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Comparative Wound Healing Efficacy of Neem Oil, Turmeric and Oxoferin® On Full Thickness Cutaneous Wounds in a Rabbit Model

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Abstract: *Wound is attributed as a loss or tearing of cellular, functional and anatomical continuity of live tissues. Complex biological phases occur during wound healing are coagulation, epithelization, granulation, collegenation and finally remodeling of tissues. Azadirachta indica (Neem) and Curcuma longa (Turmeric) are two traditional medicinal plants used as herbal medicine in many parts of the world, which show anti-inflammatory, immuno-modulatory, anti-carcinogenic, blood purifier and wound healing properties. Tetrachlorodecaoxide (TCDO; Oxoferin®) is a proved drug for wound care which contains a chlorite that shows the wound healingand immuno-modulation activity. The proposed study was conducted to evaluate and compare wound healing efficacy of Azadirachta indica and Curcuma longa with a standard drug for wound healing i.e. Oxoferin®. In this study 20 adult healthy rabbits of either sex ware selected. Three full thickness skin wounds of equal size (approximately 2x2cm) were created on each rabbit on both sides of trunk (thoraco-lumber region) and marked according to wound site. All three trial drugs topically applied to same experimental model in rotational manner. Wounds treated with neem oil designated as group A, wounds that received turmeric treatment were considered as group B while Oxoferin® were categorized as group C (control group). Healing checked out on daily basis by observations and measurements. The evaluation criteria of this study based on wound contraction rate, wound healing time, tensile strength and histo-pathological evaluation. Data thus generated was analyzed using Analysis of Variance (ANOVA) model.*

Keywords: *Histopathology, Oxoferin,Traditional medicine, Tensile strength and Wound Healing*

I. Introduction

Wound is termed as a loss or tearing of cellular and anatomical or functional continuity of living tissues [1]. Wounds are mainly categorized into surgical, traumatic, diabetic, venous, arterial and pressure wounds. Different phases which constitute wound healing process are coagulation, epithelization, granulation, collagenation and remodeling of tissues [2]. In full thickness skin wound the healing occur with fibroblast proliferation from margins into the damaged tissue area to promote angiogensis which initiate re-epithelization process [3]. Delayed and improper healing of wound is the main concern in these days all over the world due to contamination and infections of wound site [4]. A number of medicinal plants also have been used for management and treatment o wounds and over the years [5]. In last few years traditional medicinal plants have been evaluated for their pharmaceutical actions, one of which is *Azadirachta indica* (Neem). It is a native plant of India and many tropical and subtropical countries. Biological active substances isolated from *Azadirachta indica* include Azadirachtine, meliacin, gedunin, valassin, nimblolides, nimbin, nimbidin and salanin. It has been reported that neem extracts contained anti-bacterial properties that can be used against food borne pathogens and spoilage micro-organisms [6]. One of the other traditional medicinal plants is *Curcuma longa* (Turmeric,Haldi), which has wound healing properties due to its anti-inflammatory nature. It also increase fibroblast activity which enhance early synthesis of collagen fibers [7]. The major curcuminoids of *Curcuma longa* are demethoxycurcumin (also known as curcumin II), bis-demethoxycurcumin (curcumin III) and cyclocurcumin [8]. Turmeric also has been reported to exhibit properties like as an antiseptic, cure for skin diseases, wound healing, respiratory distress, cure for poisoning, eliminate waste product from body, remedy for treatment of sprains and swellings caused by injury [9-10].There are many drugs which are used as standard for treatment of all types of wounds, one of these is Tetrachlorodecaoxide (TCDO; Oxoferin®, Brookes Pharmaceutical Labs (Pvt.) Karachi Pakistan) which contains chlorite which is radiation protective agent that shows immuno-modulation and wound healing efficacy [11]. It forms a TCDO-hemo complex with heme part ofhemoglobin, myoglobin and peroxidases. This mechanism in turn activates the macrophages and modulates the process of phagocytosis to engulf pathogens and cell debris which is present on the wound surface, thus

cleansing of wound surface and regenerative process at wound site is accelerated [12]. There are many reports which indicate the use of these herbal products as wound healing agents, but their evaluation in comparison with some established wound healing agent have never been done, so keeping in view the economics the proposed study has been planned with the objectives;

1- To evaluate and compare wound healing efficacy of neem oil with an established wound healing agent i.e. Oxoferin®.
2- To evaluate and compare the wound healing efficacy of turmeric with an established wound healing agent i.e. Oxoferin®.

II. Materials and Methods

This experimental trial was executed on 60 surgically inflicted full thickness cutaneous wounds on thoraco-lumber region of 20 adult, active and clinically healthy rabbits of either sex to evaluate comparative wound healing activity of neem oil and turmeric with standard reference drug Oxoferin ®

2.1 Housing and feeding management of experimental rabbit

All the experimental models were managed uniformly for two weeks before the start of experiment by using Laboratory Animal Facility of the Department of Clinical Medicine and Surgery. During acclimatization period rabbits were provided with 8-10 hours light with good ventilation as well. Room temperature was maintained at 22-26^{o}C. Each experimental model received two doses of ivermectin @ 400 microgram/kg s/c a week apart.

2.2 Experimental design, surgical intervention and wounds dimension

Animals were anesthetized by using intramuscular injection of ketamine hydrochloride (Ketarol TM, Global Pharmaceuticals, Pakistan) @ 13-30 mg/kg body weight. By using scalpel three full thickness skin e wound of size 2cm^2 were created on both sides of trunk (thoraco lumber region) with an appropriate distance (2cm) from dorsal mid line by adopting excision wound model and each animal was considered as a single experimental unit. The inflicted wounds on each rabbit were designated as; right front, right rear and left lateral wound

2.3 Treatment protocol

Each experimental model having 3 wounds was treated topically with trial drugs rotationally. Wounds which were treated with *Azadirachta indica* (Neem oil) were categorized as group A, wounds which received treatment with *Curcuma longa* (Turmeric) past in inert cream were categorized as group B, while the animals which received standard reference drug Tetrachlorodecaoxide (TCDO; Oxoferin®) solution were categorized as group C (Control Group).

2.4 Evaluation parameters for wound healing

Wound contraction rate;Contraction rate was measured with the help of a vernier caliper by tracing the edges of wound on trace paper at after every 3 days interval [13-14]. Healing time; was evaluated by the daily visual assessment and observation of wounds. Healing time starts right from the time of infliction of wound until complete regeneration of injured tissues. Estimation of healing time was done by sum of daily observations till dropping off scar tissue [15]. Histopathology; Preserved tissue samples were processed for histopathological studies [16]. Histological photograph of each sample slide were captured using Nikon Opt I Photo 2 microscope at 100X and 200X to diameter (µm) of skin layers (i.e dermis and epidermis) and collagen contents of each sample with the help of automated image analysis system Image J® version 1.43n National Institute of health (NIH),USA. Tensile strength;It is maximum stretching force per unit of cross sectional area of healed tissue before breaking point in a Tensile testing machine (Tensometer). Following the gain in tensile force the breaking strength will be calculated [17] using following formulae:

$$\text{Breaking strength\%} = \frac{\text{Mean tensile strength of strips from treated groups}}{\text{Mean tensile strength of control groups}} \times 100$$

III. Statistical Analysis

The data thus generated were analyzed statistically by using ANOVA and the difference in the means was compared by using Least Square Duncan Multiple range test LSD[18].

IV. Results

Wound healing comprises of highly integrated and dynamic series of physiological and biochemical process. In the most part of world peoples relies on the use of traditional medicine to restore their health status.

The current study was based on the hypothesis that wound healing can be halsted by the use of traditional herbal medicine.

4.1 Wound contraction rate

Contraction of surgical wounds was completed at 16th and 18thdays in case of neem oil and turmeric respectively, while in Oxoferin® treated wound contraction was terminated at 14thdays on average. At day 15, all the treatment were statistically significant *(p<0.05)* among each other. At day 16 wounds of group A treated with neem oil healed completely while turmeric applied group B healed completely on day 18. (Table-1)

Table: 1 Contraction rate (mm= %; Mean ±SE) at 3 days interval, of 3 groups *Viz.* treated with Neem oil, Turmeric and Oxoferin® (n=20).

Treatment	day 0	day3	day6	day9	Day12	Day15	Day18
Neem oil	2.000±0.000	1.660± 0.026 B	1.210 ± 0.020 AB	0.790± 0.043 AB	0.415± 0.037 B	0.095± 0.030 B	0.000± 0.000 B
Turmeric	2.000±0.000	1.735± 0.023 A	1.240± 0.017 A	0.900± 0.059 A	0.510± 0.034 A	0.200± 0.027 A	0.020± 0.009 A
Oxoferin	2.000±0.000	1.620± 0.024 B	1.170± 0.018 B	0.675± 0.041 B	0.335± 0.024 B	0.020± 0.012 C	0.000± 0.000 B
F-value	-	5.84**	3.62*	5.39**	7.36**	13.67**	4.75*

Means sharing similar letter in a column are statistically non-significant (p>0.05)

4.2 Healing Time (Days)

Regarding healing time, wounds of group A and C were applied with neem oil and Oxoferin® respectively exhibited statistically non significance difference*(p>0.05),* while turmeric showed statistically significant difference*(p<0.05)* with Oxoferin® and statistically non significance difference *(p>0.05)*with neem oil. On average neem oil treated group took 15 days for complete healing on the other hand turmeric applied group B took 18 days for the same purpose and Oxoferin® treated wounds healed at 14 days.

4.3Tensile Strength

At the end of study period Oxoferin® was statistically significant *(P<0.05)* than neem oil where as there was statistically non-significant difference *(P>0.05)* between Oxoferin® and turmeric. When tensile strength of neem oil and turmeric compared with each other they showed statistically non-significant difference (*P>0.05).* Over all mean values attained by different treatments in term of tensile strength were 78.17± 3.977, 87.36±3.240 and 90.38± 2.321 for neem oil, turmeric and Oxoferin® respectively. (Table-2)

Histopathological examinations

After performing histopathological protocol, the stained slides of tissue samples were evaluated for thickness of skin layers including epidermis and dermis and collagen contents. Arrangement and compactness of collagen fibers was observed in terms of collagen content percentage. Number of capillary bed formation, neuvascularization, glandular arrangement and proliferation of inflammatory cells were also observed.

4.4 Thickness of epidermis

Histopathologically analyzed results suggested that Oxoferin® has a statistically significant *(p<0.05)* difference as compared to both turmeric and neem oil , while there was statistically non significant *(P>0.05)* difference was observed in case neem oil and turmeric with each other. (Table-3)

4.5 Collagen Contents %

Regarding statistical analysis of collagen contents % and compactness all the three treatments viz.neem oil, turmeric and Oxoferin® were statistically nonsignificant*(P>0.05)* among each other at the end of study period. Collagen fibers of turmeric were almost have a similar arrangement and compactness with as in case of Oxoferin®, while loose arrangement of collagen contents was observed in neem oil treated wound. Oxoferin® showed superior and well arranged collagen contents as compared to neem oil and turmeric. (Table-4)

4.6 Thickness of Dermis

Regarding thickness of dermis, histological comparison of healed tissue suggested that Oxoferin® showed statistically significant difference *(p<0.05)* when compared with neem oil and turmeric, while the comparison in case of neem oil and turmeric suggested statistically non-significant difference with each other.(Table-5)

Table: 2 Tensile strength (NF; Mean ±SE) of three groups *viz.* Neem oil, turmeric and Oxoferin® (n=10) at the end of study period.

Treatment	Mean ± SE
Neem Oil	78.17 ± 3.977 B
Turmeric	87.36 ± 3.240 AB

Oxoferin	90.38 ± 2.321 A

Table: 3 Thickness of Epidermis (mm; Mean ±SE) of three groups *viz*. Neem oil turmeric and Oxoferin® (n=10) at the end of study period.

Treatment	**Mean ± SE**
Neem Oil	17.75 ± 1.579 B
Turmeric	13.77 ± 1.412 B
Oxoferin	26.15 ± 2.109 A

Means sharing similar letter are statistically non-significant (P>0.05).

Table: 4 Collagen content (%; Mean ±SE) of three groups *viz*. Neem oil,Turmeric and Oxoferin® (n=10) at the end of study period.

Treatment	**Mean ± SE**
Neem Oil	77.25 ± 1.408 A
Turmeric	77.48 ± 1.412 A
Oxoferin	79.29 ± 1.860 A

Table: 5 Thickness of Dermis (mm; Mean ±SE) of three groups *viz*. Neem oil, Turmeric and Oxoferin® (n=10) at the end of study period.

Treatment	**Mean ± SE**
Neem Oil	1308.04 ± 85.12 B
Turmeric	1269.42 ± 76.32 B
Oxoferin	1576.39 ± 55.23 A

Means sharing similar letter are statistically non-significant (P>0.05).

V. Discussion

Wound healing is highly integrated and physiological process to restore damaged cellular and functional structure of body. Many researcher proposed that wound healing process can be enhanced by herbal drug formulation, having antiseptic, anti inflammatory and antioxidant properties [19-20]. The result of current study showed that topical application of turmeric and neem oil exhibited a significant difference in contraction rate by decreasing healing time and comparable in this context. Regarding contraction rate of neem oil on full thickness cutaneous wounds, neem oil almost showed a statistically non-significant difference throughout and at the end of study period as compared to Oxoferin®. Significant effect on wound contraction rate of neem oil might be due to its neovascularization effect of neem oil (*Azadirachta indica*), which is considered as one of important factors for wound healing as reported by Emeka *et al.* [21]. Healing time of neem oil treated wounds exhibited statistically non-significant difference with that of Oxoferin. Good results regarding healing time were matched with the findings of Somashekar *et al*. and Sunil *et al*. [19 and 20]. They reported that good results in wound healing by topical application of neem oil (*Azadirachta indica*) might be due to its herbal preparation possessing antiseptic, anti inflammatory and anti oxidant properties. Regarding histopathological evaluation of neem oil treated wounds, collagen contents % was statistically significant as compared to Oxoferin®. Collagen fibers are not properly organized in comparison with Oxoferin® suggested poor healing in case of collagen contents %. This poor effect due to collagen fibers in wound healing should be correlated with findings of Cohen and Burns [22] who reported that collagen played a central role in the healing of wounds. Skin layers thickness i.e dermis and epidermis showed statistically non significance difference when compared with Oxoferin®, the effect of skin layer thickness on wound healing might be due its astringent and antimicrobial property, which seems to be responsible for significance increase in wound contraction and rate of epithelialisation also explained by Dash *et al*. and Shivananda *et al*. [23 and 24]Tensile strength of neem oil treated cutaneous wounds non-statistically difference at the end of study period after complete healing as compared to Oxoferin® treated wounds. Tensile strength of healed tissue represents the integrity of wound healing and accelerated by fibroblast proliferation, collagen synthesis, and neovascularizationas reported by Habibipour *et al.* [25]. The difference of healing time of turmeric and Oxoferin® was statistically significant showing that turmeric efficacy of wound healing is less than the standard drug Oxoferin®. Statistically significant reduction in healing time was revealed by Oxoferin® can be attributed to generation of dense collagen contents and improved angiogenesis as reported by Yadav *et al*. [26]. Contraction rate of turmeric treated wounds was exhibited statistically significant results showing less contraction rate at the end of study period among all the treated groups. This comparison was not evaluated previously on wound healing, while the better effect of turmeric ointment on wound healing was recorded in comparison with normal saline by Purohit *et al*. [27].Turmeric was showed statistically non significance difference with standard reference drug Oxoferin®

that is comparable with each other at the end of study period. Our statistically non significance difference on tensile strength was correlated with the findings of Jagetia *et al.* [28]. Histopathological evaluation of collagen contents for turmeric treated wounds was showed statistically non-significant difference with standard drug Oxoferin®, showing less collagen contents. Significance difference of turmeric collagen contents was compared with the findings of Gadekar *et al.* [29]. He evaluated turmeric through curcumin transdermal patches formulation on rat full thickness cutaneous wounds. Our findings was related with Henhena *et al.* [30] who reported that healing of wounds relates directly to the formation of collagen content which is also a active component of proliferation tissue. When the healing time of neem oil was compared with Oxoferin® then both experimental drugs were revealed statistically significant difference with each other being a neem oil showed less healing time as compared to turmeric at the end of study period. It might be related with the findings of Bhardwaj and Rajput [31]. They described that neem oil has ability to help the body rapidly to create collagen fibres for closing of wounds in minimum time. The better findings of my research regarding healing might be in accordance with the findings of Bhardwaj and Rajput [31] who determined the less healing time by comparing pure neem oil and neem oil in paraffin base with turmeric powder. Contraction rate of neem oil treated cutaneous wounds was exhibited statistically significant difference with that of turmeric treated wounds after the end of study period at day 18 showing better trend in contraction rate wounds treated with neem oil as compared to turmeric. Similar results was also documented by Narendhirakannan *et al*. [32] who analyzed the aqueous and ethanolic extractions of seven traditional medicinal plants including *Azadirachta indica* and *curcuma longa* and found a better healing by the use of neem oil followed by turmeric and other different extracts of plants. Regarding histopathological evaluation of neem oil treated wounds, collagen contents %, was showed statistically non significance difference when compared with turmeric treated wounds showing good percentage of collagen content in case of turmeric as compared to neem oil. When epidermis and dermis thickness of neem oil treated cutaneous wounds were compared with turmeric treated group it was also showed statistically non significance difference with turmeric treated group. These significance results were correlated with the findings of Bhardwaj and Rajput [31]who reported the better histopathological findings were revealed in the wounds treated neem oil as compared to turmeric powder. Tensile strength of neem oil treated wounds and turmeric was showed statistically non significance difference, turmeric being better results as compared to neem oil. This indicates the large amount of fibroblast proliferation, collagen synthesis, and neovascularization at wound site which resulted in an increased wound tensile strength and accelerated healing.

VI. Conclusion

It was concluded that the neem oil and turmeric exhibited wound healing activity in full thickness cutaneous wounds in a rabbit model. When both neem oil and turmeric were compared with a much costly wound healing agent i.e. Oxoferin® it showed comparatively low ranked results in terms of contraction rate, tensile strength and healing time. Whereas almost similar healing efficacy as compared to Oxoferin®, in terms of sufficient collagen fibers bridging at the wound site along with rich capillary beds at healing area. When comparison was done between neem oil and turmeric then neem showed better healing efficacy. However, further studies should be conducted on their antimicrobial, anti-inflammatory and toxicological aspects as an alternatives of Oxoferin® like much costly drugs with an economically low-priced agent for wound healing or wound management.

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