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In-Vivo Evaluation of Phytosomal Gel of The Petroleum Ether Extract of Root Bark of *Onosma echiodes* for Wound Healing Activity in Rats.

Jeeja Pananchery*, Chhaya Gadgoli

Saraswathi Vidya Bhavan's College of Pharmacy, Shankara nagar, Sonarpada, Kalyan-Shil Road, Dombivli East- 421203

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*Corresponding author
Jeeja Pananchery

Email:
jeejapananchery@gmail.com

ABSTRACT

The present study is aimed at evaluation of phytosomal gel of the petroleum ether extract of root bark of *Onosma echiodes* for wound healing activity in rats. Extract of root bark of *O. echioides* was standardized by isolated naphthoquinone dimer using HPTLC. Phytosomes (equivalent to 2% w/w of naphthoquinones) of the standardized extract were prepared by thin film hydration technique. The wound healing efficacy of the formulation was evaluated in rats by inflicting excision and incision wounds followed by treatment of the wounds topically. The parameters evaluated for healing included determination of breaking strength and tensile strength of healed skin for incision model and percentage wound contraction, hydroxyproline content, granulation tissue free radicals and catalase in excision wound model. The formulation treated group showed a significant healing ($p<0.005$) of both the excision and incision wounds with respect to wound contraction and tensile strength respectively, as compared to vehicle treated group. The oxidative stress of the granulation tissue was also found to be reduced as indicated by reduced lipid peroxidation and increase in catalase activity. The phytosomal gel of *O. echioides* effectively exhibited wound healing effect.

Keywords: *O. echioides;* phytosomes; naphthoquinone; wound healing; hydroxyproline; antioxidant activity.

INTRODUCTION

Preparations of phytomedicine has been used for health maintenance subsequently prehistoric periods. The phytomedicine's possess a lot of therapeutic uses. Earlier century the phytochemical and phyto-pharmacological disciplines are developed recognized for traditional remedies; biological activities as well promote the advantages of herbal remedies. Most of the bioactive phytochemicals like flavonoids as well terpenoids are highly polar in nature that's why poorly absorbed in lipids, since there is a difficulty to cross the highly lipid-soluble biological membrane, that's leads to poor bioavailability. Numbers of methods are developed for the refining of the bioavailability like the inclusion of solubility as well bioavailability modulators, modifications in structure as well as entrapment with lipophilic carriers. One such approach is the phytosome technology. The phytosome technology is innovative method established by Indena in an attempt to combat the issue of poor bioavailability. The term "phyto" means plant and "some" means cell like. This novel preparation comprises of incorporating a standardized plant extract into phospholipids to produce lipid compatible molecular complexes with enhanced absorption and bioavailability. *O echioides* (*Boraginaceae*) also known as Ratanjot, Mahaarangi have therapeutic potential against wounds, helminths infections as well alexipharmic properties. Many plants of the genus O family *Boraginaceaeare* present in nature (Shoaib *et al*., 2019). The root bark of *Boraginaceae* yields a liposoluble red dye. Scientifically, these plants have been screened for their medicinal properties in various pharmacological models. Mainly the root extracts of *O. echioides* are applied as antiseptic for skin injuries, contusion and eruptions. The two main active components namely shikonin and alkannin (enantiomeric natural dyes) along with their oligomers is present in *O. echioides* root bark (SHOAIB *et al*., 2017). Shikonin is the major component and possess wound healing, anti-cancer activity and has shown apoptosis and cell cycle arrest in MDA-MB-231 and HCT-116 cancer cell lines (Barreto *et al*., Kumar *et*

al., 2007, Kumar *et al*., 2013). Current (semisynthetic/ synthetic drugs) treatment strategies available for wound healing has several side effects while several shreds of evidences suggested that herbal formulations have therapeutic potential against wound (Singh *et al*., 2021).

Wound healing efficacy play substantial role in several models like incision as well excision (Shrivastav *et al*., 2018, Ali-Seyed and Siddiqua, 2020). Hence, in the light of abovementioned information on phytosomal formulation of *O echioides*, present study was undertaken to evaluate the wound healing potential of phytosomal formulation of *O. echioides* using incision and excision wound model in Wistar rats.

MATERIALS AND METHODS

Animals

Healthy non-pregnant and nulliparous female Wistar rats of approximately same age and weighing between 150-200 gm were acquired from central animal house facility and maintained with controlled conditions of humidity (60-65%), temperature (20±2°C), light and dark cycle 12h. Food and water were provided ad libitum. All methods were performed by recommendations provided by government authorities. The experimental protocol is approved by Institutional Animals Ethics Committee (Reg No. CRY/1920/007"and CRY/1920/001.)

Chemicals

O echioides was obtained from a local market, Mumbai and authenticated at Khalsa College, Mumbai. The voucher specimen is deposited in the Institute for reference (Specimen no. PYC # 261211). Ketamine hydrochloride, xylazine, Ehrlich reagent, Perchloric acid, Chloramine T, Standard Hydroxyproline, phosphate buffered saline (PBS), citric acid monohydrate, ethylene glycol monomethyl ether, Triton X-100 were obtained from Research Laboratories, Mumbai at analytical grade.

Extract preparation

Dried root bark of *O. echioides* was powdered in mixer and the powdered crude drug (100 g) was extracted with petroleum ether (60-80^{o}C) using Soxhlet Extraction apparatus for 18-20h. using a rotary flash evaporator, *O. echioides* extract was concentrated to a syrupy mass consistency and heated in vacuum oven till achieving dryness. The major colouring compound was isolated by column chromatography using Toluene: formic acid (99:1).

The isolated major colour compound was dissolved in methanol and UV spectrum was recorded using UV visible spectrophotometer (Elico SL 159 UV-VIS). The isolated colour compound was scanned from 200 to 800 nm to determine λ_{max} determination. Quantification of the isolated Color Compound in Pet. ether extract of dried root bark of *O. echioides* was carried out by High Performance thin Layer Chromatography (HPTLC).

Preparation of phytosomes

Phytosomes were prepared using a thin film dispersion technique. Briefly, petroleum ether extract of root bark of *O echioides*, soyalecithin, and cholesterol in a 1 : 2 : 2 ratio were dissolved in a mixed solution of chloroform and methanol (1 : 1 ratio) which was added to a round bottom flask and then dried using a rotary evaporator to form a thin lipid film. The dried film was then hydrated using 10 mL phosphate buffer (pH 5.5) at 35°C for 30min. Free *O.echioides* was removed by filteration through a 0.45 μm membrane filter. The measured volume of phytosomal formulation (equivalent to 2% w/w of the red dye equivalent to 40 mg of major colouring compound) was centrifuged by using cooling centrifuge for 90 mins at 4°C for 8,000 rpm. The semisolid mass of phytosomes (equivalent to 2% w/w of the red dye equivalent to 40 mg of major colouring compound) was separated from supernatant and mixed in the caprylic capric triglyceride and dimethicone copolymer gel base (Awasthi *et al*., 2011, Direito *et al*., 2019).

Acute oral toxicity studies

Healthy female Wistar (Non–Pregnant and nulliparous) rats were orally fed with Ether Extract of *O echioides* diluted in Vegetable oil at the dose of 5, 50, 300, 2000, 5000 mg/kg body weight for 14 days. This study required a total of six female rats to be tested at one dose level. Animals were observed individually at least once during the first 30, 60, 120, 180 and 240min after dosing, with special attention and once daily thereafter, for a total period of fourteen days. All observations of toxic signs were systematically recorded for each animal in the daily observation record format (Sharma and Sahu, 2016).

Wound healing activity

Animals were randomly divided into four groups each containing six. Group I: Normal - normal saline; Group II: Control- vehicle; Group III: Cipladine; Group IV: Phytosomal gel of *O. echioides.*

Incision, excision and burn model wounds were treated with *O echioides* extracts every day for 14 days study period (Dev *et al*., 2019).

Incision wound model

All selected animals were clean-shaven on the dorsum portion using electric clipper and anesthetized with 1:1 ketamine hydrochloride and xylazine. Impression on dorsal region was created exactly 1cm away from vertebral column and 5cm away from ear. A linear five cm long paravertebral incision was created through entire thickness of the shaved skin. After complete haemostasis, wounds were closed with sutures (three interrupted sutures) placed 1 cm apart. The sutures were removed on 10th day after wound creation and the animals were treated daily with the phytosomal gel of *O. echioides* extract for fourteen days. Group I animals were allowed to heal naturally. Rats were observed individually at least once daily for total study duration. All observations of clinical signs were systematically recorded(Paul *et al*., 2020).

Excision wound model

All selected animals were anesthetized with 1:1 using ketamine hydrochloride and xylazine. Using toothed forceps and pointed scissors, a circular area of 300-400 mm^2 excision wound was created. A two mm depth were created by cutting out layer of skin from the shaven dorsum area. Wound was left open in the case of Group I animals, whereas the standard drug cipladine and phytosomal gel of *O. echioides* extract were applied topically on excision wounds in specific groups. Rate of wound contraction and epithelialization period were estimated in this model. Using a transparent sheet, permanent marker and millimetre-based graph paper, the wound areas on days 0,3,6,9 and 15 were measured for wound contraction and the percentage in contraction was calculated by,

$$A= \frac{B-C}{B}\ x\ 100$$

A = % wound contraction; B= Initial wound area; C= final day wound area

At the end of the study the skin was divided into different parts for histopathology, biochemical parameters and antioxidant enzymes.

Measurement of tensile strength

The degree of wound healing is indicated by the tensile strength of a healing skin. Tensile strength signifies how much the restored and healthy tissue endure to breaking and characterizes the quality of healed tissue. All rats were anesthetized with 1:1 using ketamine hydrochloride and xylazine.

$$\frac{Tensile\ Strength}{Total\ Breaking\ Load} = Cross-sectional\ area \quad (220)$$

After suture removal, the healed tissue was excised from all animals. On 14th day of study, the breaking strength and tensile strength of the wound will be measured by using Tensiometer (Parente *et al*., 2012).

Biochemical parameters

A portion of the excised skin from normal control, diseased vehicle, standard and test extract groups were assessed for different biochemical parameters and to evaluate the healing properties of *O. echioides* (Jain *et al*., 2018).

Estimation of hydroxyproline

The whole healed tissue was excised and dried in glass vials in a 110°C oven for 48 h. The lyophilized sample of about 5 mg was hydrolysed with 5ml of 6 N HCl at 110°C for 18 to 20 h in a sealed tube. After hydrolysis, sample was evaporated to dryness in water bath (or 24h at 110°C). To each sample, 2ml of PBS sealed with parafilm was added and incubated for 1 hour in a 60^0C water bath. All samples were centrifuged and the supernatant were analysed for hydroxyproline (Paul *et al*., 2020).

Estimation of collagen

Hydroxyproline is converted to its equivalent collagen through multiplication by the factor 7.46.

Catalase

Initial step in catalase estimation is the addition of 10% tissue homogenate to 1% Triton X100 (pH-7) and further diluted with phosphate buffer pH-7 (1:100). To the cuvette, 2ml of tissue sample was added and the reaction was initiated by addition of 1 ml of H2O2. The kinetics of the reaction was observed for 30 sec spectrophotometrically and monitored at 240 nm (229- 230) and H_2O_2 (mMol) decomposed/min/gm skin tissue weight was calculated by, Abs at 0 sec – Abs at 15 sec X Factor of skin tissue weight in the tissue sample analysed Abs of 30 mM H_2O_2 (Shinde et al., 2021).

Lipid peroxidation assay

Estimation of lipid peroxidation was carried out by dissolving TCA-TBA-HCl reagent (15 gm TCA, 0.375 gm TBA in 100 ml of 0.25 N HCl.) The assay was performed by adding tissue homogenate of about 1 ml to 2 ml of TCA: TBA: HCl reagent. After addition, all tubes were vortexed for few seconds in boiling water bath. Prior centrifugation of samples

for 15 min, all the tubes were cooled to room temperature and supernatants were pipetted out in cuvette and OD was measured at 535 nm against blank. Calculation: n moles of MDA/ gm of tissue =OD X 1.56 M^{-1} cm^{-1} (Shinde *et al*., 2021, Shinde *et al*., 2020).

Histopathological Study

Healing tissues were obtained from and processed for histopathological study. Wound tissue specimen (five µm thickness) sections were prepared by microtomy and stained with haematoxylin and eosin (H&E) dye. All the four animal groups were assessed for any histological changes (Jain *et al*., 2020).

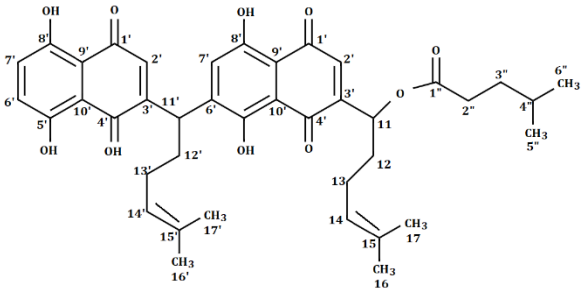
Statistical analysis

Data were expressed as Mean ± SEM, data were analyzed by one-way ANOVA and Bonferroni's post hoc test for comparison through a graph pad, prism software, and version 6.0, USA. The value of P< 0.05 was considered significant.

RESULTS AND DISCUSSION

Extract preparation

The concentration of major colouring compound in extract was 32.6% w/w (Figure 1). Preparation of phytosome was optimized by varying concentration of soyalecithin and cholesterol. The optimum concentration obtained was 1:1 ratio of soyalecithin: cholesterol as it yielded maximum entrapment extract equivalent to major colouring compound. The Phytosomes were incorporated into gel base and used for further wound healing property.



Fiigure 1. Structure of isolated major colouring compound (Nikita *et al.,* 2015)

Acute toxicity study

Before wound healing activity and assessment, preliminary toxicity of PET of *O. echioides* were carried out. Oral administration of test extract did not cause any mortality when administered up to a dose of 2000 mg/kg body weight. The LD_{50} of *O. echioides* extract was found to be >2000 mg/kg body wt. There were no significant alterations in food, water consumption and did not provoke any behavioural symptoms and signs of toxicity throughout fourteen days study period. Hence, the extract was found to be safe up to 2000 mg/kg for the present study.

Incision wound model

Rats treated with phytosomal gel of *O. echioides* extract showed significant increase on wound breaking when compared to the control and vehicle treated groups (Table I). There was not much significant wound breaking strength difference in rats treated with standard drug and test extract groups. The average tensile strength in vehicle treated group was 32.33±1.51 whereas in standard drug treated group and in extract treated group it was 40±1.79 and 42±5.06 respectively. Further, the breaking strength in the vehicle treated group was 1.62±0.08 and standard drug and extract treated group had 2.0±0.09 and 2.1±0.25 respectively. *O. echioides* extract treated rats showed better outcome and equivalent activity as compared to the standard treatment group. Phytosomal gel of *O. echioides* group has substantially increased the breaking strength value.

Excision wound model

The contraction percentage and the closure of original wound area was measured on third, sixth, ninth, twelfth and fifteenth days respectively. Phytosomal gel of *O. echioides* treated animals showed significant reduction in wound area on days 3,6,9,12,15 and faster rate of epithelialization when compared with the control group of animals. Groups treated with Phytosomal gel of *O. echioides* did not cause any substantial difference in the rate of wound contraction when compared to the standard drug. Whereas, the control and vehicle treated groups on day 3, 6, 9, 12 and 15 have not contributed to a significant contraction of the wound area. The percentage of wound contraction of four different groups namely control, standard and test was 85%, 98% and 98.3% (Table II).

Hydroxyproline, collagen in wound tissue

Phytosomal gel of *O. echioides* showed a two-fold surge in hydroxyproline content than the control group of animals (Table III). Similarly, substantial increase in hydroxyproline content was also detected in standard treatment model.

Table I. Percent wound contraction

Group	Breaking Strength (g)	Tensile Strength (N/cm^2)
Normal Control	1.58±0.04	31.26±1.09
Disease Control	1.62±0.08	32.33±1.51
Standard	2.0±0.09	40±1.79
Test	2.1±0.25	42±5.06

Table II. Percent wound contraction

Group	0 Day	3rd Day	6th Day	9th Day	12th Day	15th Day	% Inhibition
Disease Control	375.17±7.22	339±8.39	270.33±6.50	165.67±6.15	87.67±14.36	52.67±8.62	85.10±2.20
Standard	378.5±5.47	323±4.52	234.50±4.85	136.50±5.79	54.67±6.68	7.50±2.17	98.02±0.58
Phytosomal Gel	379.17±5.23	234.17±6.8	199.50±12	130.17±6.05	51.67±6.50	6.17±3.31	98.37±0.89

Figure 2. Effect of wound contraction on diseased control, standard and phytosomal gel

Surge in hydroxyproline levels is eventually responsible for increase in collagen content. In the present study, test extracts treated group showed 13-15% increase in the level of hydroxyproline as compared to normal control group. Collagen content increased significantly ($p<0.001$) in the groups treated with phytosomal gel of of *O. echioides* (508.7±55.79) than the control group (444.12±9.88) which infers more collagen deposition in the extract treated than the Group I animals, whereas Group II and Group III showed 319.29±35.61 and 529.16±43.31 respectively. Wistar rats receiving phytosomal gel of *O. echioides* extract treatment showed 13.5% increase in collagen content as compared to Group I rats. Whereas, only 3% increase in collagen content was observed in standard treatment group in comparison to the tested extract animals (Figure 2).

Tissue antioxidant parameters

Disease control group animals showed significant decrease in the catalase content when compared to normal group. The extract treated rats showed 29.13% and 10% increase in catalase as compared to disease control group. On the other hand, the LPO content got much decreased in phytosomal gel of *O. echioides* as well as standard drug treated groups (91.26±7.43 and 133.90±5.53) when compared to the control group animals. A significant ($p<0.001$) increase in LPO levels were observed in the vehicle treated when compared to the normal control, standard and test extract groups respectively. LPO of phytosomal gel of *O. echioides* tested rats was decreased by 61% as compared with the diseased control group (Figure 3).

Histopathological observations

Histopathological examination of wound area in vehicle treated showed mild inflammatory reaction in dermal layer with haemorrhagic changes in the epidermal layer which was evident with infiltration of neutrophils. Histopathological evaluation revealed that the course of healing of the wounded tissue with Phytosomal gel of *O. echioides* treatment was near equivalent to the standard drug treated rats. The tissue obtained from the tested extract showed few macrophages and very less oedema, significant increase in collagen deposition, fibroblasts and newly generated blood vessels.

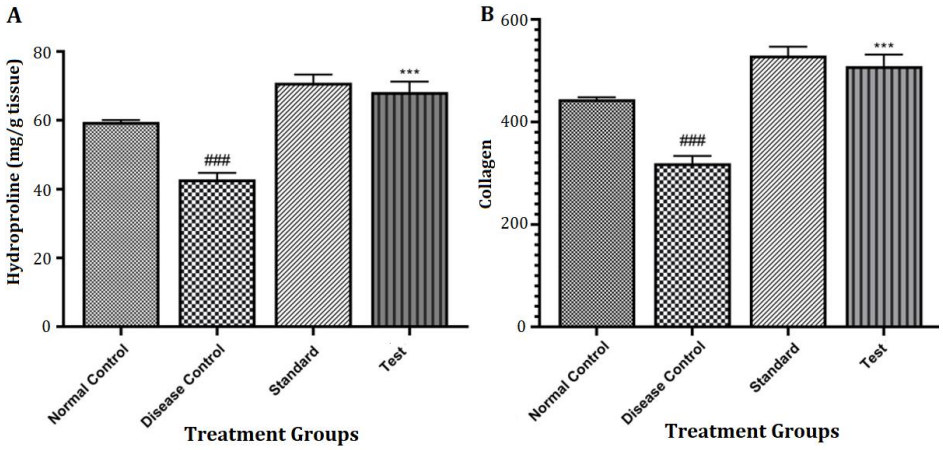


Figure 3. Effect on Hydroxyproline, collagen assay in wound tissue

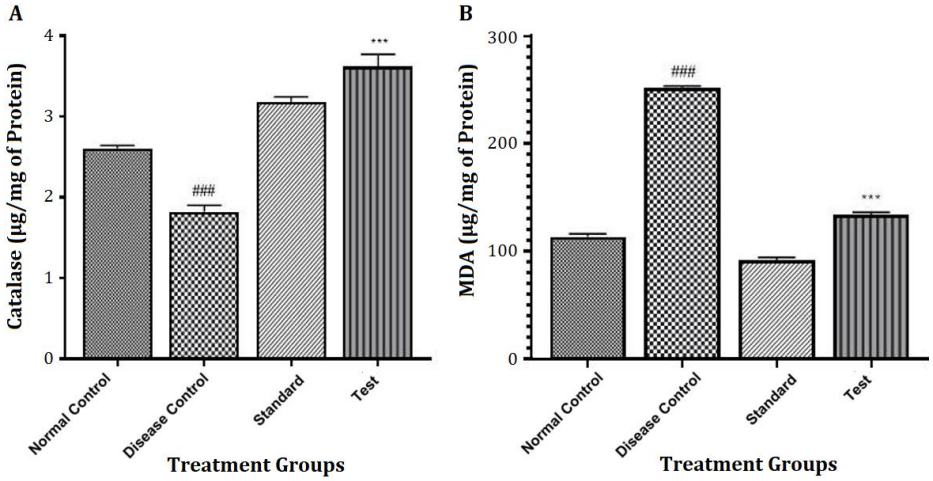


Figure 4. Effect on tissue antioxidant parameters

From the examination it was evident that there is damage to the epithelial cells of control group animals with further destruction of fibroblast and collagen fibres. Further the histopathological examination revealed that there was full thickness epidermal regeneration covering entire wound area (Figure 4).

Wound healing process comprises of different stages namely epithelialization, contraction, granulation and collagenisation/ connective tissue deposition to restore cellular structures and tissue integrity (Harding *et al*., 2005, Wu *et al*., 2007, Ghosh *et al*., 2012). The process of wound healing depends on the biosynthesis in a regulated manner, subsequent maturation and collagen deposition to a larger extent (Schultz *et al*., 2011). Inflammation, proliferation, maturation and remodelling are the very important stages in wound healing process (Bennett and Schultz, 1993a, Bennett and Schultz, 1993b). Initial phases in tissue repair process include haemostasis and inflammation where inflammatory cells promote proliferation and migration of endothelial cells. Proliferation is followed by epithelialization, angiogenesis and collagen deposition and results in neovascularization of connective tissue cells and extracellular matrix synthesis with keratinocytes and collagen leading to re-epithelialization (Rumalla and Borah, 2001). The wounded tissue undergoes contraction leading to smaller amount of scar tissue in the maturation phase and granulation tissue is formed and is made of collagen, oedema, fibroblasts and new blood vessels (Bennett and Schultz, 1993a).

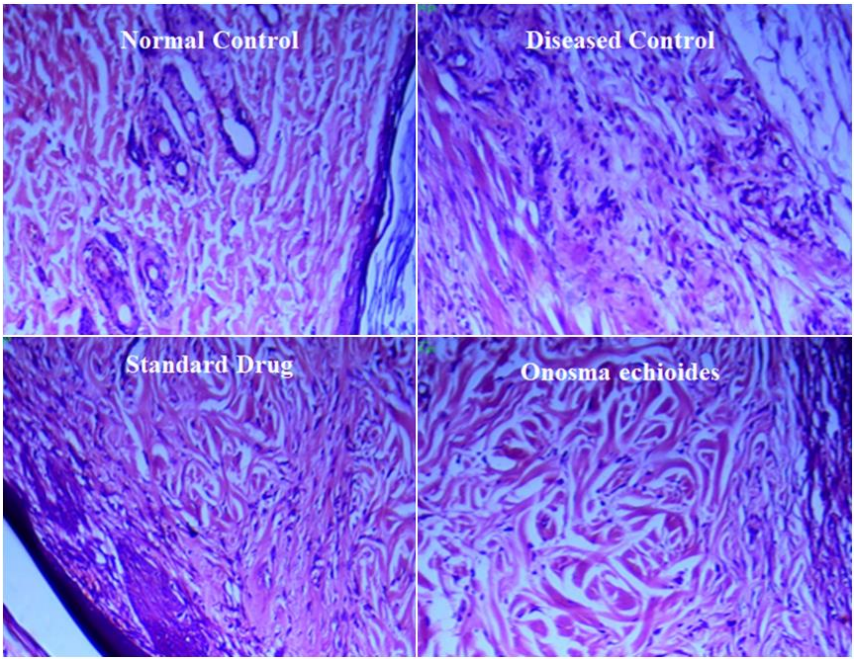


Figure 5. Effect on histological analysis

To understand the fundamental process of tissue repair, animal wound healing models play an important biological tool to develop and validate strategies for wound treatment. For skin wound healing research, rats have been widely used and often selected because of its availability, small size and low cost with an effort to replicate human wound healing complications resulting from injuries, infection and scarring, ischemia, dehiscence, venous or pressure ulceration (Dorsett-Martin and Wysocki, 2008, Dorsett-Martin, 2004). With these models it is possible to standardize the type, size, shape and depth of wound injury (Dorsett-Martin and Wysocki, 2008, Parente *et al*., 2012, Grada *et al*., 2018).

In the present investigation, incision and excision models have been chosen to assess the phytosomal gel of *O. echioides* on wound healing efficacy. In an incision wound model, animals treated with phytosomal gel of *O. echioides* exhibited better wound breaking strength compared with normal and vehicle treated group. Increase in wound breaking strength is mainly because of a surge in collagen synthesis and stabilization of the fibres. Synthesized and deposited collagen molecules at the wound area are cross linked to form collagen fibres. Wound strength is attained through stable intra- and intermolecular crosslinks and remodelling of collagen (Cai *et al*., 2017).

In excision wound models, phytosomal gel of *O. echioides* exhibited significant and faster wound healing activity compared with control and vehicle treated group. Excision biopsy of day 15 showed skin structures with normal epithelialization, formation of granulation tissue and synthesis of collagen, in standard and phytosomal gel of *O. echioides* as test groups. However, similar effect was not observed in the control group animals and lags behind in the formation of granulation tissue. Increased wound contraction by phytosomal gel of *O. echioides* may be due to the simulation of inflammatory α-chemokine, interleukin (Guo *et al*., 2017), that might enhance the intracellular communication (gap junction) in fibroblasts and hastens the maturation of granulation tissue (Moyer *et al*., 2002, Jiang *et al*., 2012, Guo *et al*., 2017).

In different phases of wound healing, inflammation, maturation of collagen and scar formation run concomitantly but independent of each other (Jiang *et al*., 2012). Hydroxyproline content of the granulation tissue revealed that animals treated with phytosomal gel of *O. echioides* indicated a significant increase than Group I and Group II animals thus signifying an increased

collagen turnover. There will be a rapid increase in collagen synthesis in the injured area soon after an injury. Collagen is the predominant extracellular protein in the granulation tissue and facilitates support and strength to a healing wound. Free hydroxyproline and its peptides are liberated with the breakdown of collagen. Hence, hydroxyproline measurement can be used as an indicator for collagen turnover. Furthermore, wet and dry tissue increase also indicated the existence of elevated protein content (Dwivedi *et al*., 2017). Wistar rats treated with phytosomal gel of *O. echioides* extract showed significant improvement in Group IV animals demonstrating faster collagen turnover leading to a faster wound healing with concurrent improvement in the breaking strength and the results attained were equivalent and comparable to that of standard drug.

Many studies have reported and acknowledged that delay in wound healing process is connected with reactive oxygen species; hence antioxidant enzymes and their role in free radicals' elimination play important part in wound healing. Experimental and clinical studies indicate that wound undergoes extensive oxidative stress by LPO activity and neutrophils-derived oxidants that contribute evidently to tissue damage during wound inflammation (Brand-Williams *et al*., 1995). Excessive reactive oxygen species production will cause oxidative stress resulting in cytotoxicity and delayed wound healing. Eradication of ROS might be an imperative approach in wound healing management. Hence, estimation of antioxidants in granulation tissue is appropriate as they have been reported to decrease the free radicals and accelerate wound healing (Gupta *et al*., 2002). The phytosomal gel of *O. echioides* treated group exhibited improved antioxidant activity by extermination of free radicals and lessening oxidative stress. The extracts were capable of upholding the redox balance by minimizing LPO and augmenting the antioxidant enzyme catalase in wound site.

According to MacKay *et al*, wound healing is augmented when a plant extract with medicinal properties is applied by the topical route (MacKay and Miller, 2003). Moreover, rats treated with phytosomal gel of *O. echioides* exhibited significant improvement in the tensile strength (incision wound model) when compared to Group I animals. Increase in tensile strength denotes improved wound healing, increase in collagen deposition and stabilization of fibres (Swamy *et al*., 2007). From our investigation, the maximum efficacy was observed in Group II animals with respect to biochemistry, antioxidant assays, wound reduction percentage and histopathology. Group II animals also exhibited promising wound healing efficacy and near comparable with the standard drug treated animals in all the parameters evaluated.

The key components of phytosomal gel of *O. echioides* plant are shikonin, alkannin and their derivatives namely deoxyshikonin/deoxyalkanin, alkaloids, phenolic compounds and naphthoquinones (Buchbauer *et al*., 1995). Other important components include ferulic, vanillic acids and flavonoids which may be responsible for its wound healing, analgesic, anti-inflammatory and antibacterial properties without causing gastric damage (Pastar *et al*., 2014). A study by Ambreen Shoaib *et al*. demonstrated that the n-hexane root bark extract is highly efficient in diabetic neuropathy management and proved to have potential benefits when tested on neuroblastoma cell lines (Shoaib *et al*., 2019). Numerous in vivo animal studies have confirmed Shikonin and its derivatives for wound healing efficacy. Ozaki *et al*. studied the potential effects of shikonin/acetylshikonin on rats and all tested preparations endorsed wound healing property (Ozaki *et al*., 1994). Proliferative, anti-inflammatory and microbial resistance of phytosomal gel of *O. echioides* plant extracts are appreciated in clinical practice as wound healing agents.

CONCLUSION

The study thus demonstrated the wound healing activity of incision, excision models, which involved observation of different physical, biochemical and histological, parameters. Outcomes of our present investigation indicated the wound healing efficacy of phytosomal gel of pet.ether extract of root bark of *O. echioides* by effectively increasing tensile strength of incision, excision and burn wound by stimulating wound contraction, promising effects on antioxidant status, decreased free radical generated tissue damage, connective tissue constituent formation and faster collagen deposition. The findings justify that phytosomal gel of pet.ether extract of root bark of *O. echioides* enhanced the wound healing and provided scientific evidence and could be applied in the management of wound healing.

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