

Efficacy of 'Anuradha Oil 11' on incision wound healingSuresh B Patankar¹, Anupama S Gorde¹, Rajesh G Raje¹¹Ace Hospital and Research Centre, Pune, Maharashtra, India**Corresponding Author****Suresh B Patankar**

E-mail ID: sureshpatankar51@gmail.com

Submission: 03.08.2022

Acceptance: 27.09.2022

Publication: 30.09.2022

https://www.doi.org/10.56136/BVMJ/2022_00076**Abstract****Background:** The herbs/plants are used in traditional and folk medicines worldwide for treating wounds and burns. Animal models help to understand the tissue repair process and corroborate various approaches to formulate therapy for wound healing.**Objective:** To determine the efficacy of 'Anuradha Oil 11' (AO11) on incision wound healing in Wistar rats. **Methods:** There were four groups formed based on the treatment received as Test group (AO11), Control group, Standard I Hydroheal group, and Standard II Curcumin group. Six female rats were used per group in this study. Linear-length paravertebral incisions were made through the shaven skin. Three surgical sutures were placed, each one cm apart. A 0.1 mL of test material AO11, 0.1 mL of standard I hydroheal, and 0.1 mL of 1% solution of standard II curcumin respectively were applied on the wound once daily for nine days. The control group wound remained untreated. On day nine (post-wound), all the sutures were removed. On day 11, all the animals were anesthetized, and the breaking strength of previously wound-treated skin was measured using a tensiometer.**Results:** All the animals appeared normal and showed no clinical signs of intoxication till the end of the study. The test group showed a higher tensile strength (48.3 g/mm²) required to open the wound when compared to the control group (38.14 g/mm²). The increase in tensile strength was statistically significant between the test (p<0.01) and both the standard groups (p<0.05). There was no statistically significant change in the body weight of all the animals. No mortality was observed in any of the groups. **Conclusion:** The study concluded that the test material AO11 showed an increase in efficacy compared to control and is safe for use.**Keywords:** Curcumin, hydroheal, incision, tensiometer, wound**Introduction**

Wounds may be defined as a cut or breaks in the continuity of any tissue. Wound healing helps restore anatomically and functionally disrupted skin⁽¹⁾. It is a complex and dynamic process that restores damaged tissues using several sequential events⁽²⁾. Some diseases like diabetes, immune-compromised conditions, ischemia, and conditions like malnourishment, aging, local infection, and local tissue damage due to burning or gunshot often delay wound healing⁽²⁾. Wound healing involves continuous cell-cell and cell-matrix interactions that allow the process to proceed in three overlapping phases, viz. inflammation (0–3 days), cellular proliferation (3–12 days), and remodeling (3–6 months)⁽¹⁾. The typical treatment includes honey, silver nanoparticles, copper complexes, stem cells, broad-spectrum antibiotics, etc. Newer techniques like VAC (Vacuum-Assisted-Closure) and Hyperbaric Oxygen Therapy (HBOT) have been attempted for wound healing with encouraging results but are costly along with some limitations⁽³⁾.

The herbs/plants are used in traditional and folk medicines worldwide for treating wounds and burns. These plants produce secondary metabolites like alkaloids, flavonoids, glycosides, and tannins that possess various bioactivities. These phytochemicals can induce and promote the healing and regeneration of lost tissue by multiple mechanisms⁽⁴⁾.

These phytomedicines are affordable and safe, although many are yet to be explored for their wound healing properties⁽⁵⁾.

Different types of models have been developed and used for studying various mechanisms of wound healing activities⁽⁶⁾. Animal models help to understand the tissue repair process and corroborate various approaches to formulate therapy for wound healing⁽⁷⁾. The incision wound model is a virtuous animal due to its accuracy and reproducibility and facilitates multiple investigations. The wound(s) can be made anywhere on the body surface as well in most of the body organs. The shape of the wound depends on the purpose of the study. This model can investigate the wound healing process and influence different systemic and local compounds as well as dressing. This model has been extensively used in skin and every other organ available for surgery in the last century. The outcome measurement is primarily wound strength, collagen content, and histology⁽⁸⁾.

The present study was conducted to establish the efficacy of the herbal formulation 'Anuradha Oil 11' (AO11) on incision wound healing when applied by the dermal route to female Wistar rats once daily for nine days following the Drug Discovery and Evaluation, Pharmacological Assay, H. Gerhard Vogel (Ed.), 2002, Protocol⁽⁹⁾.

Materials and Method

Animals

Wistar female rats, weighing 162-255 gm, aged 10 to 12 weeks, procured from APT Testing and Research Private Limited, Pune, were used for the proposed study. They were housed three per cage under a controlled temperature of 20-24°C with relative humidity between 30-70% and 12 hours light and dark cycle.

They were fed with pellets of balanced animal food of Nav Maharashtra Chakan Oil Mills, Pune and water ad libitum.

Test material AO11 - The formulation AO was prepared using Sesame oil and pig fat combination in a certain proportion as a base. This formulation was prepared after giving repeated treatment of hot water extract of the following plant materials: *Curcuma longa* (Rhizome), *Glycyrrhiza glabra* (Rhizome), *Hamiltonia suaveolens* (Stem bark), *Typha angustifolia* (Flowers), *Azadirachta indica* (Leaf).

Method

The study was conducted in 2011-2012 at National Toxicology Institute (NTC), Pune. Four groups of animals as Test, control, Standard I Hydroheal, and Standard II Curcumin were formed; each containing six female rats. The female rats were shaved on the dorsal area of the trunk with electric clippers [WAHL animal clipper KM10 (blades made in the USA)]. A five cm length linear paravertebral incision was made through the shaven skin. One skin clip was made with the help of a scissor on the back of each animal. Three surgical sutures were placed, each one cm apart. A 0.1 mL of test material AO11 (AMAI Charitable Trust's, ACE Hospital and Research Centre, Pune), 0.1 mL of standard I hydroheal, and a 0.1 mL of 1% solution of standard II curcumin were applied daily once for a period of nine days. The control wound remained untreated. On day nine, post wound, all the sutures were removed.

On day 11, all the animals were anesthetized [ketamine (Unijules Life Sciences Ltd.) and xylazine (Indian Immunologicals Limited)], and the breaking strength of previously wound treated skin was measured by using a tensiometer (Make: Veekay Test Lab, Model Veekay Sr. No. 11421). This breaking strength was converted to the tensile strength using the formula⁽¹⁰⁾:

$$\text{Total strength} = \frac{\text{Maximum Breaking Strength in grams}}{\text{Area of wound}}$$

$$\text{Length} = 50.0 \text{ mm and breadth} = 1.0 \text{ mm}$$

Clinical signs of intoxication and at the site of application were observed in all the groups. Animals were weighed individually on days 0, 7, and 11 of the study. To measure the food consumption, 100g of feed was given to the cage, and at 24 hours, the food remaining was measured. This quantity of

feed consumed was divided by the number of animals in each cage, and the amount consumed per animal was calculated. Reduced feed consumption is one of the early signs of toxicity.

Statistical analysis

The values were expressed in terms of mean±SD. Statistical analysis performed was a one-way analysis of variance method (Dunnett's Multiple Comparison Test) using GraphPad prism 5 software⁽¹¹⁾.

The Institutional Animal Ethics Committee of the NTC, Pune (IPU-0152.07) approved the experimental protocols.

Results

Clinical Findings

All the animals appeared normal and showed no clinical signs of intoxication at the site of application till the end of the study in all the groups; control, test, standard I (hydroheal), and standard II (curcumin). There was no statistically significant change in the body weight and the amount of food consumed by all the animals, as depicted in Tables 1 and 2. No mortality was observed in any of the groups. The animals did not show any other clinical signs like lachrymation, salivation, urination, diarrhea, tremors, and many other signs of toxicity.

Table 1: Mean and SD of body weight with days of follow-up

Groups	Days		
	Day 0	Day 7	Day 11
Control	210.1 (28.22)	213.9 (25.30)	217.3(23.27)
Test	189.0 (8.967)	200.3 (9.416)	205.8 (11.47)
Standard I Hydroheal	179.1 (5.748)	190.7 (5.279)	194.8 (7.367)
Standard II Curcumin	195.3 (24.26)	211.5 (27.71)	216.1 (25.87)

* Data shown as mean (SD).

Table 2: Food consumption (g/cage) with days of follow-up

Groups		Days		
		Day 0	Day 7	Day 11
Control	Cage 1	50.5	45.0	44.0
	Cage 2	47.5	44.5	42.0
Test	Cage 1	39.0	51.5	50.5
	Cage 2	48.0	54.5	47.5
Standard I	Cage 1	42.5	43.5	39.0
Hydroheal	Cage 2	49.0	49.5	48.0
Standard II	Cage 1	44.0	42.5	48.5
Curcumin	Cage 2	42.0	45.5	50.0

Tensile Strength Data

The mean tensile strength data of all the groups are depicted in Table 3. There was a statistically significant increase in tensile strength required to open the wound in the standard I (hydroheal) group and standard II (curcumin) group ($p < 0.05$), when compared with the control group. The test group showed a comparable increase in the mean tensile strength ($p < 0.01$) required to open a wound compared to the standard I and II.

Table 3: Mean tensile strength measured on day 11

Groups	Breaking strength load in Kg	Tensile strength gm/mm ²
Control	1.907 (0.207)	38.14 (4.15)
Standard II Curcumin	2.066 (0.1883)	46.32 (4.17)
Standard I Hydroheal	2.267 (0.186)	45.30* (3.72)*
Test	2.417 (0.445)	48.30** (8.89)

Data shown as mean (SD). * $p < 0.05$, ** $p < 0.01$. Three readings were taken to evaluate the mean.

The test group (0.1 mL) showed 41.14%, the standard I hydroheal group (0.1 mL) showed 30.56%, and the standard II curcumin group (0.1 mL of 1% solution) showed a 0.642% increase in tensile strength required to open the wound when compared to the control group as depicted in Table 4.

Table 4: Tensile strength compared to control measured on day 11

Group	Increase in % Breaking Strength	Increase in % Tensile Strength
Standard II Curcumin	0.760	0.642
Standard I Hydroheal	29.54	30.56
Test	38.11	41.14

Figure 1 shows the comparison between the test and control groups.

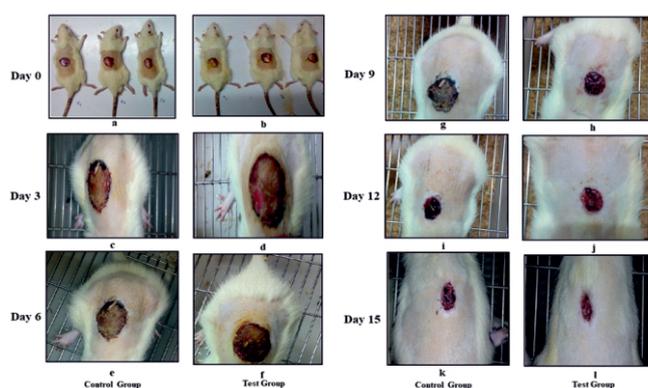


Figure 1: Comparison of control versus test group

Discussion

Wounds are common health concerns involving an intricate healing process comprising three imperative phases; inflammatory, proliferative, and remodeling. Wounds, if ignored, may turn infectious; hence need to be treated carefully and immediately. At times, chronic wounds develop free radicals and consequently result a delay in the wound healing process. Farfetched advancements in medicine have made numerous wound healing drugs available in the market, but these remedies are expensive with undesired side effects. These agents lack the potential to facilitate the wound healing process. However, many natural products have the ability to repair wounds by exerting anti-oxidant and anti-inflammatory effects and promoting tissue and collagen generation to reinstate the skin. Many phyto compounds are reported to contain cell growth factors and play a key role in fibroblast proliferation, and this property is deficient in synthetic compounds⁽¹²⁻¹⁴⁾. Plants and herbs have been reported to possess wound healing properties due to the presence of various active constituents like alkaloids, flavonoids, glycosides, terpenoids, etc.

The AO11 oil containing pig fat and without pig fat has been previously evaluated by the investigator, Patankar et al. for its safety profile⁽¹¹⁾. The oil was assessed for its acute and sub-acute dermal toxicity and mucus membrane irritation potential. The formulation was non-toxic to the hemopoietic system and non-irritant to the mucus membrane. There was an absence of renal and hepatic toxicity.

In the present study, incision wound healing efficacy of AO11 was evaluated where all the animals appeared normal and showed no clinical signs of intoxication till the end of the study of nine days. This indicates that the herbal formulation AO11 is tolerable and safe to use without harm. Usually, alteration in body weight is considered as an important parameter for the assessment of the response of an individual to the drug and might indicate its side effects. There was no statistically significant change in the body weight of any of the animals. Similar results were reported by Patankar et al.⁽¹¹⁾, wherein there exists a minor difference in the relative weight of vital organs of rats from both groups, indicating an insignificant effect on the vital organs due to dermal application of test material for 28 days. The test group (48.30%) showed an increase in mean tensile strength when compared to the control group (38.14%) and both the standards, i.e., hydroheal (45.30%) and curcumin group (46.32%). Studies have reported that extracts of herbal drugs, *Bacopa monniera*, have shown antimicrobial activity and enhanced wound breaking strength; *Tridax procumbents* have shown a significant increase in tensile strength of incision wounds^(12,13).

A study by Talekar et al. has been reported wherein polyherbal formulations have shown rapid skin regeneration and wound contraction⁽¹⁴⁾. This herbal formulation comprises rhizomes of *Curcuma longa* (turmeric) and *Glycyrrhiza glabra* (Licorice), the bark of *Hamiltonia suaveolens*, *Typha angustifolia* flowers, and *Azadirachta indica* (Neem) leaves incorporated in *Sesamum indicum* oil and pig fat used as a base. *Curcuma longa* containing active constituent curcumin is a well-known traditional medicine used widely and reported as a wound-healing agent. It possesses anti-inflammatory action and the ability to enhance granulation, tissue formation, collagen deposition, and wound contraction required for tissue repair⁽¹⁵⁾. Similarly, *Glycyrrhiza glabra* is reported to have wound healing properties which may be attributed to its anti-oxidant and anti-inflammatory activities⁽¹⁶⁾. *Hamiltonia suaveolens*, *Typha angustifolia*, and *Azadirachta indica* are traditionally used wound healing agents that are evaluated and reported^(17,18). Sesame oil is used as a healing oil in folklore medicine⁽¹⁹⁾. It has beneficial wound healing and antimicrobial properties due to its lignan-containing phytoconstituents. Various pharmacological activities like anti-oxidant, antimicrobial and anti-infective are exhibited by different components of the AO11 formulation that promote wound healing.

The literature search did not have any mention of such herbal formulation tested for external and internal wounds as well. In modern medicine, wound management consists of systemic corrections of any co-morbid condition with antibiotics, local use of cleansing agents including antiseptic/antibiotic solutions, etc. However, there is a lack of local agents available to promote the wound healing process. The present formulation shows its regenerative function in wound management. Despite the limitations of conducting the study in a small sample size and lesser days of follow-up, the study shows an increase in the efficacy of the AO11 as compared to the control.

Conclusion

In conclusion, AO11 showed an increase in tensile strength compared to the control group but a decrease in tensile strength compared to standard I and II groups. Thus, it can be concluded that the test material, AO11, may be effective compared to the control group and can be safe for use. It can be used as an internal wound healing agent, including surgical wounds, body incisions, or other internal organ injuries. This formulation could be the first of its type of herbal formulation which can be used in incision wounds confirming its safety and efficacy as well.

Patent: This drug formulation has been submitted for national and US patents and has been accepted in the name of the corresponding author.

1. Indian Patent (Number: 350253) dated 17th February 2011
2. United States patent (Number: US 8,709,509 B2) dated Apr 29, 2014
3. European patent (Number: 2675528) dated 03.05.17

Conflict of Interest: Nil

Source of Support: Nil

Copyright © 2022 Bharati Vidyapeeth Medical Journal (BVMJ). This is an open access article, it is free for all to read, download, copy, distribute, adapt and permitted to reuse under Creative Commons Attribution Non Commercial-ShareAlike: CC BY-NC-SABY 4.0 license.

ORCID

Suresh B Patankar  0000-0001-8996-7127

References

1. Patel AM, Kurbetti SM, Savadi RV, Thorat AV, TakaleVV, Horkeri SV. Preparation and evaluation of wound healing activity of new polyherbal formulations in rats. *Am JPhytomedClinTher.* 2013; 1: 498-506.
2. Ogwang PE, Nyafuono J, Moses A, Omujal F, Tumusiime HR, Kyakulaga AH. Preclinical efficacy and safety of herbal formulation for management of wounds. *Afr Health Sci.* 2011; 11: 524-9.
3. Kranke P, Bennett M, Roeckl-Wiedmann I, Debus S. Hyperbaric oxygen therapy for chronic wounds. *Cochrane Database Syst Rev.* 2004; CD004123.
4. Pawar RS, Chaurasiya PK, Rajak H, Singour PK, Toppo FA, Jain A. Wound healing activity of *Sidacordifolia* Linn. in rats. *Indian J Pharmacol.* 2013; 45: 474-8.
5. Thakur R, Jain N, Pathak R, Singh Sandhu S. Practices in wound healing studies of plants. *Evid Based Complement Alternat Med.* 2011; 2011:438056.
6. Kumar V, Khan A, Nagarajan K. Animal models for evaluation of wound healing activity. *IntBulletin Drug Res.* 2013; 3: 93-107.
7. Pandey M, Worlikar P, Ghosh A, Bondekar A, Chetan S. Comparison of wound healing activity of *Jethimadh* with *Triphala* in rats. *Int J Health Allied Sci.* 2012; 1: 59-63.
8. Gottrup F, Agren MS, Karlsmark T. Models for use in wound healing research: a survey focusing on in vitro and in vivo adult soft tissue. *Wound Rep Reg.* 2000; 8:83-96.
9. Vogel HG. *Drug discovery and evaluation: pharmacological assays/H. Gerhard Vogel (ed.)*.
10. Gal P, Toporcer T, Vidinsky B, Mokry M, Novotny M, Kilik R, et al. Early changes in the tensile strength and

- morphology of primary sutured skin wounds in rats. *Folia Biologica (Praha)*. 2006; 52:109-15.
11. Patankar SB, Mujumdar AM, Sane RT, Remya U. Evaluation of safety profiles of Anuradha oil - An herbal wound formulation in laboratory animals. *Int J Pharm Pharm Sci*. 2015; 7:278-82.
 12. Murthy S, Gautam MK, Goel S, Purohit V, Sharma H, Goel RK. Evaluation of in vivo wound healing activity of *Bacopa monniera* on different wound model in rats. *Biomed Res Int*. 2013; 2013: 972028.
 13. Talekar YP, Das B, Paul T, Talekar DY, Apte KG, Parab PB. Evaluation of wound healing potential of aqueous and ethanolic extracts of *Tridax procumbens* Linn. in Wistar rats. *Asian J Pharm Clin Res*. 2012; 5:141-5.
 14. Talekar YP, Apte KG, Paygude SV, Tondare PR, Parab PB. Studies on wound healing potential of polyherbal formulation using in vitro and in vivo assays. *J. Ayurveda Integr Med*. 2017; 8:73-81.
 15. Akbik D, Ghadiri M, Chrzanowski W, Rohanizadeh R. Curcumin as a wound healing agent. *Life Sci*. 2014; 116: 1-7.
 16. Najeeb VD, Al-Refai AS. Antibacterial effect and healing potential of topically applied licorice root extract on experimentally induced oral wounds in rabbits. *Saudi J Oral Sci*. 2015; 2: 10-13.
 17. Alande SM, Kharatmol AN, Adhav AJ, Patil AR, Disouza JI. Novel topical gel formulation of *Hamiltonia suaveolens* for wound healing activity. Next generation DNA led technologies. Springer: 59-70.
 18. Maan P, Yadav KS, Yadav NP. Wound Healing Activity of *Azadirachta indica* A. Juss bark in mice. *Pharmacogn Mag*. 2017; 13: S316-20.
 19. Shittu LA, Bankole MA, Ahmed T, Aile K, Akinsanya MA, Bankole MN, et al. Differential antimicrobial activity of the various crude leaves extracts of *Sesame radiatum* against some common pathogenic microorganisms. *Sci Res Essay*. 2006; 1:108-11.