ejpmr, 2020,7(10), 29-35

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

WOUND HEALING POTENTIAL OF *AZADIRACHTA INDICA(NEEM) LEAVES*

[1]Vijaya Paul Samuel, [2]*Laxminarayana Kurady Bairy, [3]Kumar Bhat, [4]Sareesh N. N., [5]Suresh Kumar and [6]Hemant Yadav

[1]Associate Professor of Anatomy, RAK College of Medical Sciences, RAK Medical and Health Sciences University, Ras Al Khaimah, B.O. Box 11172, United Arab Emirates.

[2]Dean and Professor, Department of Pharmacology, RAK College of Medical Sciences, RAK Medical and Health Sciences University, Ras Al Khaimah, B.O. Box 11172, United Arab Emirates.

[3]Professor, Department of Anatomy, RAK College of Medical Sciences, RAK Medical and Health Sciences University, Ras Al Khaimah, B.O. Box 11172, United Arab Emirates.

[4]Associate Professor, Department of Physiology, RAK College of Medical Sciences, RAK Medical and Health Sciences University, Ras Al Khaimah, B.O. Box 11172, United Arab Emirates.

[5]Associate Professor, Department of Pharmacology, RAK College of Medical Sciences, RAK Medical and Health Sciences University, Ras Al Khaimah, B.O. Box 11172, United Arab Emirates.

[6]Associate Professor of Pharmaceutics, RAK College of Pharmaceutical Sciences, RAK Medical and Health Sciences University, Ras Al Khaimah, B.O. Box 11172, United Arab Emirates.

***Corresponding Author: Laxminarayana Kurady Bairy**
Dean and Professor, Department of Pharmacology, RAK College of Medical Sciences, RAK Medical and Health Sciences University, Ras Al Khaimah, B.O. Box 11172, United Arab Emirates.

Article Received on 27/07/2020 **Article Revised on 16/08/2020** **Article Accepted on 06/09/2020**

ABSTRACT

Azadirachta indica (*A.indica*) is a frequently used medicinal plant in indigenous medicine. A study was carried out to investigate the wound healing ability of aqueous extract of *A.indica*. In this study two wound models namely; excision and incision wounds were used. The period of epithelization and wound contraction for excision wound and tensile strength for incision wound were the selected parameters respectively. *A.indica* increased the tensile strength in case of incision wound; and decreased the epithelization period and enhanced wound contraction in case of excision wound. Further, *A.inidca* was able to reverse the decreased tensile strength, delayed epithelization period, and wound contraction due to dexamethasone treatment. In, conclusion, the aqueous extract of *A.indica* has pro-healing effect and was effective in negating the anti-healing effect of dexamethasone.

KEYWORDS: *Azadirachta indica*, Incision wound, excision wound, dexamethasone.

INTRODUCTION

Wound is described as an interruption in the physical and functional integrity of tissue and may arise consequently from exposure to various mechanical, chemical or microbial insults in our daily activities. The process of wound healing is complex containing intricate cellular and biochemical process like inflammation, granulation, collagenation, angiogenesis, wound shrinkage and renewal of epithelium that eventually lead to the restoration of integrity and function.[1] Each time there is an alteration in the curative process from the normal, it is quite common to encounter non-healing, under healing or over healing. The main purpose for treating these problems should therefore be focused on reducing the time needed for treatment as well as lessening the unforeseen consequences. Accelerating the process of healing and the quality of the tissue made are matters that highly interest the medical fraternity, given not only the significant number of injuries associated with high morbidity and mortality, but also the increasing costs related to the stabilization, preservation and improvement stages of this process. In this setting, there is a rising attention being paid to alternatives to conventional treatment, particularly the use of natural products, which are easily available and economical.[2] Roughly, most of the herbal medicines in practice are for the management of wounds and skin disorders.[3] A thoughtful consideration has to be made to include traditional medicine in wound management in light of increased public apprehension towards antibiotic resisting pathogens.[4]

Azadirachta indica(A,indica) has been used for the past several hundred years and is considered to be among the most resourceful therapeutic florae with far-reaching and highly valued biological activity. Virtually all portions of this tree has been widely used as home therapy to treat various human disorders. Previous studies have shown *A.indica* possesses components like nimbin, nimbidin, etc and many such kinds of constituents which acts on the numerous genetic pathways to bring about effective management of the disease.[5] It has also been reported

that *A.indica* possess components that can be actively used to counter inflammation, arthritis, fever, high blood sugar levels, gastric ulcer, fungal and bacterial infection as well as tomour cells.[6,7,8,9] The ethanoic extract of *A. indica* leaves was shown to possess substantial wound healing properties that was almost at par with the standard drug.[11]

Dexamethasone (DEX) are steroidal drugs (also known as glucocorticosteroids), that have been commonly used as an immunosuppressant, anti-inflammatory and anti-allergic.[12] It has been observed that DEX induces excess production of reactive oxygen species by endothelial cells and cause vascular endothelial dysfunction.[13] A lot of apprehensions have also been raised concerning the possible adverse effects on the cardiovascular system, wound healing and skeletal muscle condition by long- and short-term DEX therapy.[14,15] DEX bring about the delay in wound repair most likely during the initial stage of the inflammatory phase by delaying the recruitment of macrophages and neutrophils thereby hindering the removal of cellular debris as well as by reducing the number of interleukins, cytokines, chemokines and growth factors from reaching the site of injury.[16,17,18,19] In view of these influence of corticosteroids on wound healing, there is always a need for an agent, which can prevent these adverse effects of steroids. Therefore, a study was designed to examine the influence of the aqueous extract of *A. indica* in incision and excision wound healing in Wistar rats.

MATERIALS AND METHODS

A.indica leaves water extract preparation

Leaves for the extract was collected from the *A.Indica* trees grown in the Al Qusaidat area in Ras Al Khaimah, United Arab Emirates. Approximately 1kg of the leaves were collected and then washed with water and left at room temperature to be air-dried. A mixer was used to grind the leaves and the powered leaves were then soaked in five liters of distilled water and stored in a dark room away from light and high temperature. After 72 hours it was filtered, evaporated and dried in a hot air oven.

Animals and sample size

Healthy male Wistar rats (3months old), weighing 180-220g were used for the study. Animals for this study were obtained from, United Arab Emirates University, Al Ain, UAE. Prior to the experiment the rats were acclimatized to the laboratory condition by housing them for 7 days in a well aerated room maintained at optimum temperature. During this period the rats were served with water and food *ad libitum*. The rats were starved 12 hrs prior to wounding with water *ad-libitum*. Wounding of the rats were done aseptically under light ether anesthesia.

Experimental group and design

The wound healing profile was carried out in incision and excision wound models using male rats. There were four groups and each group consisted of six rats.

Groups for Excision wound

Group 1: Control-No drug application.
Group 2: Dexamethasone 0.17mg/kg i.m.
Group 3: Dexamethasone (0.17mg/kg) and water extract of *A.indica* leaves in simple ointment base.(1.0g day).
Group 4: Water extract of *A.indica* leaves in simple ointment base.(1.0g per day) Topical.

Excisional wound model

After overnight starvation, the rats were wounded under light ether anesthesia. Fur over the required area for wounding the animal was removed by electric clipper and sterilized with 70% alcohol. A spherical portion of the skin measuring about 500mm^2 in area was removed from the dorsal inter-scapular region.[20] Rats showing signs of infection were excised from the study. The size of wound was quantified by measuring the area of the wound planimetrically on alternate days until complete healing was noticed. The wound contraction was expressed as percentage of the initial wound size.[21] Epithelization period is noted by the days required for complete healing without leaving raw wound. *A.Indica* ointment was topically applied on the wounds of the rats in group 3 and 4 for 21 days or until complete epithelization whichever was earlier. No drug treatment was given to control group. Group 2 was injected with 0.17mg/kg of dexamethasone whereas Group 3 in addition to the topical application of *A.indica* ointment was also injected with 0.17/kg dexamethasone for 21 days or until complete epithelization which ever was earlier.

Groups for Incision wound

Group 1: Control-No drug application.
Group 2: Dexamethasone 0.17mg/kg im.
Group 3: Dexamethasone (0.17mg/kg) and water extract of *A.indica* leaves in simple ointment base. (1.0g day).
Group 4: Water extract of *A.indica* leaves in simple ointment base.(1.0g per day) Topical.

Incisional wound model

After overnight starvation, the rats were wounded under light ether anesthesia. Sterilization of the area to be wounded was achieved by shaving dorsal fur of the animal and smearing the selected area with 70% alcohol. On both sides of the vertebral column of the animal, two paravertebral incisions measuring 6 cm each were created through entire thickness of the skin. Once total hemostasis was noted, the wounds were closed by interrupted sutures with 4-0 silk thread spaced out evenly from each other by about 1cm. After cleaning the wound with cotton swabs smeared with 70% alcohol, the rats were kept in the polypropylene cages. The control group designated as Group 1 did not receive any drug. Dexamethasone was given intramuscularly to Group 2

for 10 days. *A.indica* ointment was applied on the wounds of rats in Group 3 and Group 4 for 10 days. Subsequent to removal of sutures on the 8th day following wounding, the breaking strength (defined as the strength just adequate to open the wound) of the healed wound was recorded on the 10th day.[22]

Measurement of breaking strength

To assess the wound breaking strength a pair of surgical forceps were fixed to the both margins of each skin strip. One forceps was fixed to a stand while the other one is tied to a freely suspended graduated plastic bag through a string run over the pulley. The graduated polyethylene bag was then filled with water till the wound edge was disrupted at the wound site. Tensile strength of the wound in grams was recorded by measuring the volume of water that was needed to break the wound.[23]

Data analysis

The data was analyzed using SPSS (Version 24) software and values are stated as mean ± SEM. It was analyzed by using one-way ANOVA followed by Tukey test. $P < 0.05$ was considered significant.

RESULTS

Excision wound model

There is no significant difference in wound contraction between the control group and drug treated group on day 4. However, significance (P<0.05) was observed in the percentage of wound contraction between the dexamethasone treated groups and other groups on days 8, 12, 16 and 21. On days, 12, 16 and 21 significant reduction in wound size was noted in the *A.indica* treated group when compared to the other groups suggesting that *A.Indica* favors wound contraction. Further, on days 8, 12, 16 and 21 significant decrease in wound size was observed when *A.indica* is co-administered with dexamethasone compared to dexamethasone treated group. This suggests that *A.indica* is able to nullify the unwanted effects of dexamethasone on excision wound healing. The percentage of wound contraction in control and various drug treated rats are shown in [Table 1, Figure 1].

In control group, the healing was complete in 19.5±0.71 days. The *A.indica* treated group significantly reduced the epithelization period(16.66±0.88 days) when compared to other groups (Table 1, Figure 2). Rats treated with dexamethasone took longer time for healing (27.16±0.74 days). When *A.indica* was co-administered with dexamethasone, it could significantly reverse the adverse effect of dexamethasone on excision wound healing (p<0.05).

Incision Wound Model

The breaking strength of incision wound in control group is 319.16± 19.86g. Dexamethasone treatment significantly(P<0.05) decreased the breaking strength (218.5±26.8g) when compared to control and *A.indica* treated group (Table 1, Figure 3). However, no significant difference in wound breaking strength was observed between the control group and *A.indica* group even though tensile strength was higher in the *A.indica* group. The tensile strength was significantly increased in the group treated dexamethasone plus *A.indica* when compared to the group that received only dexamethasone.

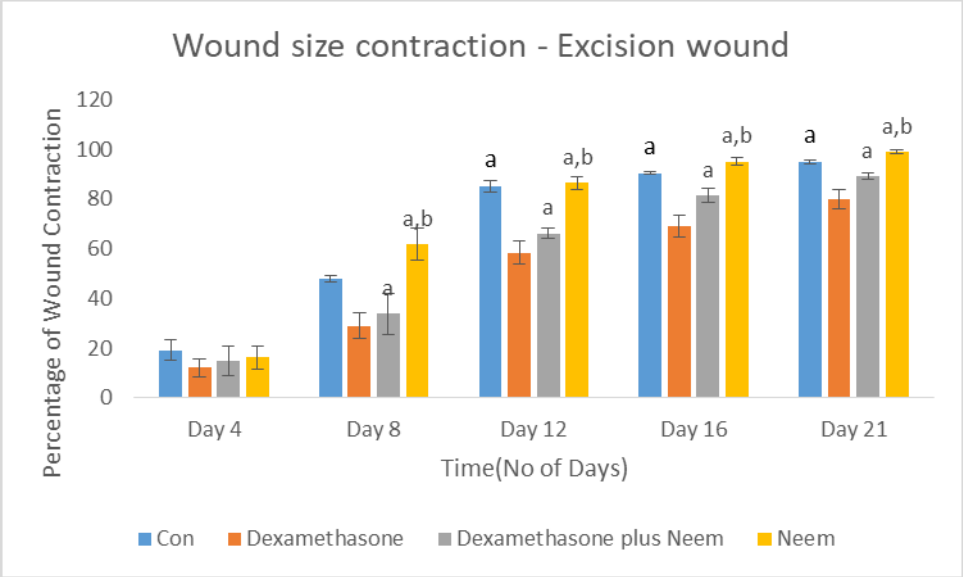

Figure 1: Effect of the aqueous extract of *A.indica* leaves on wound contraction in rats.

Data represents mean ± standard error of mean, n = 6. [a]p<0.05 vs Dexamethasone and [b]p<0.05 vs Dexamethasone plus neem.

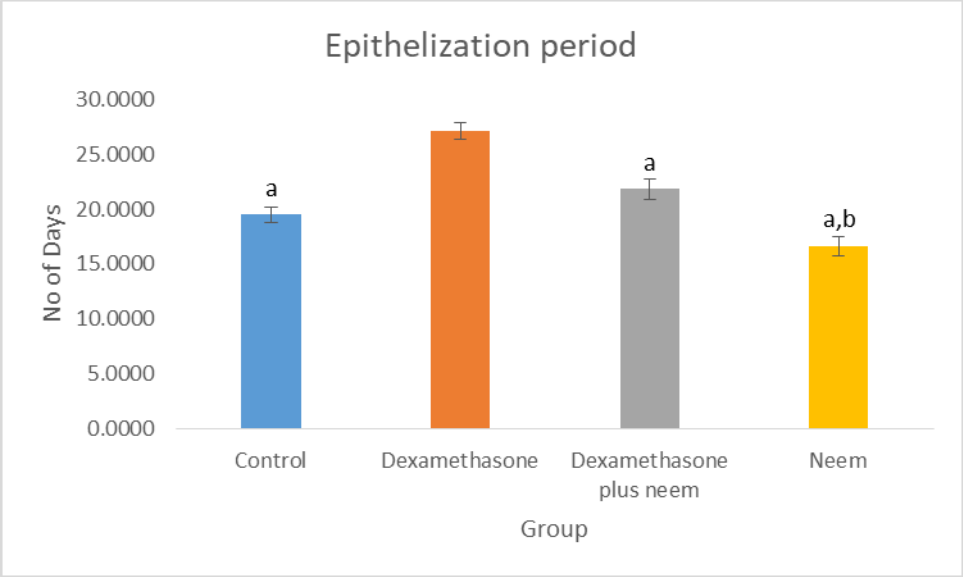

Figure 2: Effect of the aqueous extract of *A. indica* leaves on epithelization in rats.

Data represents mean ± standard error of mean, n = 6. [a]p<0.05 vs Dexamethasone, [b]p<0.05 vs Dexamethasone plus neem.

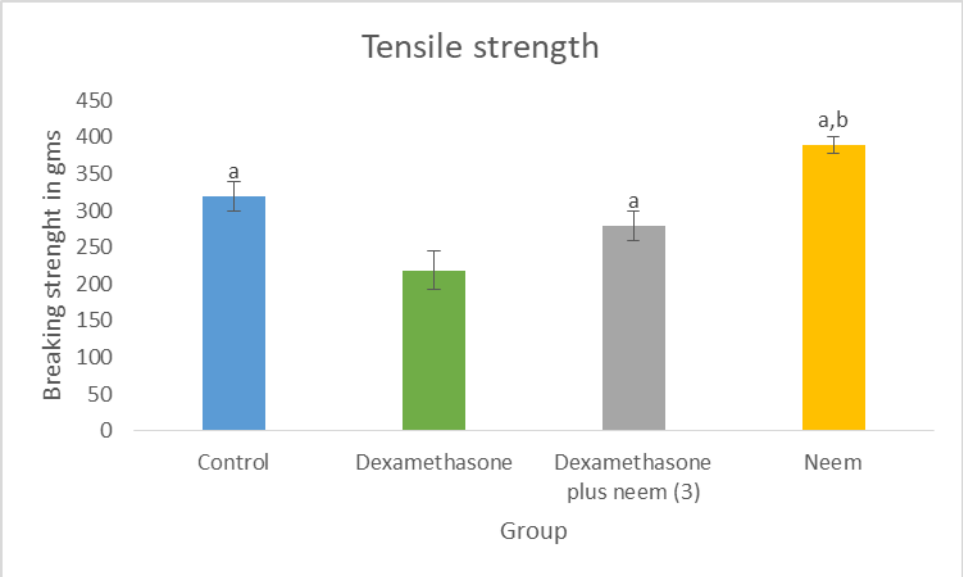

Figure 3: Effect of the aqueous extract of *A.indica* leaves on wound breaking strength in rats.

Data represents mean ± standard error of mean. N=6. [a]p<0.05 vs Dexamethasone and [b]p<0.05 vs Dexamethasone plus neem.

Table 1: Summary of the results of excision and incision wound models studied to determine the effect of aqueous extract of *A.indica* leaves on normal and dexamethasone suppressed rats.

Groups	Excision Wound Model						Incision Wound Model
	Percentage of Wound Contraction					Period of Epithelization (in days)	Tensile strength(g)
	4th day	8th day	12th day	16th day	21st day		
Control	19.21±4.05	48.03±1.31	85.1±2.14	90.45±0.55	95.05±0.75	19.5±0.71	319.16±19.86
Dexamethasone	12.09±3.5	29.01±4.98	58.44±4.44	69.2±4.41	80.09±3.84$	27.16±0.74*	218.5±26.8[a]
Dexamethasone plus *A.indica*	14.83±6.12	33.93±1.2	66.17±2.27	81.41±0.36	89.08±3.02@	21.83±0.94**	279±20.67[b]
A.indica	16.19±4.55	61.91±6.30	86.36±2.68	95.08±1.33	99.06±0.63	16.66±0.88***	389.6±10.99

*P<0.05 vs Control, **P<0.05 vs Dexamethasone, ***P<0.05 vs control. aP<0.05 vs control, bP<0.05 vs Dexamethasone. $P<0.05 vs control, @P<0.05vs Dexamethasone.

DISCUSSION

The cells of the skin, mainly the keratinocytes, fibroblasts, endothelia of blood vessels, immune cells as well as their associated extracellular matrix take part in complex interaction between each other during the wound healing process following skin injury. The process of wound healing mainly involves three collaborative phases namely inflammation, proliferation and remodeling and the cellular and extracellular components of the skin eventually has a major role in all the three phases. The epidermal keratinocytes migrate to the place of injury, undergoes proliferation and differentiate itself to bring about re-epithelization. Concurrently with the tissue-remodelling phase, myofibroblasts will act to bring about contraction of the wound few days after injury.[24] In the present study, the group that received aqueous extract of *A.Indica* leaves has shown pro- epithelialization effect and favored the rate of wound closure, when matched to the other groups.

It was also noted that, rats treated with the water extract of *A. Indica* has increased tensile strength probably due to good amount of mature collagen deposition. This concurs with various other wound healing studies conducted using various parts of the *A.indica* plant. Preeti et al reported that rats treated with *A.Indica* extract had increased levels of hydroxyproline in their granulation tissue of the wound.[25] One of the most important component of the collagen fibers in the granular tissue is hydroxyproline and its presence in significant amount is reflected as an indirect indicator of enhanced wound healing.[26] The important component of the connective tissue is collagen, which occupies a major role in the healing of wound by providing the appropriate environment for the renewing tissue as well as structural framework and strength. During the wound healing process, the fibroblast produces collagen fibers that will aid the wound in attaining tensile strength. This also indicates that the leaf extract of *A,Indica* in our study would have expedited the healing process by facilitating the movement of the fibroblasts, epithelial cells and synthesis of their associated matrix at the wound site. Thus, the leaf extract of *A.Indica* would have helped in the healing process by prevailing in one or other stages of wound healing namely inflammation, macrophagia, collagenation, contraction and epithelization as these stages are closely interlinked.

In our study, *A.india* could reverse the adverse of dexamethasone on excision wound parameters namely wound contraction and epithelization. Similar to our finding there are reports that dexamethasone delays excision wound healing. It is a well-known fact that glucocorticoids mediate their anti-inflammatory and immunosuppressive effects by suppressing the activation of pro-inflammatory cytokines, chemokines and growth factors while augmenting endothelial NO formation, because of which the number of macrophages and neutrophils recruited and migrating to the wound site is reduced. It has long been recognized that steroidal drugs suppress wound healing by delaying the inflammatory process of wound healing without which the healing will be in disarray. Studies have revealed that DEX increases the ROS producing ability of macrophages and dendritic cells.[27] However, it is intriguing to note that the free radicals play a significant role in host defense against harmful bacteria occupying the wound site by local cell signaling thereby enticing the neutrophils and macrophages to the site of injury.[28] Moreover, ROS indirectly plays a significant role in granular tissue formation through neovascularization by activating endothelial cell division and migration. However, immoral production of ROS can lead to oxidative stress, which can have a deleterious effect on wound closure.[28] Adequate evidence based on numerous studies also point to the fact that enhanced production of the ROS, lipid peroxidation and ineffective removal of cellular debris is critical in modulation of fibroblast proliferation.[28] Hence, a clear-cut equilibrium between ROS and antioxidative mediators is crucial for comprehensive wound healing. Studies have suggested that, *A. indica* exhibits its numerous pro-healing effect mainly through some of the chemical components it contains. Sufficient evidence shows that the anti-inflammatory, antioxidant, antimicrobial and immunomodulatory properties attributed to *A.indica* is primarily due to chemical components such as alkaloids, triterpenoids, and their glycosides, limonoids, flavonoids, fatty acids, and steroids found within it in adequate amounts.[25]

Antioxidants alleviate and remove free radicals, even before they bring about the undesired effect in biological cells and activates antioxidative enzyme that control injury caused by free radicals/reactive oxygen species.[29] Studies done on the leaf and bark extracts of *A.indica* have revealed that they possess within them substantial amount of phytochemical constituents that exhibit significant antioxidant properties.[29] Removal of the free radicals from the site of wound is considered to be one of the main process involved in wound healing and it has been established that flavonoids, an antioxidant found in many medicinal plants aids immensely in free radical scavenging. Previous studies have shown that leaf extract of *A. Indica* is a good source of flavonoids[30] and has most probably contributed to the enhanced wound healing observed in our study. It is difficult to ascertain from our study which of the other chemical components in the leaf extract of *A.Indica* contributed in enhancing the healing process particularly in the dexamethasone administered rats. However based on the improved tensile strength, wound contraction and epithelization observed in groups treated with *A.indica* we can conclude that it possesses significant pro-healing properties. It can also be said from our study that in addition to its antioxidant property, its role in the proliferation of fibroblast and formation of collagen fibers along with neovascularization would have contributed to the healing process particularly in the group, it was co-administered with Dexamethasone.

CONCLUSION

The topical application of water extract of *A. Indica* leaves promotes wound healing in both normal and dexamethasone treated animals. These results could substantiate, at least to a degree, the addition of this plant in the treatment of wound healing in herbal medicine. Nevertheless, more studies are essential to disclose its exact mechanism(s) of action and further studies are required to separate the active compounds accountable for these pharmacological activities that will be supportive in portraying this plant as a curative object for healing wounds and treating several diseases.

ACKNOWLEDGEMENT

The authors acknowledge the President of RAK Medical and Health Sciences University, Dr.S.Gurumadhva Rao for his support in carrying out this project.

LIST OF REFERENCES:

1. Clark RA. Wound repair: overview and general considerations. In: Clark RA, Henson PM, editors. The Molecular and Cellular Biology of Wound Repair. 2nd Edition. New York: Plenum Press, 1996; 473-88.
2. Biondo SMLP, Henning JL, Boen BRO, Prado JLD, Costa LRD, Robes RR, Ioshii SO. Comparative analysis of the effects of honey, copaiba oil-resin and a commercial product on second intention healing, in rats. Rev Col Bras Cir, 2019; 46(5).
3. Pirbalouti AG, Koohpayeh A, Karimi I. The wound healing activity of flower extracts of Punica granatum and Achillea kellalensis in Wistar rats. Acta Pol Pharm, 2010; 67(1): 107-10.
4. Anjaria J, Parabia M, Dwivedi S. Ethnovet heritage: Indian ethno-veterinary medicine - an overview. Pathik enterprise, Ahmedabad, 2002; 610-12.
5. T. R. Govindachari, G. Suresh, G. Gopalakrishnan, B. Banumathy, and S. Masilamani, "Identification of antifungal compounds from the seed oil of Azadirachta indica," Phytoparasitica, 1998; 26(2): 109–116.
6. U. Bandyopadhyay, K. Biswas, A. Sengupta et al., "Clinical studies on the effect of Neem (Azadirachtaindica) bark extract on gastric secretion and gastroduodenal ulcer,"LifeSciences, 2004; 75(24): 2867–2878.
7. B. Sultana, F. Anwar, and R. Przybylski, "Antioxidant activity of phenolic components present in barks of Azadirachta indica, Terminalia arjuna, Acacianilotica, and Eugeniajambolana Lam. trees," FoodChemistry, 2007; 104(3): 1106–1114.
8. P.E.Ebong, I.J.Atangwho, E.U.Eyong, andG.E.Egbung, "The antidiabetic efficacy of combined extracts from two continental plants: Azadirachta indica (A. Juss) (Neem) and Vernonia amygdalina(Del.) (AfricanBitterLeaf)," The American Journal of Biochemistry and Biotechnology, 2008; (4)3: 239–244.
9. R. Paul, M. Prasad, and N. K. Sah, "Anticancer biology of AzadirachtaindicaL(neem):aminireview,"CancerBiol ogyand Therapy, 2011; 12(6): 467–476.
10. Clark RA. Wound repair: overview and general considerations. In: Clark RA, Henson PM, editors. The Molecular and Cellular Biology of Wound Repair. 2nd Edition. New York: Plenum Press, 1996; 473-88.
11. Akerele O. Nature's medicinal bounty: don't throw it away. World Health Forum, 1993; 14(4): 390-5.
12. Pirbalouti AG, Koohpayeh A, Karimi I. The wound healing activity of flower extracts of Punica granatum and Achillea kellalensis in Wistar rats. Acta Pol Pharm, 2010; 67(1): 107-10.
13. Anjaria J, Parabia M, Dwivedi S. Ethnovet heritage: Indian ethno-veterinary medicine - an overview. Pathik enterprise, Ahmedabad, 2002; 610-12.
14. Biswas K, Chattopadhyay I, Banerjee RK, BandyopadhyayU. Biological activities and medicinal properties of neem (Azadirachta indica). Curr Sci, 2002; 82(11): 1336-45.
15. Nagesh HN, Basavanna PL, Kishore MS. Evaluation of wound healing activity of ethanolic extract of Azadirachta Indica leaves on incision and excision wound models in Wister albino rats. Int J Basic Clin Pharmacol, 2015; (4): 1178-82.
16. Naveen K C, Ike R H, Irra R. Effect of Neem Leaves Extract (Azadirachta Indica) on Wound Healing Ethical Considerations. Althea Medical Journal, 2015; 2(2): 199-203.
17. Maślanka T. Effect of dexamethasone and meloxicam on counts of selected T lymphocyte subpopulations and NK cells in cattle - in vivo investigations. Res Vet Sci, 2014; 96: 338–46.
18. Iuchi T, Akaike M, Mitsui T, Ohshima Y, Shintani Y, Azuma H, Matsumoto T. Glucocorticoid excess induces superoxide production in vascular endothelial cells and elicits vascular endothelial dysfunction. Circ Res, 2003; (9)2: 81–87.
19. Kaasik P, Umnova M, Pehme A, Alev K, Aru M, Selart A, Seene T. Ageing and dexamethasone associated sarcopenia: peculiarities of regeneration. J Steroid Biochem Mol Biol, 2007; (105): 85–90.
20. Jain, A., Witbreuk, M., Ball, C. et al, Influence of steroids and methotrexate on wound complications after elective rheumatoid hand and wrist surgery. J Hand Surg Am, 2002; (27): 449–55.
21. Wicke, C., Halliday, B., Allen, D. et al, Effects of steroids and retinoids on wound healing. Arch Surg, 2000; (135): 1265–70.
22. Shanbhag, T., Shenoy, S., & Rao, M. C. Wound healing profile of Tinospora cordifolia. Indian Drugs, 2005; 42(4): 217-21.
23. Kumar N, Prakash D, Kumar P. Wound healing activity of *Solanum xanthocarpum* Schrad. and Wendl. fruit. Indian J Nat Prod Resour, 2010; (1): 470–5.

24. Deyun Chen, Haojie Hao, Xiaobing Fu, and Weidong Han. Insight into Reepithelialization: How Do Mesenchymal Stem Cells Perform? Stem Cells International, 2016, Article ID 6120173, 9 pages.
25. Maan P, Yadav KS, Yadav NP. Wound Healing Activity of *Azadirachta indica* A. Juss Stem Bark in Mice. *Pharmacogn Mag*, 2017; 13(Suppl 2): S316-S320.
26. Iwona Otrocka-Domagała , Katarzyna Paździor-Czapula , Michał Gesek . BMC Vet Res, 2019 Feb 11; 15(1): 56.
27. Marina D Kraaij [1], Sandra W van der Kooij, Marlies E J Reinders, Karin Koekkoek, Ton J Rabelink, Cees van Kooten, Kyra A Gelderman. Dexamethasone increases ROS production and T cell suppressive capacity by anti-inflammatory macrophages. Molecular Immunology, 2011 Dec; 49(3): 549-57.
28. Manciula LG, Berce C, Tabaran F, Trombitaș V, Albu S. The Effects of Postoperative Astaxanthin Administration on Nasal Mucosa Wound Healing. *J Clin Med*, 2019; 8(11): 1941.
29. Mohammad A. Alzohairy. Therapeutics Role of *Azadirachta indica* (Neem) and Their Active Constituents in Diseases Prevention and Treatment. Evidence-Based Complementary and Alternative Medicine, 2016; Article ID 7382506, 11 pages.
30. Amal Kumar Ghimeray, Cheng-Wu Jin, Bimal Kumar Ghimire and Dong Ha Cho. Antioxidant activity and quantitative estimation of azadirachtin and nimbin in Azadirachta Indica A. Juss grown in foothills of Nepal. African Journal of Biotechnology, 8(13): 3084-3091.