Research Article

Swertiamarin ointment: A traditional approach in cutaneous wound healing

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Abstract

Objective: To explore the wound healing activity of *Enicostema littorale* leaf extract (Gentianaceae). **Materials and Methods:** Ethanolic leaf extract and its active secoiridoid, Swertiamarin are used for this study. There is a significant wound healing activity observed for the group of Wistar rats treated with Swertiamarin. Scientific cure is proven by measuring the wound area in each test group animals from 0 to 15 days. **Results:** The topical application of ethanolic extract and Swertiamarin ointment results in decrease of the wound area in 15 days. Swertiamarin is effective in comparison with crude ethanolic extract. Biological estimation also proved that the Swertiamarin treated group had a high percentage of wound contraction score than the control group. **Conclusion:** Swertiamarin formulated herbal ointment is an efficient cutaneous ointment for wound healing activity in excision animal models.

Keywords: Swertiamarin, Secoiridoid, *Enicostema littorale*, wound healing

Introduction

Therapeutic plants are assuming a huge role in relieving different human ailments from olden days onwards among them, *Enicostema littorale* Blume is one of the woody annual herbs of the family Gentianaceae. It is a cosmopolitan species and is available in India (Figure 1). The bitter plant is a vital indigenous drug for different ailments like wounds, diuretic condition, tooth decay, severe fever, skin problems, stomach disorder and higher blood glucose levels. Earlier workers have reported in *E. littorale*, active principles like iridoids, alkaloids, saponins, catechins, sterols, phenolic acids, triterpenoids, flavonoids and xanthenes (Ghosal et al., 1974). *In vitro* examinations and *in vivo* models have given a basic biological evolution for its different ethanopharmacological applications (Vigneswaran et al., 2017). Wound healing is a complex process initiated for reclamation of the function and integrity of the tissue damaged by an injury (Kumar et al., 2007). Selection of potential wound healing phytochemicals from selected plants is one of the promising areas in ethanopharmacological sciences. Due to this important medicinal application, this plant was selected for the present pharmacological investigations

especially the isolation and structure determination and activity of a pure amorphous Swertiamarin is found in this plant. Swertiamarin is a secoiridoid that is said to be involved in wound healing activity. No direct work has been done so far on Wound healing potential of Swertiamarin, an active constituent of *Enicostema littorale* leaves. The study also aims to investigate and isolate the compound Swertiamarin, a secoiridoid and to study the wound healing properties of the plant for healthcare and potential commercial applications.

This species is globally distributed in West Indies, tropical Africa, India and Sri Lanka. It is found all over India upto an altitude of about 450 m. from Punjab and the Gangetic plains southwards, mostly available in coastal regions (Ved et al., 2016)

Materials and methods

Chemicals and reagents

The solvents/chemicals utilized in the extraction and analysis was analytical grade, n-hexane, methanol, chloroform and n-butanol obtained from SD Fine Chemicals Private Limited, Mumbai, India. HPLC grade solvents were obtained from Merck Germany. A standard of Swertiamarin was procured from Sigma-Aldrich India.

Plant material

Healthy leaves of the plant were collected from Virudhu nagar, Tamil Nadu, India during April 2013. The plant was authenticated by comparing with the Herbarium, The

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Figure 1. *Enicostema littorale* Inner box represents the flowers

voucher specimen (Ref.RRCBI-AP4667) was deposited at National Ayurveda Dietetics Research Institute (NADRI), Bangalore -560 011, India. The material was shade dried, powdered by pulveriser and stored in airtight containers.

Extraction and isolation

One kilogram of the powdered material is refluxed with ethanol at soxhlet apparatus for 24h in batches of 300g each. The extract was filtered, pooled and the solvent was removed under reduced pressure at $45 \pm 5^\circ\text{C}$ using rotary flash evaporator (Buchi, Flawil, Switzerland) and the yield was 20%.

The constituent Swertiamarin was isolated from the ethanol extract of the leaves. The ethanolic extract was reconstituted with 20% methanol and fractionated successively with non-polar to polar solvents, viz., hexane, chloroform, ethyl acetate and n-butanol. Each fraction was collected and checked by thin layer chromatography. The last n-butanol fractions were collected and concentrated under reduced pressure. The fractions were recovered in higher concentration and excess of ethyl acetate was added to it. The white precipitate developed was recrystallised from methanol to get an off white solid. The amorphous compound was tested and the structure was confirmed by Mass, ^1H NMR, ^{13}C NMR spectroscopy and the interpretation is discussed under results.

HPLC Analysis

Accurately 100mg of the ethanol extract was weighed and completely dissolved in HPLC methanol diluted to 100mL to obtain a concentration of 1.0 mg/mL. The prepared ethanol extract solution was passed through a $0.45\mu\text{m}$ filter to acquire a clear solution. The 25 mg of analytical standard Swertiamarin was prepared in the same manner as like sample solution to obtain the concentrations $250\mu\text{g/mL}$ of Swertiamarin. A gradient HPLC (Shimadzu HPLC Class VP series) with two LC-10 AT VP pumps (Shimadzu), UV visible detector SPD-10A, an

SCL-10A VP system controller (Shimadzu) connected with a reverse phase Luna® $5\mu\text{m}$ C18 ODS column (250 mm X 4.6 mm) was used for this study. The mobile phase constitutes mixture of 0.5% acetic acid in water (Mobile Phase A) and 100% acetonitrile (Mobile Phase B). Both the mobile phases were filtered through a $0.2\mu\text{m}$ thickness membrane filters and pumped from the solvent reservoir, flow rate is maintained at the rate of 1mL/min. About $20\mu\text{L}$ of the sample was injected by using a Rheodyne syringe (Model 7202, Hamilton) and the HPLC peaks were observed at UV/PDA detector at 236 nm. Further analysis was carried out to compare the HPLC chromatogram of *Enicostema littorale* ethanol extract against analytical standard Swertiamarin.

Procedure: As per the chromatographic conditions column was equilibrated with mobile phase. $20\mu\text{L}$ of methanol was injected as blank run. $20\mu\text{L}$ standard preparations in five replicates were injected separately. $20\mu\text{L}$ of sample was injected in duplicates. Again $20\mu\text{L}$ of the standard was injected as a bracketing standard. The HPLC was run for 35 minutes and the responses of major peaks were recorded.

The relative standard deviation (%RSD) for peak and peak retention time for five injections of standard was maintained less than 2% & 1% respectively.

HPTLC finger print analysis

HPTLC chromatography is an advanced technique which is used to detect the phytochemicals present in two wavelengths (254 nm and 366 nm). CAMAG HPTLC System, equipped with a Linomat V with CAMAG $100\mu\text{L}$ syringe sample applicator, a model Camag twin trough glass chamber (20cm × 10cm) and a thin layer chromatography (TLC) scanner and Camag TLC scanner 3 software Win Cats 4.3.1 version were used in the study. 15 μL of ethanolic extract and Swertiamarin were applied to tracks in TLC aluminium sheets (10 cm × 10 cm) of silica gel $\text{G}_{60}\text{F}_{254}$. HPTLC fingerprinting profile is attempted using different mobile phases for the separation of ethanolic extracts along with the isolated compound, Swertiamarin. The suitable mobile phase is ethyl acetate: methanol: water (7.7:1.5: 0.5). The plates were developed up to 90 mm after chamber saturation conditions. After air drying the solvent, a photograph of the plates were taken at two different wavelengths and derivatised with p-anisaldehyde sulphuric acid reagent.

Preparation of p-anisaldehyde sulphuric acid reagent

Methanol (85 mL) was taken in 500 mL measuring cylinder and 10 mL acetic acid (glacial) was added and thoroughly mixed with a stirring glass rod. Conc. Sulphuric acid

(H₂SO₄) about 5 mL was added into the mixture followed by adding 0.5 mL p-Anisaldehyde to the mixture. It was ready for derivatisation of HPTLC plate.

In-vivo studies on wound healing

Animals

Healthy Wistar albino rats of either sex weighing 250-280g were procured from Central Animal house, Srimad Andavan Arts and Science College, Trichy, Tamil Nadu. They were maintained at standard house conditions. The animals were fed with diet pellets (HLL, Bangalore) and watered *adlibitum*. The animals were transferred to the lab at least 2h before start of the experiments. The protocols for conducting the wound healing activities in rats were approved by Institutional Animal Ethics Committee. (IAEC) (Reg. No.: SAC/IAEC/BC/2015/Ph.D-002 dated 14.07.2015) as per the guidelines of Committee for the purpose of control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Preparations of ointments

For topical administration, the test samples were prepared in an ointment base (vehicle) which consists of petroleum jelly B.P. It was weighed in a beaker and then melted using thermostatic water bath. *Enicostema* ethanolic extract 15% (w/w) and isolated pure Swertiamarin 3% (w/w) are weighed and added respectively to the molten ointment base and then homogenized well. The drug formulations were prepared every 5th day and the drugs were topically applied on the surface of the wounds of the experimental animals.

Acute dermal irritation study

The study was conducted as per Organisation for Economic Co-operation and Development (OECD; French) OECD guidelines 402: acute dermal irritation and corrosion (OECD 2017). The skins were observed for inflammation, swelling, redness and other related signs as per the method described earlier after 4 hours of the treatment (Sanwal and Chaudhary, 2011).

Experimental design in excision wound model

Four groups of animals having six rats each were used for each of the excision wound model. The animals of group 1 were considered as the control, animals of group 2 and group 3 were treated with an ethanol extract and Swertiamarin ointment respectively. The animals of group 4 were treated with the reference standard, soframycin ointment (Sanofi India Limited, Mumbai, India).

The experimental rats were inflicted with excision wound model and were anaesthetized with slight vapour breathing of under diethyl ether in anaesthesia chamber. An excision wound was created and the dorsal side of rats was shaved with an extremely sharp edge razor blade. Excision wound of size 2.5cm² areas of

skin in long, 0.2cm² in deep inside was created by utilising surgical scissors. Haemostasis was achieved by smearing the wound with cotton swab absorbed in ordinary saline. The wound was left undressed to the open condition. (After creating the wounds, all the animals in the groups were kept independently for 0-3 days). The drugs were topically applied daily till the wound closure starting from the date of operation (Rao et al., 2000). The parameters studied were percentage of wound contraction and its biochemical parameters. The wound closure was measured periodically to observe the percentage of wound contraction. This result reveals that, epithelialisation time that the developing a new epithelial tissue to cover the wound. The study has been conducted for the period of 0-15 days.

Measurement of wound contraction

The level of wound size was dictated by the decrease in wound size. The injury measurement was estimated routinely by following injury site with follow paper and estimated graphically (Saha et al., 1997). After excision (0 to 15 days), the staying wound was estimated by utilizing vernier calliper. The surface region of the injury was estimated while leading the analysis. The decrease of wound size was checked from the underlying and last zone of the injury (Shenoy et al., 2011). The standard deviations and mean deviations were given in cm.

wound reduction % =

$$\frac{\text{Area of initial wound} - \text{Area of final wound}}{\text{Area of initial wound}} \times 100$$

Biochemical estimations

The following biochemical estimations were made on the experimental and control rats: (i) Tensile strength of skin (Saha et al., 1997) (ii) Analysis of Granuloma weight (Lee, 1968) (iii) Analysis of Platelet Derived Growth Factor, (iv) Analysis of Hydroxyproline (Woessner, 1961) (v) Analysis of Hexosamine in granulated tissue (Wagner, 1979), (vi) Analysis of enzymes glutathione peroxidase (GPx) (Rotruck et al., 1973) (vii) Assay of Catalase (Maehly and Chance, 1972) (viii) Analysis of the serum Ascorbic acid (Omayi et al., 1979) (ix) Analysis of nucleic acid content (Giles and Myers, 1965), (ix) Analysis of RNA content (Yoichi Endo, 1970). The rats were sacrificed after 15 days of study, after taking the blood samples.

Statistical analysis

The results are mentioned as the mean $\pm$ SD of six animals in each group. The data were evaluated by the null hypothesis in one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls t-test employing SPSS for windows. $P < 0.05$ was considered as statistically significant.

Results and discussion

Characterization of the compound

Earlier workers reported that aerial parts of *Enicostema axillare* were the richest source of Swertiamarin (Rana, 2014). Column chromatography was used mainly for the isolation and separation of Swertiamarin and it gave a poor yield (Vaidya et al., 2009; Rai and Thakur 1966; Anwar et al., 1996; Vishwakarma et al., 2004). Also, it is hygroscopic in nature and it is difficult to store the pure molecule.

The structure of the isolated compound (Figure 2) obtained by fractionation was a off white amorphous powder, weight is 2.5 gm, with melting point 110°C; The characterization of this compound was carried out using spectroscopic techniques. The molecular mass of the compound was obtained by using Mass Spectrometry. The mass spectrum showed parent molecular ion m/z at 375(M+H) APCI, Positive ion mode which confirms the assignment of molecular formula of $C_{16}H_{22}O_{10}$.

Figure 2. Structure of Swertiamarin

HPLC Studies

Swertiamarin from ethanolic extracts of leaves of *Enicostema littorale* was determined by using HPLC. HPLC chromatogram of ethanolic extract gives main peak due to the presence of Swertiamarin along with other minor peaks (Table 1). The Swertiamarin content was varying in ethanol extract and n-butanol fraction with 20%w/w and 50% w/w on dry basis respectively. The isolated compound Swertiamarin was eluted at RT 15.488 min, (Figure 3). The Swertiamarin content in dried *Enicostema littorale* extract is 2.2% (w/w) on raw material basis.

Content of Swertiamarin =

HPTLC Fingerprinting Profile

HPTLC fingerprinting at two different wavelengths and after derivatisation with p-Anisaldehyde sulphuric acid reagent as

Table 1. HPLC chromatogram and its peak purity %

Peak No	Ret. Time	Name	Peak Area	Peak purity%
1	11.975	RT 11.975	9026	0.139
2	14.224	RT 14.224	4598	0.071
3	14.725	RT 14.725	1114	0.017
4	15.154	RT 15.154	1388	0.021
5	15.488	RT 15.488	6240566	96.352
6	15.956	RT 15.956	50534	0.780
7	16.233	RT 16.233	105071	1.622
8	17.509	RT 17.509	64531	0.996
TOTAL				100

Figure 3.(A) & (B) HPLC chromatogram of the ethanolic extract and isolated compound Swertiamarin

shown in the representation figure 4, R_f 0.20 confirmed the presence of swertiamarin.

Structure elucidation of Swertiamarin

Mass fragmentation pattern

Atmospheric-pressure chemical ionization- Mass

$$\frac{A1 \times \text{Concentration of standard Swertiamarin (mg/ml)} \times \text{Potency of Standard Swertiamarin}}{A2 \times \text{Concentration of sample (mg/ml)}}$$

A1=Area of sample peak corresponding to Swertiamarin; A2=Area of standard peak corresponding to Swertiamarin

Figure 4. HPTLC photograph representation of *E.littorole* extracts, different wavelength at UV254nm, UV366nm & After derivatisation with Anisaldehyde H_2SO_4 reagent. **Track Legends:** T1-*E.littorole* ethanol extract; T2-*E.littorole* hexane fraction; T3-*E.littorole* chloroform fraction; T4-*E.littorole* n-butanol fraction; T5-Swertiamarin before crystallisation; T6-Isolated compound Swertiamarin

spectrometry (APCI-MS) analysis of swertiamarin confirmed that, pseudo molecular ion peaks at m/z shows 375 $[M+H]^+$. It confirms the molecular mass of the compound is 374 (Figure 5) and also shows the loss of glucose $[M^+ - \text{glucose}, m/z 179]$ showed 195 mass. Also supported that the mass fragmentation pattern at m/z 177 showed loss of one molecule of water. This is represented as in figure 5 & figure 5a.

¹H NMR Spectrum

The structure of Swertiamarin was characterised by NMR. ¹H NMR spectrum data recorded at 300 MHz (in CD_3OD) and shown in Figure 6. The spectral values are compared with the literature values (Rana, 2014; Anwar et al., 1996; El-Nagar and Beal, 1980). The expansion of proton NMR recorded spectrum also depicted in figure 7.

¹³C NMR (CD_3OD) data of Swertiamarin

The spectrum displayed 16 signals. Out of 16 signals six

Figure 5. APCI-MS Spectrum of swertiamarin

Our studies showed an improved rate of wound contraction and

reduction in healing time in animals treated with ointment containing *Encicostema littorale* leaf extracts in excision wounds group 3 was found to be the most effective and quickest when compared to the ethanolic extract group 2 when tried in wounds. The wound healing rate was significantly greater than that of the control and almost

Table 2. ¹H-NMR and ¹³C-NMR spectral data of [δ, (ppm) in CD₃OD] of pure Swertiamarin from *Enicostema littorale* Blume

C/H No.	¹³ C-NMR (Anwar, 1996)	¹³ C-NMR (Nagar and Beal 1980)	¹³ C-NMR (Rana, 2014)	¹³ C-NMR (δ, 75 MHz)	¹ H-NMR (δ, 400MHz) (Rana, 2014)	¹ H-NMR (δ, 300MHz)
1	98.29	97.7	99.1	99.237	5.59 (1H,d,J=1.2Hz,H-1)	5.728 (1H,d,J=1.5Hz, H-1)
2	-	-	-	-	-	-
3	-	150.7	154.7	154.962	7.50 (1H,s,H-3)	7.635 (1H,s,H-3)
4	109.13	109.9	108.9	108.038	-	-
5	32.01	63.1	64.9	62.726	-	-
6	62.69	32.6	33.7	33.901	1.73 (2H,m)	1.726 (2H,m)
7	62.99	64.6	65.9	66.122	4.61(1H,m),4.28(1H,m)	4.629 (1H,m), 4.337(1H, m)
8	131.49	131.5	133.8	133.006	5.35 (1H,d,J=8.0Hz)	5.336 (1H,d,J=7.8Hz)
9	50.21	50.8	51.9	52.142	2.83(1H,m)	2.897 (1H,m)
10	121.26	121.3	121.2	121.343	5.37 (1H _b ,m,-10), 5.25 (1H _a ,m-10)	5.382 (1H _b ,m,-10), 5.27 (1H _a ,m-10)
11	169.32	164.8	168	168.189	-	-
1'	98.92	97.0	100.2	100.379	4.45(1H,d,J=7.6,H-1')	4.649(1H,d, J=7.8 H-1')
2'	75.14	70.7	74.5	74.606	2.82(1H,t)	2.897(1H,dd)
3'	77.14	72.4	78.5	78.712	3.15(1H,m)	3.241(1H,m)
4'	70.06	68.2	71.4	71.561	3.03(1H,m)	2.931(1H,m)
5'	77.86	71.9	77.8	77.958	3.15(1H,m)	3.241(1H,m)
6'	60.66	61.6	62.6	62.726	3.43(1H _b ,m) 3.67(1H _a ,m)	3.402(1H _b ,m) 3.691(1H _a ,m)

Figure 9. Tensile Strength of skin and Granuloma weight in experimental animals on excision wounds. Values are Mean±SE, n = 6; * p < 0.05 statistically significant when compared to Group I.

graph is depicted in Figure 9. This implies that, enhanced collagen maturation increased cross-linking of collagen fibers. The increased weight of the granulation tissue also gave an indication of the presence of higher protein content (Swamy et al., 2007).

The time required to finish epithelialisation of the excision wound is a critical factor to survey the wound healing process. It was observed that the interim time taken to finish epithelialisation of the excision wound in Swertiamarin treated group was not as much as the animals treated with ethanol extract and the Swertiamarin treated group are represented in Figure 10. Significant decrease in the period of epithelialisation and

Figure 10. Effect of epithelialization in experimental animals on excision wounds. Values are Mean±SE, n = 6; * p < 0.05 Statistically significant when compared to Group I

increase in excision wound contraction rates were observed in control group of animals. This is also proved by Swamy et al. (2007). The hydroxyproline content was also significantly elevated compared with that of the control (Figure 11). This is also proved by Li et al. (2016) Treatment with Swertiamarin or losartan improved the Hydroxyproline content significantly.

In the present study the hexosamine level was found to increase in *E. littorale* ethanol extract and Swertiamarin ointment treated groups for 15 days study of wound healing performance (Figure 12).

The PDGFs have crucial roles during development, but

Figure 11. Effect of Hydroxyproline content in granulated tissue of experimental animals on excision wounds (Values are Mean±SE, n = 6;* p< 0.05 Statistically significant when compared to Group I.)

Figure 13. Platelet derived growth factor in experimental animals on excision wounds. Values are Mean±SE, n = 6;* p< 0.05 - Statistically significant when compared to Group I.

Figure 12. Levels of Hexosamine in granulated tissue of experimental animals on excision wounds (Values are Mean±SE, n = 6;* p< 0.05 statistically significant when compared to Group I.)

Figure 14. Excision wound expressed as percentage of wound contraction in control and treated groups

there is limited evidence for normal physiological functions in the adult. Increased PDGF activity has been linked with several diseases and pathological conditions, however causal pathogenic roles of the PDGFs have been established for some diseases, providing prospects for therapy using PDGF antagonists. PDGF receptor-inhibiting substances are now extensively tested in preclinical models as well as in human clinical trials. In addition, recombinant human PDGF-BB has been introduced in the clinic as a wound healing therapy. The PDGFs have pivotal roles during advancement, yet there is restricted proof for typical physiological capacities in the adult. Increased PDGF activity has been connected with many diseases. Causal pathogenic role of the PDGFs have been built up for a few ailment, giving prospects to treatment utilizing PDGF. PDGF receptor-inhibiting substances are present widely analysed in preclinical models and in addition in human clinical models (Andrae et al., 2008). It indicates that, PGDF have shown wound healing therapy is represented as figure 13.

Haematological profiles in experimental animals also analysed and the RBC and Haemoglobin content significantly increased when compared to control group. This also clear indication that, animals treated with *E. littarole* ethanolic extract and Swertiamarin treated group was showing improvement in all respects and the results presented in table 3.

Wound healing is a complex process and involves quite a lot of physiological and structural

changes beginning with wound closure, repair and replacing of injured tissues with new tissue formation (Ibrahim et al., 2017). A few biochemical parameters were contemplated in the animals subjected to wound healing in order to understand the biochemical changes connected with wounding and wound healing potential. Swertiamarin and soframycin show a significantly higher DNA Content compared to the control and hence it can be assumed that there is a greater amount of DNA synthesis needed for new cells and for repairing DNA of existing cells (Table 3). Lipid peroxidation (LPO) is the method in which free radicals

Table 3. Levels of Haematological profiles in granulated tissue of experimental animals on excision wounds

Parameters		Control group (1)	<i>E. littorale</i> ethanol extract treated group (2)	Swertiamarin treated group (3)	Soframycin treated group (4)
Haematological profiles	RBC (10^6 Cells/mm ³)	2.85±0.033	3.27±0.038 *	4.65±0.044 *	5.54±0.067 *
	WBC (10^3 Cells/mm ³)	9.92±0.0822	6.40±0.0530*	5.7±0.1123*	3.59±0.065*
	Hb (%)	7.30±0.07	9.34±0.09*	10.72±0.07*	13.59±0.02*
Nucleic acid content (mg/g of tissue)	DNA	5.42±0.47	9.75±0.84*	14.08±1.22*	17.33±1.50*
	RNA	7.9±0.50	14.3±0.8*	20.61±1.2*	25.3±1.5*
Non- antioxidant enzymes	LPO(nmol MDA/g tissue)	119.25±1.41	86.11±1.25*	61.11±0.82*	42.78±1.83*
	GSH(μg/g tissue)	3.1±0.09	3.8±0.26*	5.4±0.24*	6.8±0.40*
Antioxidant enzymes (U/mg protein)	SOD	4.51±0.35	5.41±0.21*	8.12±0.63*	11.73±0.91*
	CAT	6.00±0.64	8.29±0.58*	10.37±0.53*	13.58±0.45*
	GPx	2.44±0.06*	4.05±0.05*	7.46±0.04*	8.20±0.02*
Ascorbic acid (mg/dl)		1.36±0.0458	2.32±0.078*	3.07±0.1030*	3.55±0.119*

*p<0.05 Statistically significant when compared to control group 1.(n=6)

Table 4. Effect of Swertiamarin ointment on rate of wound contraction by excision wound in experimental animals

Groups	Wound Contraction (cm ²)		% of Wound Contraction
	0 Day	15 th Day	
Group 1	2.05±0.04	1.57±0.07	23.58
Group 2	2.08±0.03	1.18±0.03*	43.20
Group 3	2.15±0.04	0.62±0.03*	71.32
Group 4	2.20±0.05	0.35±0.04*	84.09

*p< 0.05 statistically significant when compared herbal ointment treated (groups 2, 3) with excision wound control group 1; Values are expressed as Mean ± SEM n=6

"take" electrons from the lipids in cell films, causing cell damage. The end products of lipid peroxidation are reactive aldehydes, such as malondialdehyde (MDA), the major bioactive marker. It is due to its numerous biological activities resembling activities of reactive oxygen species (Table 3).

Gentamicin treated rats demonstrated antioxidant activity to counteract mitochondrial and in addition post-mitochondrial parts of renal tissues as confirmed by increasing the levels lipid peroxidation and antioxidant enzymes like G-glutathione peroxidase (GPx) and Super oxide dismutase (SOD) (Saranya et al., 2013).

A better wound healing pattern with complete wound closure was observed in rats treated with soframycin ointment group on 15 days for 87.21%. There was a significant reduction in wound area from 0 day to 15 days in treated rats and also the closure rate was

much faster when compared to control group animals (Figure 14).

With reference to the percentage of wound closure, the results indicated that Swertiamarin ointment (71.32%) showed wound closure followed by *Enicostema littorale* ethanol extract (43.20%). The results clearly indicated that the Swertiamarin ointment influences the wound closure to a greater extent when compared to the control (Table 4).

Conclusion

The findings indicated that the wound healing potential of Swertiamarin ointment on experimental rats (Figure 15). The topical application containing Swertiamarin ointment significantly induced the wound contraction and distinct wound closure. It might be inferred that Swertiamarin ointment is exceptionally powerful on open injuries and shown as a potential herbal drug formulation. It also had a distinct influence on the collagen level which is the precursor protein for wound healing mechanism. Further Swertiamarin ointment accelerated epithelialisation and increased wound contraction in the later stage of the wound healing process which has been evidenced by the excision studies. Hence, based on the stated findings it can be concluded that Swertiamarin ointment with genuine formulation may be used as a viable topical treatment and a practical remedial agent for wound healing.

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Figure 15. Wound healing activity of *Enicostema littorale* on excision wounded Wister rats. A: 0 day; B: 15th day

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Conflict Of Interest

The authors do not have any conflict of interest.

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