

Effect of *Verbascum Thapsus* L. on normal and dexamethasone suppressed wound healing

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Summary

Ethanolic extract of roots of *Verbascum Thapsus* were investigated in normal and dexamethasone depressed healing in incision, excision and dead space wound models in albino rats. The root extract of *Verbascum Thapsus* have shown the maximum breaking strength compared to control group. The rate of epithelialization and wound contraction in excision model was better than control groups. There was significant increase in granulation tissue weight and hydroxyproline content in dead space model compared with control group. The antihealing effect of dexamethasone was also reverted by the administration of ethanolic extract of *Verbascum Thapsus* in all the wound models .The result indicated that root extract of *Verbascum Thapsus* has a significant wound healing activity and also promotes healing in dexamethasone depressed healing conditions.

Key words: Wound healing Activity, *Verbascum Thapsus*, Ethanolic extract, Dexamethasone.

INTRODUCTION

A wound may be defined as a break in the epithelial integrity of the skin or may also be defined as a loss or breaking of cellular and anatomic or functional continuity of living tissue [1]. Wound healing studies are mainly aim to detect various means and factor influencing healing process, so they could be either used or avoid in clinical practice to favorably alter the healing process [2]. Although many indigenous tribes around the world have long suspected that many plants might have medicinal wound healing properties, and has not really got the attention of orthodox medical practitioners as a potential source of a healing agent which may prove to be useful in the treatment of wounds [3].

Some of plants possessing prohealing activity have been scientifically analyzed. The wound healing potential of *Tridax procumbens* [4], *Trigonella foenumgraecum* [5], *Leucas lavandulaefolia* [6] and *Aloe vera* [7] have shown promising healing activity.

Till today, there are no effective drugs are available which can successfully reverse the dexamethasone depressed healing. Dexamethasone is a potent anti-inflammatory glucocorticoids used in transplantation and allograft rejection [8]. Glucocorticoids are known to suppress wound healing [9].

Verbascum thapsus (Great or Common Mullein) is a species of mullein, native to Europe, northern Africa and Asia, and introduced in the Americas and Australia. It is a hairy biennial plant that can grow to 2 m or more tall. Its small yellow flowers are densely grouped on a tall stem, which bolts from a large rosette of leaves. Traditionally *V. thapsus* has been used to cure headaches, fevers, cramps, burns, and a host of other ailments (including cold feet). The plant does contain coumarins and other

toxins so it should be used wisely. [10] Great Mullein has been used as an alternative medicine for centuries and in many countries throughout the world. It has been employed for the treatment of asthma and other pulmonary complaints. The seeds are reported to be aphrodisiac and narcotic in nature³. The present study reported the isolation of iridoid glycosides, three iridoid [11] one phenylethyl glycoside [12,13,14], two sesquiterpenes [15,16], one diterpene [17], and one biflavonoid [18] from the whole plant of *V. thapsus*.

However, no simultaneous wound healing & reversal of Dexamethasone suppressed healing activity on the roots of *Verbascum thapsus* was scientifically available. Therefore, the present study has been carried out to explore the wound healing and reversal phenomena of Dexamethasone suppressant activity by *Verbascum thapsus*

MATERIALS AND METHODS

Materials

The root of *Verbascum thapsus* was collected from adjoining areas of Modasa (Sabarkantha), in July, 2010, and was authenticated by Associate Professor Dr. M. S. Jangid, Department of Botany, College campus, Modasa, Hemchandracharya North Gujarat University, Patan (Gujarat), India, by carrying out macroscopic and microscopic evaluation.

Preparation of the root extract

Roots of the plant were dried in shade. The dried root barks was powdered (3 kg), defatted with petroleum ether (60–80 °C) and soaked in ethanol (95%) and kept aside for 4 days. After 4 days, the ethanolic layer was decanted off. The process was repeated for four times. The solvent from the total extract was distilled off.

The preliminary phytochemical analysis

The preliminary phytochemical studies were performed for testing different chemical groups present in ethanolic extract [19] The freshly prepared root extract of *Verbascum thapsus* was qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extract was performed for alkaloids, flavonoids, phenolic compounds and tannins, carbohydrate, proteins and amino acids. Saponin was also tested. These were identified by characteristic color changes using standard procedures.

Animals

Wistar albino rats of either sex weighing between 180 and 200 g were obtained from VBTCP, Umrakh, India. The study was approved by the Institutional Ethics Committee for animal experimentation VBTCP, Umrakh, India, (VBTCP/ IEAC/CPCSEA/ 07/ 19/10) and all the procedures on animals were carried out as per CPCSEA guidelines, India. These animals were used for the wound healing activity studies. The animals were stabilized for 1 week. They were maintained in standard conditions at room temperature, $60\pm5\%$ relative humidity and 12 h light dark cycle. They had been given standard pellet diet and water *ad libitum* throughout the course of the study. The ethanolic extract of *Verbascum thapsus* was administered orally (p.o.); whereas dexamethasone (Dexona Vials) was administered intra peritoneal (i.p.).

Excision wound model:

Under light ether anaesthesia an impression of 500 sq mm was made on the shaved back of the rat as described [20]. The skin of the impressed area was excised carefully. Animals are kept in separate cages. The day on which wound was made

consider as day‘0’ (Zero) (**Table- 1**). Wound area was traced and measured planimetrically with the help of sq mm graph paper. Number of days required for falling of the eschar without any residual raw wound gave the period of epithelization. The parameters which was observed was period of epithelialization (Reepithelialization).

Incision wound model

The rats were anesthetized by administering ketamine (0.5 ml/kg b. w. i.p.). Incision wounds of about 6 cm in length and 2mm in depth were made with sterile scalpel on the shaved back of the rats 30 min later the administration of ketamine injection. The parted skin was kept together and stitched with black silk at 0.5cm intervals. Surgical thread (no. 000) and a curved needle (no. 9) were used for stitching. The continuous thread on both wound edges were tightened for good closure of the wounds. The wounds of animals in the different groups were treated with drug by oral administration as described above, for the period of 10 days. The wounding day was considered as day 0. When wounds were cured thoroughly, the sutures were removed on the 8th post-wounding day and the tensile strength of the skin that is the weight in grams required to break open the wound/skin was measured by tensiometer on the 10th day reported.

Tensile strength was calculated using the following formula :

$$\text{Tensile strength} = \frac{\text{Breaking strength (g)}}{\text{Cross-sectional area of skin (mm}^2\text{)}}$$

Dead space wound model

The dead space wounds were created by making a small transverse incision in the lumber region on either side of vertebral column in each animal. Two polypropylene tubes (2.5cm × 0.5 cm) were inserted subcutaneously one on either side of vertebral

column and pushed cephal head for 3-4 cm for the final implantation to harvest the granulation tissue. The animals were treated with the extracts from 0 day to 9th post-wounding day considering wounding day as zero. Granulation tissue formed on the implanted tubes was carefully dissected out on the 10th post-wounding day and the tensile strength was measured by continuous constant water flow technique [21] Mean value gives the breaking strength for a given group. The tissue was dried in oven at 60⁰C for 24 hours and the dry weight was noted. The acid hydrolysate of the dry tissue was used for the estimation of the hydroxyproline content in the tissue [22].

Statistical Analysis

The mean value \pm SEM was calculated for each parameter. Results were statistically analyzed by one-way-analysis of-variance (ANOVA) followed by posthoc scheffe's test. $P < 0.05$ was considered as significant.

RESULTS

Phytochemical screening

Phytochemical screening of the plant extract revealed the presence of saponins, flavonoids, carbohydrates and tannins.

Toxicity studies

In performing preliminary test for pharmacological activity in rats, ethanolic extract did not produce any significant changes in the behavioral or neurological responses upto 400 mg/kg body weight. Acute toxicity studies revealed the non-toxic nature of the ethanolic extracts of *Verbascum thapsus*. The result obtained from the LD50 study indicates that ethanolic extract of *Verbascum thapsus* is safer to use in animals even at a dose of 400 mg/kg p.o.

Incision Model

In incision wound model the drug treated group (Group 2) showed significant breaking strength compared to control group (**Table 1**). In dexamethasone treated (Group 3), showed significant decrease in breaking strength. But comparatively the ethanolic root extract showed a promising reversal of dexamethasone depressed healing in rats by increasing the breaking strength in incision wound model (Group 4).

Dead Space Model

In dead space wound model, the ethanolic extract significantly increased the hydroxyproline content which is a marker of collagen content and thus healing process (**Table 1**). In this model, the dry and wet granulation tissue weight and granulation tissue breaking strength was increased compared with control group and dexamethasone group.

Excision Model

In excision wound model, the ethanolic root extract of *Verbascum thapsus* showed a promising result by decreasing the period of epithelialization (**Table 1**) and increasing the percentage wound contraction (**Table 2**) compared to control group of animal. The dexamethasone treated group showed increase in epithelialization period and percentage wound contraction was decreased. But the ethanolic extract has significantly reverted the period of epithelialization by increasing it and percentage wound contraction was also increased (**Table 2**).

TABLE 1: The effect of Ethanolic extract of *Verbascum thapsus* in absence and in presence of Dexamethasone treated rats in incision, excision and Dead space wound models [Values are Mean \pm SD for 6 rats].

Groups	Incision wound breaking Strength	Excision wound period of epithelialization (days)	Wet Tissue Weight (mg/100g rat)	Dry Tissue Weight (mg/100g rat)	Granulation Tissue Breaking Strength (g)	Hydroxyproline Content (mg/g tissue)
Control	270.7 \pm 24.38	23 \pm 1.57	245 \pm 21.45	29 \pm 2.0	288 \pm 14.78	12.67 \pm 3.21
Verbascum thapsus Ethanolic Treated	442.2 \pm 33.66 ^a	16 \pm 1.21 ^a	345 \pm 15.50 ^a	47 \pm 1.5 ^a	399 \pm 17.8 ^a	49.34 \pm 7.12 ^a
Dexamethaone Treated	205 \pm 23.67 ^c	35 \pm 5.23 ^{ax}	180 \pm 11.25 ^{bx}	20 \pm 3.4 ^x	180 \pm 9.6 ^{ax}	9.67 \pm 2.67 ^x
Ethanolic Extract + Dexamethasone Treated	340 \pm 15.67 ^c	25 \pm 1.35 ^{xp}	260 \pm 17.62 ^{xp}	35 \pm 5.0 ^{yr}	268 \pm 11.3 ^{xp}	19.54 \pm 6.23 ^x

[P Values: ^a < 0.001, ^b < 0.01, ^c < 0.05 Vs Control, ^x < 0.001, ^y < 0.01, Vs *Verbascum thapsus*, ^p < 0.001, ^r < 0.05]

TABLE 2: The effect of Ethanolic extract of *Verbascum thapsus* in absence and in presence of Dexamethasone treated rats in excision wound models [Values are Mean \pm SD for 6 rats].

Groups	4 th Day	8 th Day	12 th Day	16 th Day	20 th Day	22 nd Day	24 th Day	28 th Day
Control	10 \pm 2.36	45 \pm 2.23	69 \pm 1.78	85 \pm 4.1	96 \pm 4.0	99 \pm 1.6	100 \pm 00	100 \pm 00
Verbascum thapsus Ethanolic Treated	30.45 \pm 4.8 ^a	65.67 \pm 4.8 ^{cx}	88.78 \pm 3.23	99.67 \pm 1.2 ^{bx}	100 \pm 00	100 \pm 00	100 \pm 00	100 \pm 00
Dexamethaone Treated	5.7 \pm 3.2 ^{ax}	15.43 \pm 5.8 ^{ax}	38.34 \pm 5.5 ^{ax}	59.67 \pm 5.67 ^{ax}	71.34 \pm 4.67 ^{bx}	87.12 \pm 3.2	93.32 \pm 3.4	98.9 \pm 1.56
Ethanolic Extract + Dexamethasone treated	8.89 \pm 2.1 ^{ax}	25.78 \pm 2.34 ^{ax}	49.78 \pm 3.46 ^{ax}	79.78 \pm 5.5 ^{cy}	88.45 \pm 3.43 ^r	98.65 \pm 3.33	100 \pm 00	100 \pm 00

[P Values: ^a < 0.001 , ^b < 0.01 , ^c < 0.05 Vs Control, ^x < 0.001 , ^y < 0.01 , Vs *Verbascum thapsus*, ^p < 0.001 , ^r < 0.05]

Conclusion:

Wound healing process consists of different phases such as granulation, collagenization, collagen maturation and scar maturation which are concurrent but independent to each other. Hence in this study three different models were used to assess the effect of ethanolic *Verbascum thapsus* root extracts on various phases. The result of the present study showed that *Verbascum thapsus* possesses a definite prohealing action. The breaking strength of the incision wounds was increased in ethanolic root extract of *Verbascum thapsus*. Deposition of newly synthesized collagens at the wound site increases the collagen concentration per unit area and'''''' hence the tissue tensile strength [4]. Similarly, in dead space model there was a significant increase in granuloma tissue breaking strength in ethanolic extract treated groups in dead space wound model. The higher breaking strength indicates better healing of wounds. Higher hydroxyproline content was seen with extract treatment. The increased amount of hydroxyproline in test groups shows increased collagen content, since hydroxyproline is the direct estimate of collagen synthesis. Therefore, it supports the wound healing activity of *Verbascum thapsus*. The preliminary phytochemical analysis of the *Verbascum thapsus* root extract showed the presence of tannins, alkaloids and saponins. Any one of the observed phytochemical constituents present in *Verbascum thapsus* may be responsible for the wound healing activity. In excision wound healing model the ethanolic extract of the root of the plant *Verbascum thapsus* showed significant increase in percentage closure of excision wounds by enhanced epithelization. This enhanced epithelization may be due to the effect of *Verbascum thapsus* extracts on enhanced collagen synthesis.

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