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STUDY ON THE IN VIVO WOUND HEALING ACTIVITY OF THEMEDA TRIANDRA FORSSK

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ABSTRACT

A study was conducted to evaluate the in vivo wound healing potential of Themeda triandra Forssk. The extraction of Themeda triandra Forssk was carried out using a Soxhlet apparatus, a method known for extracting active compounds from plant materials. Once the extract was obtained, an ointment formulation containing the extract was prepared for application. The objective of the study was to assess the wound healing activity of the ointment when applied topically to wounds. The experimental setup involved creating controlled wounds on an appropriate animal model. The ointment containing the Themeda triandra Forssk extract was then applied to the wounds. After the application, the size and condition of the wounds were measured and recorded daily to track the healing progress. These measurements were essential in quantifying the rate of wound closure and the overall effectiveness of the ointment in promoting tissue repair. The results of the study indicated that the application of Themeda triandra Forssk ointment significantly accelerated the healing process. The wound area progressively reduced over the course of the study, demonstrating the plant's potential as a natural remedy for wound healing. The observed results suggest that Themeda triandra Forssk. possesses bioactive compounds that aid in tissue regeneration and

wound closure, In conclusion, the study highlights the promising therapeutic potential of Themeda triandra Forssk As a natural wound healing agent. Further research could explore the specific bioactive components responsible for these effects and their mechanisms of action in wound healing.

KEYWORDS: Wound healing potential, Themeda triandra Forssk.

1. INTRODUCTION[1-5]

Traditional plant-based medicine, historically plants have been used for treating various disease in different forms, from raw parts to complex extracts. Many current medications are derived from plants, requiring isolation and modification of active compounds. Plant base therapies are common in rural areas of developing nations. ancestors discovered the healing properties of plant through experimentation. A wound is a disruption of the body's tissues, affecting cellular and anatomical continuity. wound care practices are constantly changing due to factors like antibiotic resistance. The decline in antibiotic effectiveness has led to a resurgence of traditional and alternative wound treatments. wound healing process involves complex biological processes ton restore tissue structure and function. Healing can be classified as normal, delayed, or excessive. there are 6 million chronic wound patients worldwide.

WOUND HEALING

Wound is the disruption of a body part's cellular and anatomical continuity is the simplest definition of a wound, which may result from an injury to the tissue that is physical, chemical, thermal, microbiological, or immunological.

CLASSIFICATION OF WOUNDS

1. Acute wounds: It occurs commonly due to accidents such trauma or burns. Acute wound should heal in short duration

2. Chronic wounds: Patients with chronic wounds required prolonged periods of dressings and this can cause a significant financial health care system's burden.

WOUND HEALING MECHAMNISM

Both regeneration fibroplasia in wound healing involves cell growth, differentiations. Thepresence of a basement membrane is critical for orderly epithelial tissue renewal.

SKIN STRUCTURE AND FUNCTION

The skin serves as the body's external protective barrier, shielding against mechanical trauma, UV light, and infection. It also regulates body temperature, conserves fluids, and plays asensory role. The epidermis has a stratified structure with multiple layers, while the dermisprovides structural support and contains support and contains blood vessels and nerves.

INFLAMMATION

The local response of living mammalian tissues to an external agent's harm is known as inflammation.

1. Infective agents like bacteria, viruses.
2. Immunologivcal agents like cell-mediated and antigen antibody reactions.
3. physical agents like heat, cold, mechanical trauma.
4. Chemical agents like organic and inorganic poisons.

GENERAL PROCESS OF WOUND REPAIR

The wound healing has four phases,

HEMOSTASIS: It is the first phase of the length of this phase was 0 to 2 days. in this phasethe thrombin converts to fibrinogen and produces coagulation from, dilated vessels.

INFLAMMATION PHASE: This phased typically lasts for several days and involves the recruitment of white blood cells to the wound site. These cells help to clear debris, bacteria, and other foreign materials from the wound. Inflammation is characterized by redness, swelling, heat, and pain.

PROLIFERATION: During this phase, new tissue begins to grow to fill the wound space. Fibroblasts, a type of cell, produce collagen, a protein that provide structural support to the new tissue. Blood vessel also grow into the wound to supply nutrients and oxygen. This phase can last for several weeks.

REMODELING: In this phase, the newly formed tissue is remodeled and strengthened. Collagen fibers are recognized, and excess tissue that forms during this phase may be less flexible and pigmented than surrounding skin.

Table 1: showing the Growth factors present during wound repair.

FACTOR	SOURCE	TIMEOFAPPERENCE AFTER WOUND	RESPONCWCELL
Platelet derived growth factor	Platelets	Within minutes	Fibroblast, vascular smooth muscle Cells
Monocyte derived growth factor	Monocytes	24 to 48 hrs.	Fibroblasts
Macrophages derived endothelial growth factor	Macrophages	24 to 48 hrs.	Endothelial cell
Endothelial cell derived growth factor	Endothelial	5 to 7 days	Fibroblasts
Transforming growth factor-B	macrophages	With in minutes	Fibroblast

HORMONES

Wound hormones

These inhibitors are known as 'chalones'. Chalones diffuse out the injured differentiated cells and permit proliferation of undifferentiated stem cells.

Autacoids

Histamine is the one of the mediators of inflammation. It has been found that healing tissue produces large amounts of histamine. Histamine depletion, retards wound healing resulting in lowering tensile strength.

DRUGS

A number of chemical agents or drugs like aspirin, indomethacin, tolmetin, mefenamic acid, cytotoxic agents, radiation, and immunosuppressant's have been proved experimentally to adversely affect healing process.

Lidocaine/prilocaine creams as topical aesthetic agent had no adverse effect in incision wound model, furthermore it may have some beneficial effects on wound healing. Steroids adversely affect wound strength, wound contractions and union of skin grafts. Steroids can inhibit wound healing, even if applied topically by inhibiting formation of granulation tissue.

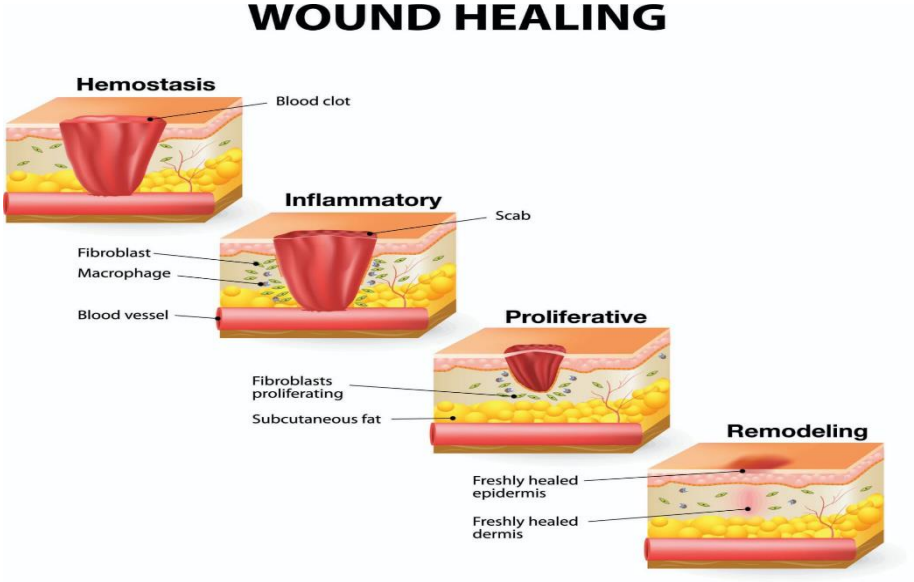

Fig No.1: Showing the phase of wound healing.

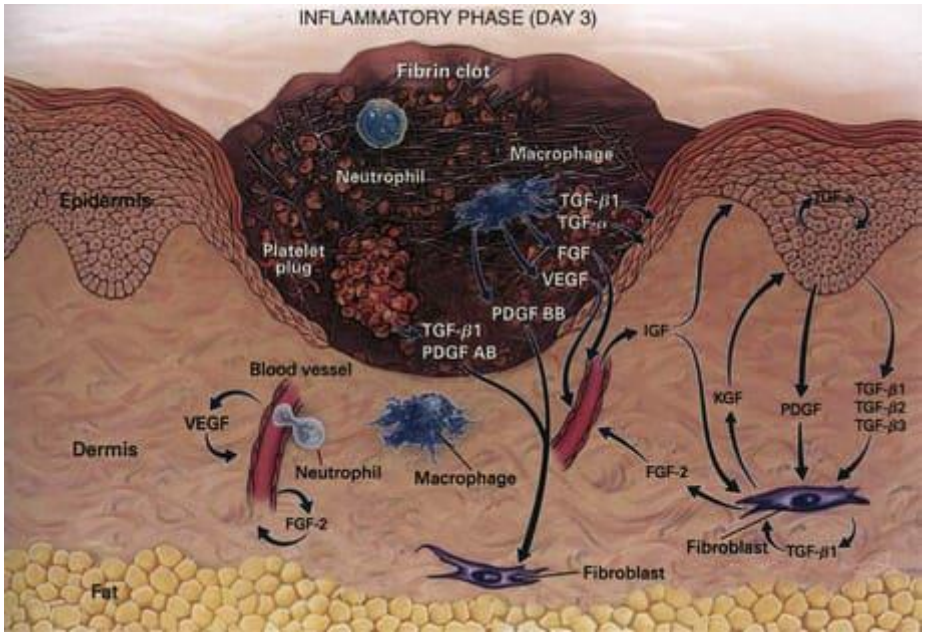

Fig No. 2: showing the inflammatory phase.

2. PLANT PROFILE[6-10]

BOTANICAL NAME – *THEMEDA TRIDANDRA FORSSK*

Synonym- themeda Australis, anthistiriainberbis, Themeda imbibes, anthistiria australis, Anthistria japonica.

TAXONOMICAL CLASSIFICATION OF THE PLANT

Kingdom – Plantae

Division – Angiosperms

Class – Monocots

Sub class – Commelinids

Order – Poales

Family – Poaceae

Sub family – Panicoideae

Tribe – Andropogoneae

Genus – Themeda

Species – Triandra

Plant type – Grass

The themeda is a genus of the plant having grass family native to Asia, Africa, Australia and aphasia. There are about 18 to 26 species; many of these are native to southeast Asia.

VERNACULAR NAMES

English – Red oat grass, kangaroo grass

Afrikaans – Rooi grass

Kannada – Bettanchihulla, bheemanahandhihullu

Tamil – Pedda yerra, Kalla kasurn

HABITAT

The plant Themeda triandra Forssk. forssk is found or wide spread in grass land and open woodlands. The grass grows well in clay soil having rain 500-800 mm/year. The flowering and fruiting time is October to July.

PLANT DESCRIPTION

Morphology: Themeda Tandra is a perennial grass with a distinctive appearance. It has a fascicle structure, with a thickened axis bearing numerous spikelets. The spikelets are arranged in pairs and have a unique shape, with a wedge-shaped callus and aa long, brown awn.

Distribution: Kangaroo grass is widely distributed, occurring in south Africa, east Africa, Australia, Tasmania, Papua New Guinea, south East Asia, and India, it is found in various states, including Odisha, where it is common in hilly regions.

Cultivation and collection: The document provides information on the cultivation and collection of Themeda triandra Forssk. It discusses the temperature and rainfall requirements for seed germination and growth. The plant typically grows at altitudes up to 300 meters.

TRADITIONAL USES ABOUT THE PLANT

- A decoction of the root is drunk as a treatment for dysmenorrheal.
- In Africa vit is used for the thatching and basketry.
- The seeds of the useful part T. triandra are used as a famine food in Africa.
- Useful part of the plant-leaves stems roots and seeds.

Fig no. 3 Themeda triandra Forssk.

3. MATERIALS AND METHODS[11-15]

COLLECTION AND AUTHENTICATION OF PLANT

The Themeda triandra Forssk. forssk plants were collected in the month of September 2022 from the mountain class region of Andra Pradesh. Then the whole plants were taken and leaves were plucked, washed and dried in shape at room temperature. The leaves took two months to get completely dried then it was grinded coarsely to get a rough texture and packed in air-tight container.

EXTRACTION OF PLANT MATERIAL

The extraction method depends upon the variation of length of the extraction period solvent used in the process of extraction, pH of the solvent, temperature and particle size of the plant. For Themeda triandra Forssk. forssk plant extraction Soxhlet apparatus are used.

PROCESS OF EXTRACTION

REQUIREMENTS: coarsely dried plant materials, Soxhlet apparatus, heating mantle.

SOLVENT USED: 95% (w/v) ethanol.

PROCEDURE

whole coarsely powered plant materials were put in the cellulose thimble chamber of the Soxhlet apparatus. After that the thimble was covered with the help of cotton. Then the heating mantle was switched on and the solvent was covered with the help of cotton. Then the heating mantle was switched on and the solvent was allowed to start evaporating and the vapors moved towards the condenser. Then the condense dripped into the reservoir containing the thimble. Once the level of solvent reaches the siphon it poured back into the flask and then the cycle began again. The process was continued for 72hrs. After the process has been finished, the ethanol was evaporated using a rotary evaporator leaving a small yield of extracted plant materials in the glass bottom flask.

ANIMAL HANDLING

Animal of either sex, same age group, and approximately of similar weight were engaged following an acclimatization period of 2-14 day. The animals were maintained at a well-ventilated animal house under standard controlled conditions at a temperature 22±1ºc to 30±1ºc, relative humidity 35±5 to 65±5% and kept under 12/12 h light / dark cycles with free access to food and water without restraint. The animals were put individually in clean, sterile polyvinyl or propylene cages.

PHYTOCHEMICAL INVESTGATIOIN OF THE EXTRACT

The ethanolic extract of Themeda triandra Forssk.forssk were subjected to qualitative analysis for the various Phyto constituent like flavonoids, tannins, glycosides, saponins, terpenoids.

Test for flavonoids

✔ 10 ml of ethyl acetate was heated over a steam bath for 3 minutes with a fraction of the powdered plant material. After filtering the mixture, 4 milliliters of the filtrate were constantly shaken with 1 milliliter of diluted ammonia solution, observation of yellow coloration indicate a positive test for flavonoids.

Teat for Tannins

✔ About 2.5g of the plant extract was dissolved in 5 ml of distilled water and was filtered and then ferric chloride reagent added to the filtrate. A blue- black, green, or blue-green precipitate was taken as evidence for the presence of tannins.

Test for Saponins

✔ The extract was diluted with distilled water and made up to 20 ml. The suspension was shaken in graduated cylinder for 15 minutes. 2 cm layer of foam indicates the presence of saponins.

Test for Terpenoids

✔ About 5 ml of each extract was mixed in 2ml of chloroform and concentration sulphuric acid (3ml) was carefully added to form a layer. A reddish-brown coloration of the interface was formed to show positive reaction.

Test for steroids

✔ About 2 ml of anhydride was added to 5.0 g ethanolic extracts of each sample with 2ml of sulphuric the color changed from violet to blue or green in some samples indicating the presence of steroids.

Test for Glycosides

✔ Extracts were hydrolyzed with dilute HCL and then subjected to test for glycosides.

Legal's test

✔ Extracts treated with sodium nitroprusside in pyridine and sodium hydroxide.

ACUTE ORAL TOXICITY STUDY

This test determines the toxicity of a substance when administered orally. Rats are used as the test subjects. The animals are given increasing doses of the substance of the substance until a certain number survive or die. The study is terminated when one of three stopping criteria is met.

PREPARATION OF TEST DRUG (OINTMENT)

The herbal extracts which were formulated for topical application in rats was prepared in the form of ointment. For the preparation of ointment, a simple ointment base was first prepared by the addition of PEG 400, and Ceto stearyl alcohol. After formation of ointment base the ethanolic extract of t. triandraforrsk was mixed to it and stirred gently by the help of mortar and pestle.

4. EXPERIMENTAL METHODS FOR WOUND HEALING ACTIVITY[16-22]

The wound healing activity can be studied broadly by four models such as:

I. Incision wound healing
II. Excision wound model
III. Dead space wound model
IV. Burn wound model

Incision wound model

Rats were prepared by removing hair and anesthetized before creating incision on either side of the spinal column. Three groups were established; a control group receiving only ointment base, a test group receiving an ethanolic extract, and a standard group receiving povidone iodine. Ointment was applied daily for 16 days, with sutures removed on day 8. Tensile strength was measured on day 10 to assess wound healing efficacy.

Dead space wound model

A dead space wound is created in rats by subcutaneously implanting sterilized grass pith under anesthesia. This model is used to study wound healing processes and the effectiveness of different treatments. The rats are then observed for a wound healing, a various treatment can be applied to assess their impact on the healing process. Male albino rats were subjected to partial thickness burn wounds using a heated blade. The rats were then divided into three groups; a control group, a test group receiving Themeda triandra Forssk., and a standard drug group, all groups received their respective treatments once daily for 10 days. On day 10, the granulation tissue formed on the wounds was excised, weighed, and analyzed for hydroxyproline content to assess wound healing.

Burn wound model

Male albino rats of Wistar strain (150-200gm) body weight were selected and maintained at uniform temperature and diet in well ventilated cages. Partial thickness burn wound were inflicted on overnight starved animal under light ether anesthesia using a metal rod (1.5 cm in diameter) heated to 80–850-degree c and expose for 20 sec. after 24hrs the dead tissue was excised using sterile surgical blade. Wound contractions and epithelialization period were evaluated in burn wound model.

EXCISION WOUND MODEL

In this excision wound model the animals were grouped as follows.

GROUP I: was served with only ointment base topically for 16 days.

GROUP II: Received topically application of 10% betadine iodine ointment twice a day for excision wound model for 16 days.
GROUP III: received topical application of 10% w/w of the extracts in simple ointment base twice a day for excision wound model for 16 days.

Rats were anesthetized and grouped. Excision wounds were created on their backs. The wounds were prepared with alcohol and a circular piece of skin was removed. Homeostasis was achieved and the drug was applied topically twice a day until the wounds healed.

Following an evaluation of the injured areas, the wound contractions was determined as a percentage of the decrease in injured areas on 4^{th}, 8yh, 12^{th} and 10 days until complete re-epithelization was achieved. The following parameters were then studied as follows.

EPITHELIZATION PERIOD

It was monitored by observing the number of days required for Escher to fall away, leaving no raw wound behind.

WOUND CONTRACTION

To monitor this, progressive changes in wound area were followed planimetrically. Leaving the wounding day, wounds were traced on alternate days, the animal was restrained in proper position during tracing. The tracing was then transferred to I mm^2 graph paper. From, these wound areas were read and the percent of wound contraction was calculation was calculated taking the initial size of wound ($100mm^2$) as 100% percentage wound closure can be calculated using the formula.

$$\text{Percentage of wound closure:} \quad \frac{\text{Initial area of wound} - \text{nth day area of wound}}{\text{Initial area of wound}} \times 100$$

5. RESULTS

A Well – orchestrated process involving cell migration, proliferation, and extracellular matrix remodeling is essential for wound healing. Wound care involves various measures like dressing, anti-inflammatory treatments. traditional medicines are being studied more scientifically to understand their effectiveness. Gene and stem-cell therapy are promising approaches for enhancing wound healing. Stem cells are capable of differentiating into

various tissue types and have self-renewal capacity. They are attracted to areas of wound healing, where they multiply and differentiate into cells needed for repair; stem cells can be obtained from sources like bone marrow, peripheral blood, umbilical cord blood, and adipose tissue. They can accelerate the healing response of acute and chronic wounds. Endothelial progenitor cells (EPCs) are a type of stem cell that contributes to wound angiogenesis. Factors like placental growth factor (PIGF) and VEGF regulate EPC recruitment in angiogenesis.

Evaluation of wound healing starts from the reduction in the wound's size, expressed as a percentage of the original wound size. It is measure from the day of operation until complete epithelization. Hydroxyproline is an amino acid found in collagen, the protein that gives tissue strength and stability. Measuring hydroxyproline levels can indicate collagen turnover and wound healing progress. hexosamine is a component of the ground substance involved in extracellular matrix synthesis. Its levels increased during the initial phases of wound healing and then decreases. Biomechanical strength refers to the breaking strength of the healed wound, which is measured as the force required to break the incision apart. It indicates the tensile strength of the wound tissue and reflects the degree of wound healing.

PHYTOCHEMICAL INVESTIGATION

Table 2: showing The Phytochemical Test ForThemeda triandra Forssk.

SL NO.	Phytochemical Tests	Presence of phytochemical	Activity	Mechanism of action
1	Flavonoids	+ve	Antimicrobial Antidiarrheal	Complex with cell wall, binds to adhesions. inhibit release of autocoids and prostaglandins. Inhibit GI Release of acetylcholine.
2	Terpenoids	+ve	Anti-microbial Antidiarrheal	Membrane disruption. Inhibit release of autocoid and prostaglandin
3	Saponins	+ve	Antidiarrheal Anticancer anthelmintic	Inhibit release of histamine possesses anti oxidating effects, thus reduces nitrate generation
4	Steroids	-ve	Antidiarrheal	Enhances intestinal absorption of sodium and water
5	Tannins	-ve	Antimicrobial Antidiarrheal Anthelmintics	Bind to adhesion, enzyme inhibition substrate deprivation, and complexation.
6	Glycoside	+ve	Antidiarrheal	Inhibits release of autocoids and prostaglandins.

ACYTE ORAL TOXICITY STUDY

The Themeda triandra Forssk.forssk ethanolic extract up to dose of 3000mg/kg body weight did not show any mortality. Hence 1/10th of this dose i.e. 300mg/kg body weight of Themeda triandra Forssk. were used for wound healing activity.

Table 3: showing the acute oral toxicity study of ethanolic extracts of Themeda triandra Forssk on rats.

SL NO.	ment	Dose mg/kg body weight				Inference
		1500	2000	2500	3000	
1	CONTROL	0	0	0	0	Stop dosing
2	Themeda triandra Forssk. (ethanolic extract)	0	0	0	0	Stop dosing

EXCISION WOUND MODEL

Rats were anesthetic by ketamine HCL injection before the excision under aseptic conditions, a circular wound with a diameter of roughly 2.5 cm was created on the deplicated dorsal thoracic region of rats. These wounds were monitored throughout the investigation. By placing a transparent polythene graph paper over the and sketching its outline, the areas of the wounds were instantly qualified, this over was taken as the initial wound area reading. The rats are categorized into three groups (n=6). The animal of group I treated as control and only ointment base applied topically. The animal of group II treated as test I and received ointment of Themeda triandra Forssk. forssk extract, group III received standard drug povidone iodine ointment. All samples were applied once daily for 16 days, starting from the day of wounding. The observation of percentage wound closure was made on the, 8th, 12th and 16th, post wounding days. The wound area of each animal was measured by using tracing paper method. The percentage of wound contraction like wound contraction and epithelial time were evaluated by the excision wound model.

Wound contraction and Epithelization time in excision wound model

The wound contraction was calculated as percentage reduction in wound area with respect to initial wound area while the epithelization time was noted as the number of days after wounding required for scar to fall off leaving no raw wound behind.

The percentage wound contraction was determined by using following formula:

$$\text{Percentage of wound closure:} \quad \frac{\text{Initial area of wound – nth day area of wound}}{\text{Initial area of wound}} \times 100$$

Effect of control test drug, standard drug (povidone iodine) was observed on percentage wound concentration in excision wound model on initial, on 4th, 8th, 12th, and 16th, day interval which is shown in table no.2 it has been seen that significally wound healing took place in case of animals treated with a Themeda triandra Forssk extract which is 16 days as compared to control & the standard drug which took 23 and 15 days respectively for complete wound healing the least rate wound healing was seen in control group which received no treatment and fastest rate of wound healing was se en in standard drug group where animals received standard drug which is povidone iodine.

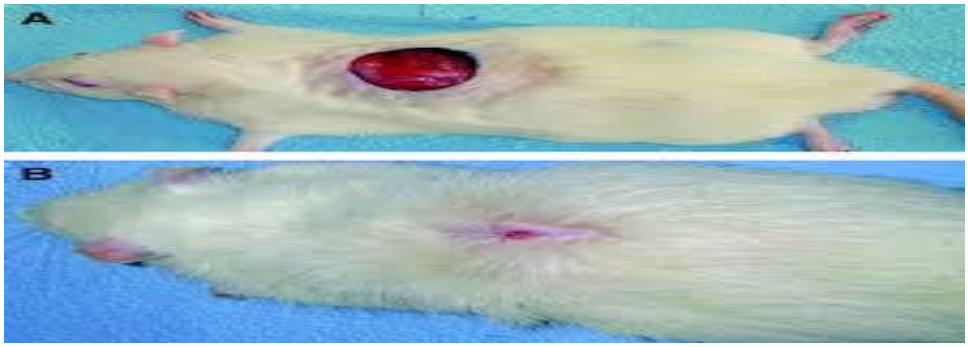

Figure-4-a. Showing the excision wound on rats b. Showing the cure of wound excision wound model.

Table 4: Effect of Triundra extract on precent wound contractions and epithelization period in excision wound model.

	AREA OF WOUND CLOSURE (sq.mm+S.E.M)					
GROUPS	**INITIAL**	**4TH DAY**	**8TH DAY**	**12th DAY**	**16TH DAY**	**EPITHELIZATION PERIOD (DAYS)**
I (CONTROL)	8.85+0.78	19.85+0.78	39.14+0.78	48.56+1.90	67.79+2.70	25
II (TEST DRUG)	7.85+5.32	31.8+0.058	44.08+0.034	2.18+0.050	87.82+0,064	20
III (STANDARD)	8.28+5.25	42.24+1.70	65.34+1.78	80.14+1.87	90.12+0.85	18

6. CONCLUSION

In the precent study ethanolic extract where prenared from and it's wound leafes Themeda triandra Forssk healing activity was studied by using establish models in rats. Toxicology studies reveled that Themeda triandra Forssk was safe and does not alter normal physiological and behaviour process even at higher dose level. To study the wound healing activity of ethaolic extract the excision wound model is selected because the technique is simple to be used for routine screening of wound healing activity. The ethanolic extract of Themeda triandra Forssk significant wound healing activity in excision wound model. As comparable to the marketed 10% w/w poviodine ointments thus it is concluded Themeda triandra Forssk is beneficial for healing of wound and it has amazing wound healing properties.

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