

## **Influence of Some Phytofractions of *Strychnos Nux Vomica* on Cutaneous Wound Healing in Albino Rats**

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### **ABSTRACT**

**AIM-** Influence of Some Phytofractions of *Strychnos Nux Vomica* on Cutaneous Wound Healing in Albino Rats.

**MATERIAL & METHODS-** As per facts, references received from literature survey, the *seeds of Strychnos nux-vomica* were selected as plant materials for investigational study. The dried *seeds of Strychnos nux-vomica* were extracted by successive solvent extraction method with the help of soxhlet apparatus. The treatment was done topically in all the groups.10 The extract of both plants was applied at a dose of 100 mg/kg/day (In the form of 10 % w/w extract ointment in steric acid) for 16 days. Wound areas were measured on days 1, 4, 8 and 16 for all groups, using a transparency sheet and a permanent marker. Recording of wound areas were measured on graph paper. The day of scar falling, after wounding without any residual raw wound was considered as the day of epithelialization.

**RESULTS-** The % yield of ethanolic, methanolic, petroleum ether, chloroform and acetone extracts of dried seeds of *Strychnos nux-vomica* was found 16.55, 4.25, 2.5, 8.50 and 7.90 % respectively. The ethanolic extract of *Strychnos nux-vomica* seeds showed presence of high amount of phenolics and tannin compounds. In Extract treated groups; ethanolic extract of *S. nux-vomica* seeds treated rats showed highest wound closure 96.44% after 11 days of continuous treatment with extract ointment, which was more than 79.86% of normal control group respectively. The chloroform extract and methanol extract treated groups also exhibited significant increase in wound closure more than both control groups. The wound area of ethanolic extract treated rats was measured about 10.33 mm<sup>2</sup> which was very much less than non treated

normal (50.33 mm<sup>2</sup>) rats. In other treatment groups the chloroform and methanol extract treated rats also showed significant decrease in wound area upto 21.67 and 27.67 mm<sup>2</sup> respectively.

**CONCLUSION-** The ethanolic extract and ethylacetate soluble fraction of ethnaolic extract of seeds of plant *Strychnos nux-vomica* were found to be effective in management of wound healing complications in normal albino rats. In excision wound model the extracts and fractions of *S. nux-vomica* showed decrease in wound area, day of scar falling and increase in % wound closure in treated normal rats as compared to non treated normal rats.

**KEYWORDS-**Phytofractions, *Strychnos Nux Vomica*, Cutaneous Wound Healing, Albino Rats, Ethylacetate soluble fraction, Ethnaolic extract

## INTRODUCTION

Human body has incredible systems with a set of mechanisms made to fight or kill pathogens. It is the internal defense system, which destroys the pathogens that have attacked the body. The external defense system prevents microorganisms from entering the body. 1 Any damage or break in the surface of the skin is wound. It may be defined as a loss or breaking of cellular, anatomical and functional continuity of living tissue.2 Amplitude of damage can be from a simple crack in the epithelial integrity of the skin or extending deeper into subcutaneous tissue with damage to other internal structures such as tendons, muscles, vessels, nerves and even bone.3 It may be caused by physical, chemical, thermal, microbial, or immunological assault to the tissue. When skin is torn, cut, or punctured, it is termed as an open wound and when blunt force trauma causes a contusion, it is called closed wound, whereas the burn wounds are produced by fire, heat, radiation, chemicals, electricity, or sunlight.17 A chronic wound is one in which the normal process of healing is disrupted at one or more points in the phases of haemostatic, inflammation, proliferation or remodelling, resulting in a delay in healing beyond the anticipated time. The time period of healing might be a couple of weeks or up to six weeks in some cases.4 The healing process of chronic wound is incomplete and interrupted by various physiological and mechanical factors, resulting in a wound that fails to proceed through the usual stepwise progression. These hindering factors include poor nutrition, insufficient oxygenation, infection, prolonged inflammation, age, diabetes and other diseases, drugs, smoking, alcoholism, and oxidative damage by excess free radicals.5 Extensive review of literature available indicated

that the plant *Strychnos nux vomica* having numerous pharmacological properties. Even though the plant is so widely used in the indigenous medicine system there are gaps in the literature from this part of the country with respect to its usage in the different alternative system of medicine including phytochemistry, antimicrobial, anticancer, microscopical and larvicidal activity of the plant. Hence efforts were taken to study the effect of phytochemicals of *Strychnos nux vomica* seeds in wound healing activity.

## **MATERIALS & METHODS**

### **Plant Materials**

As per facts, references received from literature survey, the seeds of *Strychnos nux-vomica* were selected as plant materials for investigational study. The seeds of *Strychnos nux-vomica* were purchased from authorized herbal supplier in local city market.

### **Extraction & Fractionization**

The dried seeds of *Strychnos nux-vomica* were extracted by successive solvent extraction method with the help of soxhlet apparatus. The plant material first extracted with petroleum ether then chloroform, acetone, methanol and ethanol.

### **Qualitative chemical examination**

#### **Alkaloids:**

*Dragendorff's test:* In 2 mg of the ethanolic extract 5 ml of distilled water was added, 2M HCl was added until an acid reaction occurs. To above solution 1 ml of Dragendorff's reagent was added. Formation of orange or orange red precipitate indicated the presence of alkaloids.

*Hager's test:* To 2 mg of the ethanolic extract taken in a test tube, a few drops of Hager's reagent were added. Formation of yellow precipitate confirms the presence of alkaloids.

#### **Carbohydrates:**

*Anthrone test:* To 2 ml of anthrone reagent solution, 0.5 ml of aqueous extract was added. Formation of green or blue colour indicated the presence of carbohydrates.

*Benedict's test:* To 0.5 ml of aqueous extract, 5 ml of Benedict's solution was added and boiled for 5 min. Formation of brick red coloured precipitate indicated the presence of carbohydrates.

#### **Flavonoids:**

*Shinoda's test:* In a test tube containing 0.5 ml of the ethanolic extract 10 drops of dilute hydrochloric acid followed by a small piece of magnesium was added. Formation of pink, reddish or brown colour indicated the presence of flavonoids.

### **Triterpenoids:**

*Liebermann Burchard's test:* 2 mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of a violet coloured ring indicated the presence of triterpenoids.

### **Saponins:**

In a test tube containing about 5 ml of an ethanolic extract, a drop of sodium bicarbonate solution was added. The test tube was shaken vigorously and left for 3 min. Formation of honeycomb like froth indicated the presence of saponins.

### **Steroids:**

*Liebermann-Burchard's test:* 2 mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of green colour indicated the presence of steroids.

### **Tannins:**

In 1-2 ml of the ethanolic extract, few drops of 5 per cent w/v FeCl<sub>3</sub> solution were added. A green colour indicated the presence of gallotannins, while brown colour indicated the presence of pseudotannins.

### **Procurement and selection of animals:**

Wistar albino rats of either sex weighing between 100–150 gm of either sex were obtained from central animal house of our Institution. The animals were stabilized for 1 week; they were maintained in standard condition at room temp; normal light dark cycle.

### **Acute oral toxicity Studies:**

The acute toxicity study was carried out in adult female albino rats by “fix dose” method of OECD (Organization for Economic Co-operation and Development) Guideline No.420. Fixed dose method as in Annex 2d: Test procedure with a starting dose of 2000 mg/Kg body weight was adopted. 7, 8

### **Acute Dermal Toxicity (Fixed Dose Procedure**

The acute dermal toxicity study was carried out in adult female albino rats by “fix dose” method of OECD (Organization for Economic Co-operation and Development) Guideline No.434. Extracts of the dried *seeds of Strychnos nux-vomica* was applied topically at dose level 2000 mg/kg. 9

### **Preparation of extract and active phytochemical ointment in steric acid:**

The dried extracts and fractions (10 % w/w) of the dried *seeds of Strychnos nux-vomica* was triturated in pestle mortar with steric acid ointment base. And used further in excision cutaneous wound healing model in animals.

### **Excision wound healing activity in albino rats:**

Excision wounds were used for the study of rate of contraction of wound and epithelization; all wounds were of full-thickness type extending up to the adipose tissue. Animals were anaesthetized with slight vapour inhalation of di-ethyl ether and the back side of each rat was shaved. Excision wounds sized 300 mm<sup>2</sup> and 2 mm depth were made by cutting out piece of skin from the shaven area. The entire wound was left open. Animals were closely observed for any infection and those which showed any sign of infection were separated, excluded from study and replaced. The treatment was done topically in all the groups.<sup>10</sup> The extract of both plants were applied at a dose of 100 mg/kg/day (In the form of 10 % w/w extract ointment in steric acid) for 16 days. Wound areas were measured on days 1, 4, 8 and 16 for all groups, using a transparency sheet and a permanent marker. Recording of wound areas were measured on graph paper. The day of scar falling, after wounding without any residual raw wound was considered as the day of epitheliazation.

### **Treatment Groups: For *Strychnos nux-vomica* seeds extract treated excision wound models:**

1. Group I (NC): Normal Wound Control; Normal rats topically treated with Plane steric acid ointment base.
2. Group III (NV-ETOH): Wounded rats topically treated with ointment of ethanolic extract of dried *seeds of Strychnos nux-vomica* in steric acid ointment base.
3. Group IV (NV-MEOH): Wounded rats topically treated with ointment of methanolic extract of dried *seeds of Strychnos nux-vomica* in steric acid ointment base.
4. Group V (NV-Pet): Wounded rats topically treated with ointment of petroleum ether extract of dried *seeds of Strychnos nux-vomica* in steric acid ointment base.
5. Group VI (NV-CHCl<sub>3</sub>): Wounded rats topically treated with ointment of chloroform extract of dried *seeds of Strychnos nux-vomica* in steric acid ointment base.
6. Group VII (NV-Acetone): Wounded rats topically treated with ointment of acetone extract of dried *seeds of Strychnos nux-vomica* in steric acid ointment base.

**Treatment Groups: For fractions of ethanolic extract of *Strychnos nux-vomica* seeds treated excision wound models:**

1. Group I (NC): Normal Wound Control; Normal rats topically treated with Plain steric acid ointment base.
2. Group II (NV-EAC): Wounded rats topically treated with ointment of ethyl acetate soluble fraction of ethanolic extract of dried seeds of *Strychnos nux-vomica* in steric acid ointment base.
3. Group III (NV-But): Wounded rats topically treated with ointment of butanol soluble fraction of ethanolic extract of dried seeds of *Strychnos nux-vomica* in steric acid ointment base.
4. Group IV (NV-Wat): Wounded rats topically treated with ointment of water soluble fraction of ethanolic extract of dried seeds of *Strychnos nux-vomica* in steric acid ointment base.

**RESULT AND DISCUSSION**

**% Yield of different extracts of *Strychnos nux-vomica* seeds**

The % yield of ethanolic, methanolic, petroleum ether, chloroform and acetone extracts of dried seeds of *Strychnos nux-vomica* was found 16.55, 4.25, 2.5, 8.50 and 7.90 % respectively (Table No. 1).

**Table No. 1:** % Yield of different extracts of *S. nux-vomica* seeds

S. No	Treatment	% Yield	Characterstics
1	<i>S. nux-vomica</i> Ethanol extract	16.55 %	Sticky & dark brown in color
2	<i>S. nux-vomica</i> Methanol Extract	4.25 %	Sticky & dark brown in color
3	<i>S. nux-vomica</i> Pet. Ether Extract	2.5%	White in color
4	<i>S. nux-vomica</i> Chloroform Extract	8.50%	Sticky & dark green color
5	<i>S. nux-vomica</i> Acetone Extract	7.90 %	Dark green color

### Qualitative chemical examination of different extracts of *S. nux-vomica* seeds

On qualitative phytochemical examination of different extracts of *Strychnos nux-vomica* seeds the test for carbohydrate was found positive. The ethanolic extract of *Strychnos nux-vomica* seeds showed presence of high amount of phenolics and tannin compounds (Table No. 2).

**Table No. 2:** Presence of phytochemicals in different extracts of *S. nux-vomica* seeds

S. No.	Name of Phytochemicals	<i>S. nux-vomica</i> Ethanol extract	<i>S. nux-vomica</i> Methanol Extract	<i>S. nux-vomica</i> Pet. Ether Extract	<i>S. nux-vomica</i> Chloroform Extract	<i>S. nux-vomica</i> Acetone Extract
1	Test for carbohydrates	+	+	+	+	+
2	Test for Proteins	-	-	+	-	-
3	Test for steroids	-	+	+	++	+
4	Test for Glycosides	-	++	-	++	+
5	Test for Alkaloids	+	+	+	+	-
6	Test for flavonoids	++	++	-	+	-
7	Test for phenolic & tannin compounds	+++	++	-	++	++

### Acute toxicity studies of different extracts of *Strychnos nux-vomica* seeds

The acute toxicity test was performed for both topical (Dermal) and oral administration of extracts of seeds of *Strychnos nux-vomica*. All extracts were found safe for administration of extract by oral as well as dermal (Topical) route (Table No. 3).

**Table No. 3:** Acute toxicity studies of different extracts of *S. nux-vomica* seeds

S. No.	Treatment	Acute Oral Toxicity	Acute Dermal Toxicity
1	<i>S. nux-vomica</i> Ethanol Extract	Safe	Safe

2	<i>S. nux-vomica</i> Methanol Extract	Safe	Safe
3	<i>S. nux-vomica</i> Pet. Ether Extract	Safe	Safe
4	<i>S. nux-vomica</i> Chloroform Extract	Safe	Safe
5	<i>S. nux-vomica</i> Acetone Extract	Safe	Safe

### Effect of *S. nux-vomica* seeds extracts on Excision wound parameters

As per Table No. 4, In Excision wound model the seeds extract treated rats showed significant healing of wounds as compared to non treated rats. In Extract treated groups; ethanolic extract of *S. nux-vomica* seeds treated rats showed highest wound closure 96.44% after 11 days of continuous treatment with extract ointment, which was more than 79.86% of normal control group respectively. The chloroform extract and methanol extract treated groups also exhibited significant increase in wound closure more than both control groups.

The Table No. 4 demonstrates that the wound area of ethanolic extract treated rats was measured about 10.33 mm<sup>2</sup> which was very much less than non treated normal (50.33 mm<sup>2</sup>) rats. In other treatment groups the chloroform and methanol extract treated rats also showed significant decrease in wound area upto 21.67 and 27.67 mm<sup>2</sup> respectively.

According to Table No. 4 in ethanolic extract treated group the day of scar falling i.e. final epithelization was observed on 17th day of wounding which was more than the normal control and other extract treated groups. The chloroform and methanol extract treated groups also showed faster scar falling as compared to non treated rats.

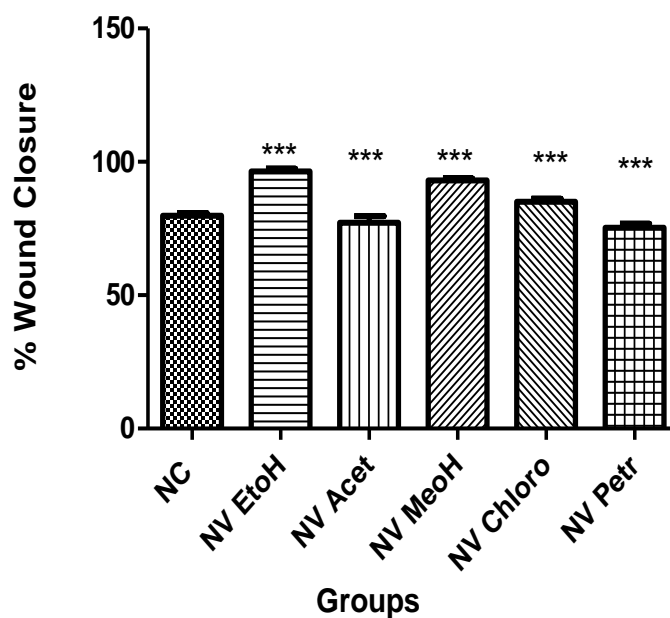
**Table No. 4:** Effect of *S. nux-vomica* seeds extracts on Excision wound parameters

Groups Parameters	Normal Control	<i>S. nux-vomica</i> Ethanol extract	<i>S. nux-vomica</i> Methanol Extract	<i>S. nux-vomica</i> Pet. Ether Extract	<i>S. nux-vomica</i> Chloroform Extract	<i>S. nux-vomica</i> Acetone Extract
% Wound Closure	79.86± 0.841	96.44± 0.919 ***	93.50± 0.522 ***	75.31± 0.954 ***	85.05± 0.321 ***	77.18± 0321 ***



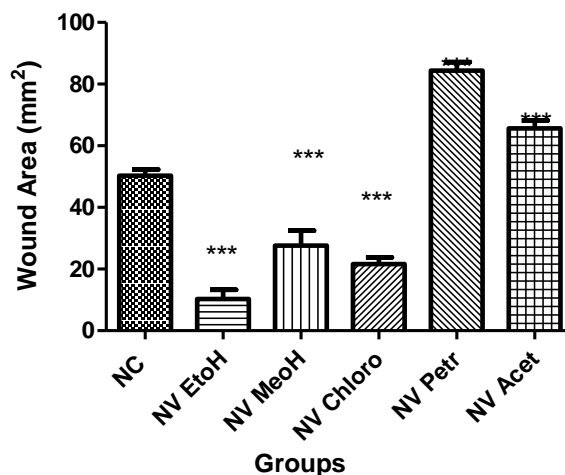
Wound Area Final (mm <sup>2</sup> )	50.33±0.8028	10.33±1.568***	27.67±1.944***	84.33±1.145***	21.67±0.8819***	65.67±1.054***
Epithelization (Days)	26.83±0.3073	17.83±0.323***	20.33±0.4216***	37.34±0.739***	24.83±0.428***	25.83±0.856***

Data are expressed as Mean ± SEM and analyzed statistically by One way ANOVA followed by Dunnett's Multiple Comparison Test, using Graph Pad Prism Software trial version. IN Dunnett's Multiple Comparison Test, Group NC was compared with treated groups. P value considered as P<0.05 Significant (\*), P<0.01 Very Significant (\*\*), P<0.001 Highly Significant (\*\*\*).



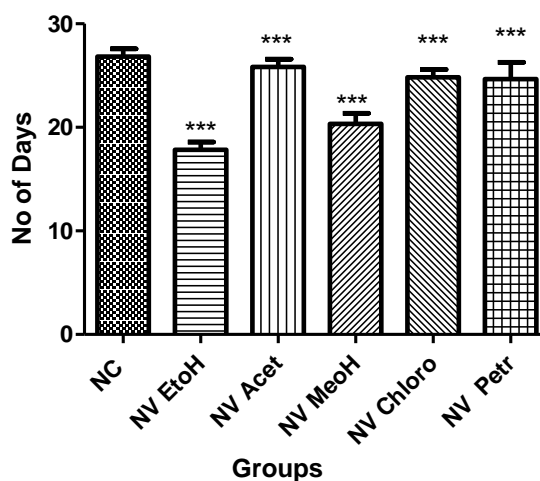
**Figure No. 1:** % Wound Closure of *S. nux-vomica* extracts

Data are expressed as Mean ± SEM and analyzed statistically by One way ANOVA followed by Dunnett's Multiple Comparison Test, using Graph Pad Prism Software trial version. IN Dunnett's Multiple Comparison Test, Group NC was compared with treated groups. P value considered as P<0.05 Significant (\*), P<0.01 Very Significant (\*\*), P<0.001 Highly Significant (\*\*\*).



**Figure No. 2:** Wound Area of *S. nux-vomica* extracts on 11th day

Data are expressed as Mean  $\pm$  SEM and analyzed statistically by One way ANOVA followed by Dunnett's Multiple Comparison Test, using Graph Pad Prism Software trial version. IN Dunnett's Multiple Comparison Test, Group NC was compared with treated groups. P value considered as P<0.05 Significant (\*), P<0.01 Very Significant (\*\*), P<0.001 Highly Significant (\*\*\*).



**Figure No. 3:** Day of Epithelialization of *S. nux-vomica* extracts

Data are expressed as Mean  $\pm$  SEM and analyzed statistically by One way ANOVA followed by Dunnett's Multiple Comparison Test, using Graph Pad Prism Software trial version. IN Dunnett's

Multiple Comparison Test, Group NC was compared with treated groups. P value considered as  $P < 0.05$  Significant (\*),  $P < 0.01$  Very Significant (\*\*),  $P < 0.001$  Highly Significant (\*\*\*).

## DISCUSSION

The wound healing is a complex process in normal individuals; it becomes more complicated when associated with neuropathic, myopathic or occurred due to chronic protein metabolic deficiency disorders. The hypoxia is a complex disorder which produces many diseases and complications by generation of free radicals or reactive oxygen species. The metabolic byproducts of glucose and lipoprotein altered the stereochemistry and functioning of all proteins in body and disturbed the normal physiological functions of proteins like growth, repair and regeneration of cells and tissue. Free radicals and reactive oxygen species play an important role in tissue and cellular damage. The use of antioxidants has been reported to promote wound healing.<sup>11</sup> The controlling of blood protein and growth factors level is a important factor to promote wound healing.<sup>12</sup> Repairing of damaged tissue occurs in a series of steps or phases including hemostasis, inflammation, granulation and maturation with remodeling.<sup>13</sup> Collagen synthesis is increased during wound healing after tissue injury. Collagen provides strength to tissue and plays a major role in homeostasis as well as in epithelialization.<sup>14,15</sup> Collagen is responsible for production of extra cellular matrix. The main contributor of extra cellular matrix is collagen and it is also responsible for increase in wound tensile strength for perfect healing.<sup>16</sup> The ethanolic extract was further fractionized in ethyl acetate, butanol and water. The ethyl acetate soluble fraction was identified as most active fraction amongst all the three fractions i.e. ethyl acetate, butanol and water soluble fractions of ethanolic extract of dried seeds of *Strychnos nux-vomica*.

In wound healing study of fractions isolated from ethanolic extract of *S. nux-vomica* seeds; the excision wound model was created to study the healing of wounds in terms of epithelialization, wound area and wound closure measurement. The ethylacetate soluble fraction of ethanolic extract of *S. nux-vomica* seeds showed faster scar falling and epithelialization as compare to control non treated normal rats. The epithelialization represents the completion of remodeling and maturation phase and is the sign of complete healing of wound. The decrease in wound area resembles increased % of wound closure in ethylacetate soluble fraction of ethanolic extract of *S.*

*nux-vomica* seeds treated rats. The faster wound closure is occurred only in condition of rapid proliferation of granuloma tissues.

## CONCLUSION

The ethanolic extract and ethylacetate soluble fraction of ethnaolic extract of seeds of plant *Strychnos nux-vomica* were found to be effective in management of wound healing complications in normal albino rats. In excision wound model the extracts and fractions of *S. nux-vomica* showed decrease in wound area, day of scar falling and increase in % wound closure in treated normal rats as compared to non treated normal rats. The herbal formulations made by above said phytofractions could be more beneficial for society, and further study related with isolation of potent chemical compounds and responsible biological quantitative confirmation from seeds of *Strychnos nux-vomica* could meet in the form of treatment for physical or chemical injury complications especially for wound healing complications in patients.

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