IN VITRO ANTIOXIDANT PROPERTIES AND WOUND HEALING ACTIVITY OF HYDROETHANOLIC TURMERIC RHIZOME EXTRACT (ZINGIBERACEAE)

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ABSTRACT

Objective: In the present study, we evaluated the ethno pharmacological effect of the different doses of Turmeric rhizome hydroethanolic extract ointment (TRO), using in vitro antioxidant activity and in vivo excision, incision and dead space wound models in Wistar rats.

Methods: The antioxidant activity and acute toxicity studies were performed. In excision wound model, period of epithelization time and wound area were monitored on days 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 using graph paper. Additionally, histological evaluation was carried out on days 3, 7, 14 and 21. Incision wound modle and dead space, were performed for breaking strength value and hydroxyproline content.

Results: The results of wound contraction and skin breaking strength a significant increased showed in wound contraction and breaking strength value rate, in all treatment groups with TRO, especially in high dose of TRO. Thus, results of histopathological evaluation showed that TRO accelerate wound healing process specifically in re-epithelization period, angiogenesis and collagen disposition in all treatment groups.

Conclusion: Data revealed that ointment formulation prepared with hydroethanolic extract of Turmeric rhizome had remarkable wound healing activities.

Keywords: Hydroethanolic extract, Turmeric rhizome, Excision wound, Incision wound, Dead space, Rat.

INTRODUCTION

Skin is one of the largest organs in the body, which injures inescapably during life; these often arise due to physical or chemical injuries and microbial infections. Following burn, organ injury and pathogens capable to entry into the body and consequently it is prone to infection. To minimize happening of such problems, skin wound healing process begins immediately [1]. Inflammation, proliferation and tissue remodeling are three phases of healing process which occurs following tissue damages [2]. Herbal preparations can be more effective and safety than conventional medicines because of their non-toxic nature which ables them to be administered over long periods [3].

Turmeric (Curcuma longa L) is one of the main spices belongs to Zingiberaceae family [4], which imparts a characteristic yellow color to curry powder and has been used to enhance color, aroma and flavor of food in most regions of southern Asia [5]. Flavonoids are responsible for many biochemical processes in plant growth and development [6]. Turmeric extract contain high levels of mineral dyes, curcumin, curcuminoids, phenolic compounds and volatile oils e.g. turmerone, atlantone and zingiberene [7, 8].

Scarce information available on effects of Turmeric rhizome hydro alcoholic extract on in vivo non infected wound healing. So the hypothesis of present study was to evaluate wound healing activities of Turmeric rhizome hydroalcoholic extract using in vivo experimental models.

MATERIALS AND METHODS

Plant and Hydro alcoholic extract preparation:

Turmeric rhizome powdered (150 g) was purchased from local herbal market in Hamadan province, Hamadan, Iran. Specimens from the plant material were deposited at the Department of Botany Sciences, the Hamadan research Agricultural and Natural resoures center, Hamadan, Iran. The powder of Turmeric rhizome was suspended in 300 ml of an aqueous ethanol solution (7:3, v/v) for 7th day at room temperature. The mixture was filtered using a fine muslin cloth followed by filter paper (Whatman No. 1). After filtration, the solvent was completely evaporated under vacuum at 40 °C in a rotary evaporator and the hydroalcoholic Turmeric rhizome extract was obtained after lyophilization [9].

Determination of ferric reducing/antioxidant power assay (FRAP)

FRAP assay was carried out according to the method previously described by Benzie and Strain [10]. FRAP reagent was prepared from acetate buffer (1.6 g sodium acetate and 8 ml acetic acid make up to 500 ml) (pH 3.6), 10 mM TPTZ solution in 40 mM HCL and 20 mM iron (III) chloride solution in proportion of 10:1:1 (v/v) respectively. The FRAP reagent was prepared fresh daily and was warmed to 37°C in the oven prior to use. A total of 50 μl sample extract was added to 1.5 ml of the FRAP reagent and mixed well. The absorbance was measured at 593 nm using microplate reader spectrophotometers (Molecular devices, VERSAmax tunable, California, USA) after 4 min. Samples were measured in three replicates. Standard curve of iron (II) sulfate solution (200, 400, 600, 800 and 1000 ppm) was prepared using the similar procedure. The results were expressed as μmol Fe (II)/100 g extract sample.

Determination of total phenolics

Total phenolic contents of all plant extracts were determined using Folin-Ciocalteu reagent as described by Singleton and Rossi [11]. Samples were inserted into different test tube and mixed thoroughly with 5 ml Folin-Ciocalteu reagent (previously pre-dilute 10 times with distilled water). After 5 min, 4 ml of 7.6% sodium carbonate (Na2CO3) was added and allowed to react for 2 hrs at room temperature. The absorbance was measure at 765 nm using microplate reader spectrophotometers (Molecular devices, VERSAmax tunable, California, USA). Samples were measured in three replicates. Standard curve of gallic acid solution (10, 20, 40, 60, 80 and 100 ppm) was prepared using the similar procedure. The results were expressed as mg GAE/100 g extract sample.

Formulation of Topical Wound Application Forms

We prepared four variants of the topical application form comprising of the study materials in ointment. All the variants...
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comprised of the base formulation consisting of Eucerin (30%) and Vaseline (70%) in about 1:2 proportions.

After the operation, all rats were divided randomly into four groups. Two groups served as controls: Group 1 (negative control) had no receive any administration and Group 2, placebo (positive control) animals were applied to the base formulation consisting of Eucerin (30%) and Vaseline (70%). Groups 3, 4 and 5 (experimental) applied respectively, with the 1.5% (w/w), 3% (w/w) and 6% (w/w) of powder extract mixed with base formulation (Turmeric rhizome extract ointment: TRO) until complete epithelialization. The respective ointments were topicaly applied once a day (0.5g), on the wound area to all animals in each respective groups, starting from the day of operation, till complete epithelialization. All rats were monitored until complete epithelialization for possibility of infection and any wound fluid or other abnormalities.

**Experimental animals**

Healthy inbred male Wistar albino rats weighing (165-180g) approximately 7 weeks of age were obtained from the experimental animal house, Veterinary college of Uremia University and used in study. All rats were divided randomly into five groups (n=8 in each). The animals were kept in separate cages for four days at room conditions to aclimatize. Animal houses were in standard environmental conditions of temperature (22±3°C), humidity (60±5%), and a 12h light/dark cycle. The animals offered on standard pelleted diet and fresh tap water. All rats were closely observed for any infection and if they showed signs of infection separated, excluded from the study and replaced. The study was approved by the ethics committee for animal experimentation by the Faculty of Veterinary Medicine, Islamic Azad University, Urmia Branch.

**Acute toxicity tests**

In order to find a safe dose for Turmeric rhizome hydroethanolic extract, acute toxicity study was conducted. twenty healthy white mixed sex Wistar rats (ten males and ten females), approximately 200 g and 6 to 8 weeks of age were randomly divided into 5 groups of 4 animals each: Control, Placebo, 4, 8g and 12g of Turmeric rhizome powder groups. Animals were under surveillance for 30 min 24, 24 and 48h after administration for the onset of clinical or toxicological symptoms. Mortality rate was recorded in a period of two-week, if any. The animals were euthanized on day 14 post-test. Hematological, serum biochemical and histological (liver and kidney) parameters were determined based on the standard methods described by others [11].

**Circular excision Wound Model**

This model of wound was used to monitor wound closure time and histopathological. After induction of anesthesia with Xylazine HCL 2% (5 mg/kg/IP); Afiasan International, Woerden, Holland) rats were fixed in a ventral posture on a surgery table. The skin of the dorsal area from the scapula to the end thoracic vertebra was shaved with an electric clipper and scrubbed. Following on, create one circular, full thickness surgical wounds (20 mm diameter) with a surgical scalpel and scissor. Then, all animals were randomly divided into five groups of eight in each. This model of wound was used to evaluate of percentage, re-epithelialization and histology (histomorphometry) of wound healing.

**Subjective evaluation**

The information of wound healing such as appearance of granulation tissue, amount of drainage on the wound surface, complete filling of the wound by granulation tissue, appearance of erythema, edema or necrosis were also recorded by visual observation during the daily wound care.

**Percentage Wound Contraction**

A transparency paper and a permanent marker were used in order to measure and record the wound size. The wound areas in all groups were recorded and measured on graph paper every other day. Later, wound contraction was calculated as percentage of the reduction in wounded area. The wound healing percentage was calculated by Walker formula after measuring the wound size [12]. The percentage of wound healing was computed at the beginning of experiments and the next 2, 4, 6, 10, 12, 14, 16, 18 and 20 days. A specimen sample of tissue was isolated from the healed skin of each group of rat for the histopathological examination. Percentage wound contraction was calculated as:

Percentage of wound size =Wound area on day X / Wound area on day zero × 100. Percentage of wound healing = 100 - Percentage of wound size.

**Histologic evaluation**

In order to specimens from skin were taken in the 3, 7, 14 and 21 days after surgery, with the rats under general anesthesia. Sample tissues, excised along with 1 to 2 mm surrounding normal skin and in a depth of approximately 3 mm, were pinned on a flat cork surface and fixed in neutral-buffered formalin 10%. Then sample tissues were routinely processed, paraffin wax embedded, sectioned at 5 µm, and stained with Masson's trichrome stain. Stained sections were microscopically (by light microscope, Olympus CX31RBSF attached cameraman) evaluated to assess the predominant stage of wound healing. Three parallel sections were obtained from each specimen. Following factors such as cellular infiltration (the number of mononuclear cells, polymorphonuclear cells and Fibroblastic aggregation), angiogenesis (the number of blood vessels and capillary buds) were quantitatively evaluated in 5 per high power fields (HPFs)(×400) [13].

**Incision wound model**

Animals were randomly divided into six experimental groups and six rats in each: Control, Placebo, 1.5%, 3% and 6% groups. All animals of experimental groups were anesthetized with the same way mentioned above and a 4-cm length incision was made through the skin and cutaneous muscle at a distance about 2 cm from the middle on right side of the deplatted back. The wound was closed at 0.5 cm intervals using 3/0 nylon (Dafilkin, B/Braun, Germany). All the groups were treated the same as mentioned in the excision model. Ointments were applied once daily for 9 days. On day 9, sutures were removed and the tensile strength of healed wounds was measured on day 10 by Strongraph mechanical test frame (Toyoseiki Tensile Testing Unit, Model R3, Japan) [14].

Tensile strength was calculated using the following formula:

Tensile strength = breaking strength (g) / cross sectional area of skin (mm2)

**Dead space wound model and hydroxyproline content estimation**

Animals were randomly divided into six experimental groups of six animals each: Group 1 was considered as the control group 2 was considered as the Placebo and groups 3, 4 and 5 were treated orally with 50 mg/kg, 100mg/kg and 200 mg/kg hydroethanolic extract suspended in 3% w/v TRO, respectively. Animals in placebo group received the same volume of phosphate buffered saline. The animals of control group had no received extract or PBS orally. Dead space wound was created using subcutaneous implanting of polypropylene tubes, 2.5 cm×0.5 cm, in the lumbar region on dorsal side [15]. All animals in experimental groups received extract from 0 to 9 day post-wounding induction. On day 10 post-wound induction, granuloma tissue created on implanted tube was carefully dissected and used to determine breaking strength and estimation of hydroxyproline content.

**Statistical Analysis**

Experimental results were expressed as means ± SEM. Statistical analyses were performed using PASW 18.0 (SPSS Inc., Chicago, IL, USA). Model assumptions were evaluated by examining the residual plot. Results were analyzed using one way ANOVA. Dunnett's test for pair-wise comparisons was used to examine the effect of time and treatments. Differences were considered significant when P < 0.05.
RESULTS

Antioxidant activity and total phenolic content

In this study, the antioxidant activity is also determined on the basis of the ability of antioxidant in this plant extract to reduce ferric (III) iron to ferrous (II) iron in FRAP reagent [16, 17]. Generally, FRAP assay was used due to its simplicity and reproducibility. The results indicated that turmeric exhibited antioxidant activity of 3.23 ± 2.55 μmol Fe (II)/g. Total phenolic contents of Turmeric rhizome extract were tested using the dilute Folin-Ciocalteu reagent. The result clearly showed that turmeric had a mean value of total phenol 15.48 ± 0.3 mg/g dried extract.

Acute toxicity tests

The Results of oral administration of Turmeric rhizome powder in rat indicated that this plant powder at dose 12g/bw had no impressed any signs of toxicity.

Wound contraction and days of re-epithelialization

The Results of the mean wound contraction percentage and epithelialization period, in the excision wound repair model after topical administration of the Turmeric rhizome hydroethanolic extract ointment in different doses, is presented in table 1.

These data revealed that rate of wound contraction percentage significantly higher at day 6, 8, 10, 12 and 14, was corresponding to (90.92 ± 0.92) extracted ointment in different doses, is presented in table 1. Results of oral administration of Turmeric rhizome powder in rat indicated that this plant powder at dose 12g/bw had no impressed any signs of toxicity.

Table 1: Effects of the TRO on percentage of circular excision wound and epithelialization period in Rat.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day2</th>
<th>Day4</th>
<th>Day6</th>
<th>Day8</th>
<th>Day10</th>
<th>Day12</th>
<th>Day14</th>
<th>Day16</th>
<th>Day18</th>
<th>Day20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>140.0±10</td>
<td>18.1±1.4</td>
<td>23.6±1.0</td>
<td>37.7±2.2</td>
<td>55.0±2.2</td>
<td>65.1±2.0</td>
<td>70.9±1.0</td>
<td>78.4±1.0</td>
<td>86.2±0.6</td>
<td>90.9±3.6</td>
</tr>
<tr>
<td>Placebo</td>
<td>41±1</td>
<td>2±1</td>
<td>7±1</td>
<td>8±1</td>
<td>1±1</td>
<td>7±1</td>
<td>3±1</td>
<td>5±1</td>
<td>4±1</td>
<td>3±1</td>
</tr>
<tr>
<td>TRO 1.5%</td>
<td>16.5±1.1</td>
<td>24.6±2.4</td>
<td>33.5±1.8</td>
<td>49.9±3.0</td>
<td>69.7±3.1</td>
<td>77.0±2.2</td>
<td>80.0±1.2</td>
<td>90.9±1.2</td>
<td>94.6±2.3</td>
<td>98.2±0.2</td>
</tr>
<tr>
<td>TRO 3%</td>
<td>18.9±1.1</td>
<td>33.8±1.2</td>
<td>43.9±1.7</td>
<td>54.0±2.0</td>
<td>73.9±2.1</td>
<td>78.8±2.4</td>
<td>84.9±2.3</td>
<td>94.5±2.1</td>
<td>98.0±0.0</td>
<td>100%</td>
</tr>
<tr>
<td>TRO 6%</td>
<td>21.3±1.0</td>
<td>28.6±1.1</td>
<td>47.5±1.4</td>
<td>59.6±1.9</td>
<td>76.0±3.1</td>
<td>82.6±2.4</td>
<td>88.5±2.0</td>
<td>96.9±2.6</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

n=6 animals in each group; TRO: hydroethanolic extracts of Turmeric rhizome extract ointment. Values are expressed as Mean ± SEM; * and ** is presented significant differences P<0.05 and P<0.01 between marked data in the same column.

Table 2: Mean Distribution of immune cells (PMN and MNC), new vessels formation and fibroblasts in different groups in Rat.

<table>
<thead>
<tr>
<th>Groups</th>
<th>PMN</th>
<th>MNC</th>
<th>New vessels</th>
<th>Fibroblast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>62.4±4.1</td>
<td>12.1±0.54</td>
<td>2.5±0.1</td>
<td>21.5±1.52</td>
</tr>
<tr>
<td>TRO 1.5% (day 3)</td>
<td>42.7±5.1</td>
<td>19.2±0.95</td>
<td>5.2±0.62</td>
<td>39.3±1.63</td>
</tr>
<tr>
<td>TRO 3% (day 3)</td>
<td>39.8±4.2</td>
<td>23.1±0.77</td>
<td>6.5±1.01</td>
<td>47.8±2.19</td>
</tr>
<tr>
<td>TRO 6% (day 3)</td>
<td>22.1±2.6</td>
<td>26.1±0.45</td>
<td>7.8±1.29</td>
<td>55.2±2.38</td>
</tr>
<tr>
<td>Untreated</td>
<td>50.2±3.4</td>
<td>14.3±0.4</td>
<td>3.2±0.28</td>
<td>35±1.26</td>
</tr>
<tr>
<td>TRO 1.5% (day 7)</td>
<td>33.2±1.4</td>
<td>15.8±0.71</td>
<td>4.1±0.71</td>
<td>58±2.12</td>
</tr>
<tr>
<td>TRO 3% (day 7)</td>
<td>29.9±0.4</td>
<td>16.5±1.6</td>
<td>4.7±0.29</td>
<td>67±2.41</td>
</tr>
<tr>
<td>TRO 6% (day 7)</td>
<td>15.1±2.8</td>
<td>19.9±0.8</td>
<td>5.6±0.68</td>
<td>81±2.25</td>
</tr>
<tr>
<td>Control (day 14)</td>
<td>32.1±1.7</td>
<td>9.2±0.67</td>
<td>2.8±0.81</td>
<td>57±2.62</td>
</tr>
<tr>
<td>TRO 1.5% (day 14)</td>
<td>18.4±1.4</td>
<td>12.4±0.58</td>
<td>1.3±0.56</td>
<td>4.9±3.91</td>
</tr>
<tr>
<td>TRO 3% (day 14)</td>
<td>15.8±0.3</td>
<td>12.8±1.41</td>
<td>2.5±0.09</td>
<td>85±4.33</td>
</tr>
<tr>
<td>TRO 6% (day 14)</td>
<td>8.9±0.6</td>
<td>14.3±1.0</td>
<td>3.6±1.0</td>
<td>94±4.81</td>
</tr>
<tr>
<td>Control (day 21)</td>
<td>12.9±0.9</td>
<td>3.3±0.3</td>
<td>0.7±0.29</td>
<td>28±1.87</td>
</tr>
<tr>
<td>TRO 1.5% (day 21)</td>
<td>5.1±0.3</td>
<td>3.8±0.81</td>
<td>0.9±0.32</td>
<td>35±2.72</td>
</tr>
<tr>
<td>TRO 3% (day 21)</td>
<td>3.9±0.5</td>
<td>3.1±0.88</td>
<td>1.9±0.68</td>
<td>42±3.42</td>
</tr>
<tr>
<td>TRO 6% (day 21)</td>
<td>1.1±0.1</td>
<td>4.3±0.79</td>
<td>2.2±1.0</td>
<td>49±3.55</td>
</tr>
</tbody>
</table>

TRO: hydroethanolic extracts of Turmeric rhizome extract ointment; PMN: polymorphonuclear cells; MNC: mononuclear cells; n=6 animals in each group; Values are expressed as Mean ± SEM; * and ** is presented significant differences P<0.05 and P<0.01 between marked data in the same column.
Note well formed collagen in deep dermis and longitudinal angiogenesis in treated animals. High dose of TRO-dependently downregulated the immune cells infiltration and enhanced collagen deposition in comparison to control sham animals, Masson trichrom staining. 400×.  

**Note:** Fibroblasts (FB), Macrophages (M), Collagen (C) bundles and vascularization with new blood vessels (BV).

**Incision wound study**

For evaluation of tensile strength of new repaired tissue, we used linear incision wound model. As shown in table 3, application of the Turmeric rhizome hydroethanolic extracts ointments onto the incised wounds, cause the significantly higher rate of mean ± SEM tensile strength in treatment groups 6% (498.08 ± 9.68) and 3% (449.81 ± 6.99) TRO compared control group (268.01 ± 4.60) on day 10 (p < 0.05).

**Table 3:** Effect of TRO on linear incision wound model in Rat. 

<table>
<thead>
<tr>
<th>Groups</th>
<th>Statistical mean ± SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>268.01 ± 4.60</td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>282.48 ± 4.75</td>
<td></td>
</tr>
<tr>
<td>TRO 1.5%</td>
<td>357.88 ± 4.88</td>
<td>*</td>
</tr>
<tr>
<td>TRO 3%</td>
<td>449.81 ± 6.99&quot;</td>
<td>**</td>
</tr>
<tr>
<td>TRO 6%</td>
<td>498.08 ± 9.68</td>
<td>***</td>
</tr>
</tbody>
</table>

n=6 animals in each group; Values are expressed as Mean ± SEM; * and " is presented significant differences P<0.05 and P<0.01.

TRO: hydroethanolic extracts of Turmeric rhizome extract ointment

**Fig. 1:** cross section from tissue 14 days after wound induction in (A) control-sham group, (B) TRO 1.5%, (C) TRO 3%, (D) TRO 6% groups in Rat.

**DISCUSSION AND CONCLUSIONS**

The phytochemical analysis of rhizome parts of Turmeric revealed presence of phenols in extract. Phenols and flavonoids have therapeutic uses due to their anti-inflammatory, antimicrobial, anti-fungal, antioxidant, wound healing and astringent properties which accelerating the process of wound contraction and epithelialization [13, 18]. Recently, curcumin demonstrated that chemo preventive and anticancer effects in several human cancers [19, 20]. The present study established the anti-oxidant activity Turmeric rhizome hydroethanolic extract. A number of studies reveal that curcumin (diferuloylmethane), is the active component of turmeric, an important role in treatment and/or has supporting role in various inflammatory conditions including arthritis, bronchitis, fever, diarrhea and relieve pain [21, 22, 23], anti-cancer effects [19, 20, 23], would healing effect [24, 25, 26].

Inflammation, proliferation and tissue remodeling are three phases of wound healing process which occur following tissue damages as closely as possible to its natural state. The healing process is activated when platelets come into contact with exposed collagen leading to platelet aggregation and the release of clotting factors resulting in the deposition of a fibrin clot at the site of injury. The fibrin clot serves as a provisional matrix and sets the stage for the subsequent events of healing. Inflammatory cells also arrive along with the platelets at the injury site providing key signals known as growth factors. Fibroblasts is the connective tissue cell responsible for collagen deposition required to repair the tissue injury. Collagen is the main constituent of extra cellular tissue, which is responsible for support and strength [2].

Hence, in the present study, to evaluate the effect of Turmeric rhizome hydroethanolic extract in deferential doses were used excision and incision wound models.

Histopathological study on day 3 or 4 after excision wound model creation, showed the coincidence significantly decrease of neutrophils count and increased the macrophages count onto the wound site; which these results were consistent other researcher findings (table 2).

Also the histopathological study on day 7 after excision wound model creation, showed a significant increase in tissue granulation formation, fibroblasts cells migration in all treatment groups, especially in treated group with 6% TRO compared to control group which may be due to increase in new blood vessel formation, increased levels of interleukin-8 secretion, due to the higher number of macrophage cells in wound site (table 2), and anti-oxidant activity of Turmeric hydroethanolic extract in treatment groups. This result were consistent other researcher findings [27, 28].

Histopathological study in two and three weeks post wound model creation, showed the collagen synthesis, collagen deposition and the quality and quantity of collagen bundles in the wound site (table 2). Increase in collagen concentration and stabilization of the collagen fibers, as a major component of extracellular tissue, may be cause the increase in tensile strength value in incision wound model [29], and also increase in rate of wound contraction in excision wound model. In our study, tensile strength and wound contraction, especially in treatment group with 6% TRO, was significantly increased compared to control group. Our results were consistent (wound size reduction), the findings Merrellia G, which was done on diabetic mice [26].

On the other hand, one of biochemical marker for tissue collagen is hydroxyproline [23, 18]. The amount of collagen synthesis onto wound, reflect to amount of hydroxyproline content in granulation tissues, which hydroxyproline is involved in the wound-healing. As shown in Table 4, in our study, topical application of
Turmeric hydroethanolic extract ointment leads to increase of hydroxyproline content of granulation tissues in all treatment groups, especially in treatment group with 6% TEO, compared to the control group.

In conclusion, according to our results, topical administration of differential doses of hydroethanolic extract of Turmeric rhizome, especially topical ointment 6% compared to other doses and two control groups, remarkable promote wound healing activity with increase in the rate of wound contraction and re-epithelialization, tensile strength value and collagen deposition in rat as an in vivo experimental wound models, and it may be suggested for treating various types of wounds in animal and human beings.

Also our result show that use of Turmeric rhizome hydroethanolic extracts in various doses had no adverse effect no mortality during the experiment, which imply that extract of this plant had no toxic activity and change on the skin such as type of irritation and even noticeable inflammation or swelling.

CONFLICT OF INTERESTS
Declared None

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REFERENCES