RF= 0.21), bluish black (RF= 0.38), blue (RF= 0.48), orange (RF= 0.56), yellow (RF= 0.62), pale yellow (RF= 0.92) respectively. The comparison of obtained RF values in available literatures confirms the presence of alkaloids, glycosides, tannins, phenolic compounds.

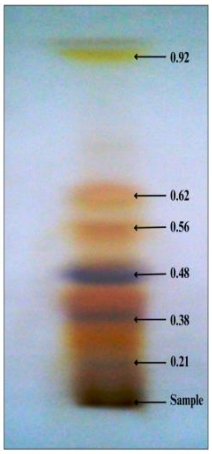

Figure-1 Thin layer chromatography of NAT extract.

5.4. Wound contraction of excision wounds

On day 4, wounds in the control, standard, and test groups were examined visually and found to have hard, pink-colored granulation tissue followed by moderate inflammation (Figure-2). The control group was reported to have 85.7% (Figure-3) better wound healing from day 4 to day 16 than the standard group.

According to (Table-4), the wound closure percentages for the control, standard, and test groups were 60.1%, 82.2%, and 85.75% on day 16. Maximum wound closure was more noticeable in the test group ($P < 0.02$) treated with NAT ointment 5% w/w fruit extract than regular saline treated group. When compared to the control group, the standard group receiving 5% povidone-iodine ointment significantly decreased the wound area on day 16 ($P < 0.0.04$). At day 16, the test group had considerably more wound closure than the control group ($P < 0.01$).

All wounds that were fully healed and showed no scab at the wound site had been considered to be at the epithelization phase. Control, standard, and test groups' epithelization times were 23, 19, and 18.8 days, respectively. Compared to other groups, the test group's epithelization duration was considerably shorter.

Table-4 Percentage of excision wound healing at different post wounding days.

Post wounding days	Control (Normal saline)	Standard (5% Povidone-iodine)	Test (5% extract ointment)
Day 0	394.0 ± 4.5 (0%)	391.6 ± 4.7 (0%)	387.0 ± 7.4 (0%)
Day 4	372.4 ± 4.4 (5.4%)	367.2 ± 4.8 (6.2%)	342.2 ± 16.1 (11.5%)
Day 8	318.6 ± 3.7	290.4 ± 6.5	266.4 ± 18.5

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	(19.2%)	(25.8%)	(31.1%)
Day 12	278.8 ± 2.0	198.0 ± 5.4	158.6 ± 9.1
	(29.2%)	(49.4%)	(59.0%)
Day 16	157.2 ± 3.7	67.2 ± 3.7	55.2 ± 2.6
	(60.1%)	(82.8%)	(85.7%)
Epithelization period (day)	23.0 ± 0.3	19.8 ± 0.3	18.8 ± 0.3

Values are expressed as Mean ± SEM; n=5, *P*<0.05.

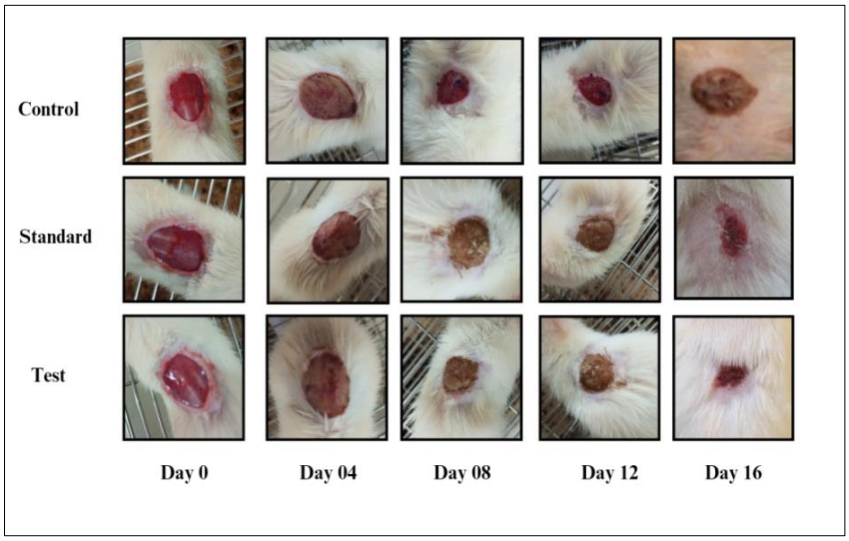

Figure-2 Excision wound healing.

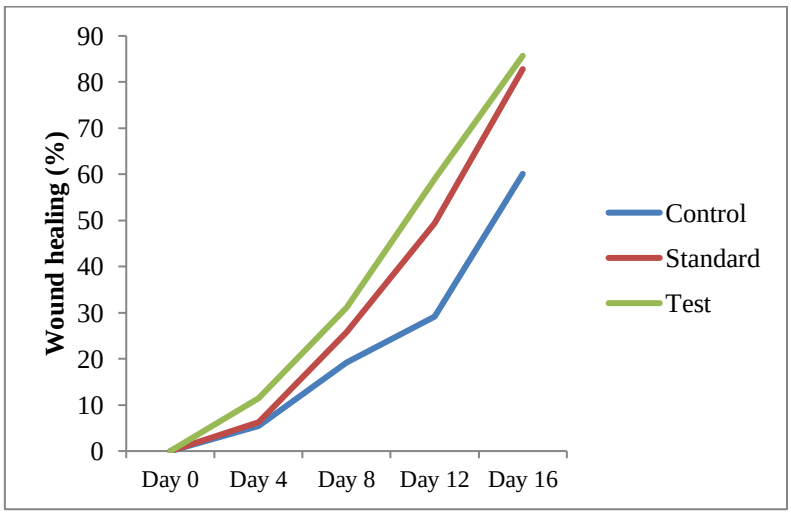

Figure-3 Percentage of excision wound healing

5.5. Degree of burn wound healing

On day 4, control, standard, and test groups were all visually evaluated, and it was found that all groups' wounds had mild inflammation and granulation tissue deposition. On day 16, the control group has a larger, brown-colored scab that is still mending unevenly over the site, but the standard and test groups have a smaller scab that is surrounded by hair growth (Figure 4).

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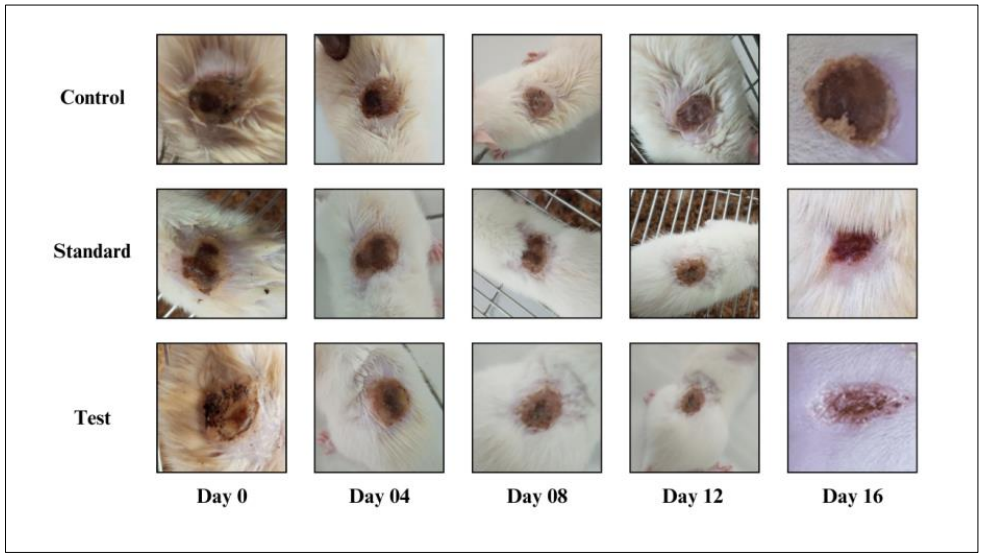

Figure-4 Burn wound healing.

When compared to the control group, the test group noted for better wound healing after using an ointment containing 5% fruit extract of NAT (Table-5) displays the development of wound contraction brought on by administration of normal saline, 1% w/w silver sulfadiazine cream, and 5% w/w ointment containing ethanolic fruit extract of NAT. Maximum wound healing was shown in the test group from days 8 to 12, whereas it was 74.9% and 78.3% healing of control group. On day 16, the test group's wound closure was 94.8%, compared to 88.4% for the standard group and 73.8% for the control group.

In comparison to the control and test groups, the normal saline-treated group exhibits delayed wound healing throughout the duration of the full study period ($P < 0.01$) and ($P < 0.03$).

The test group show highest wound closure percentage at day 16th as compared to control and standard group. In the test group, the wound was fully closed after 18.8 days, whereas it took 23 days and 19.8 days in the control and standard groups, respectively.

Compared to the standard and control groups, the test group's epithelization time was reduced by 18.8 days in significant manner. In comparison to the test group's epithelization period of 23 days, the standard group's time was much shorter (19.8 days).

Table-5 Percentage of burn wound healing at different post wounding days.

Post wounding days	**Control** (Normal saline)	**Standard** (1% Silversulfadiazine**)**	**Test** (5% Extract ointment)
Day 0	222.0 ± 3.0 (0%)	219.2 ± 5.8 (0%)	222.0 ± 3.0 (0%)
Day 4	193.2 ± 2.8 (12.9%)	185.0 ± 5.0 (15.6%)	185.0 ± 5.0 (16.6%)
Day 8	141.6 ± 2.4 (36.2%)	119.2 ± 7.3 (45.6%)	121.2 ± 6.2 (45.4%)
Day 12	84.6 ± 3.6 (61.6%)	55.0 ± 4.2 (74.9%)	48.0 ± 4.6 (78.3%)
Day 16	58.0 ± 4.2 (73.8%)	25.4 ± 3.5 (88.4%)	11.4 ± 4.9 (94.8%)
Epithelization period (day)	21.4 ± 0.4	17.8 ± 0.3	16.8 ± 0.3

Values are expressed as Mean ± SEM; n=5, $P<0.05$.

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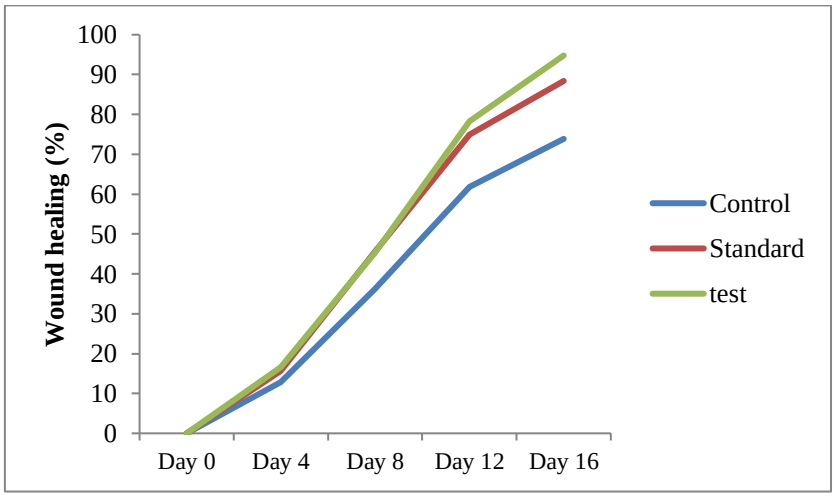

Figure-5 Percentage of burn wound healing.

Histopathology of excision wounds

On day 16th, all animals of excision wounds and burn wounds granulation tissue were removed for histopathology. Histological features of excision wounds are illustrated in (Figure-6).

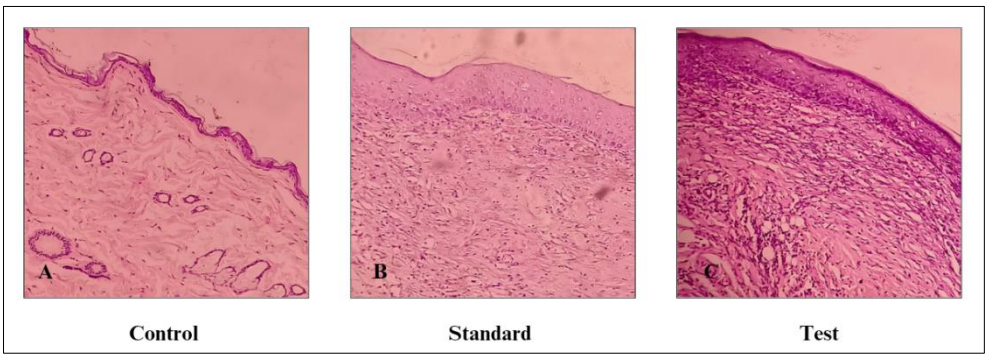

Figure-6 Histopathology of excision wounds.

The test group that received the 5% w/w extract ointment was demonstrating considerable wound healing by the presence of increased collagen, fibroblast, and neovascularization as well as a decreased number of inflammatory cells. There are fewer macrophages and the formation of mature granulation tissues seen. In comparison to the control and 5% w/w povidone-iodine treated standard groups, the lesion shows no signs of pus collection, fibrin deposition, or edema.

Compared to the test group, which had relatively abundant fibroblasts, acute edema, and poorly developed granulation tissue, the standard group showed some sign of healing, including the development of immature granulation tissue, a reduction in inflammatory cells, the synthesis of collagen, and expanding blood vessels.

The control group's histology findings show excised wounds that aren't entirely healed, along with edema and immature granulation tissue growth. There are more inflammatory cells and macrophages in the control than other groups.

5.6. Histopathology of burn wounds

On day 16 following burn injury, the test group demonstrated highest wound repair by increased number of collagen and fibroblast as compared to standard and control groups (Figure-7). Development of blood vessels and fewer numbers of inflammatory cells

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were present, and mature granulation tissues had been observed at sub-epidermal location.

Comparatively to the test group, the standard group had a moderate level of granulation tissue maturation, no edema, the presence of collagen fibers and fibroblasts, and a reduced number of inflammatory cells.

The presence of many inflammatory cells and a lack of collagen and fibroblasts were found in the control group. There was no angiogenesis and granulation tissue maturation in the epidermal area.

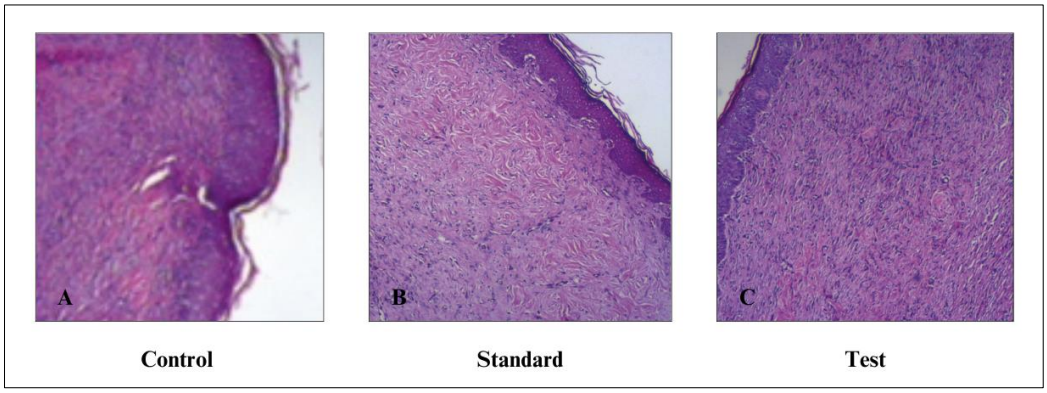

Figure-7 Histopathology of burn wounds.

6. CONCLUSION

Present study demonstrates the excision wound healing and burn healing effects of fruit extract of NAT. On the basis of results obtained in the present investigations, it can be concluded that 5% w/w ethanolic fruit extract of fruits of Nyctanthes Arbor-tristis has significant excision wound and burn wound healing activity. The result obtained from the data justifies the use of fruits of Nyctanthes arbor-tristis for treatment of both types of wounds.

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