STSH190129

AN IN-VITRO STUDY OF WOUND HEALING PROPERTY OF ARNICA 1X IN L929 CELL

INTRODUCTION :

WOUND are injuries that causes breakage in the continuity of skin or other body tissues. They include cuts, scratches , Punctured skin ,surgery sutures and stitches can also cause wounds .There are two major classification of wounds ,Open wound and closed wound [1]. An Open wound is an injury involving external or internal breakage in the body tissues Usually involving the skin. Accidents with sharp objects can cause Open wound[2]. In Closed wound tissue damage and bleeding occur in undersurface of skin. These wounds are usually caused by direct blunt trauma. Contusions, hematoma comes under closed wound [3].

WOUND HEALING is the body's response to injury in an attempt to restore normal structure and function. It involves two processes : Regeneration and Repair . Regeneration means when healing takes place by proliferation of parenchymal cells and usually results in complete restoration of the original tissues. Repair means when healing takes place by proliferation of the connective tissue resulting in fibrosis and scarring. At times, both these processes take place simultaneously .

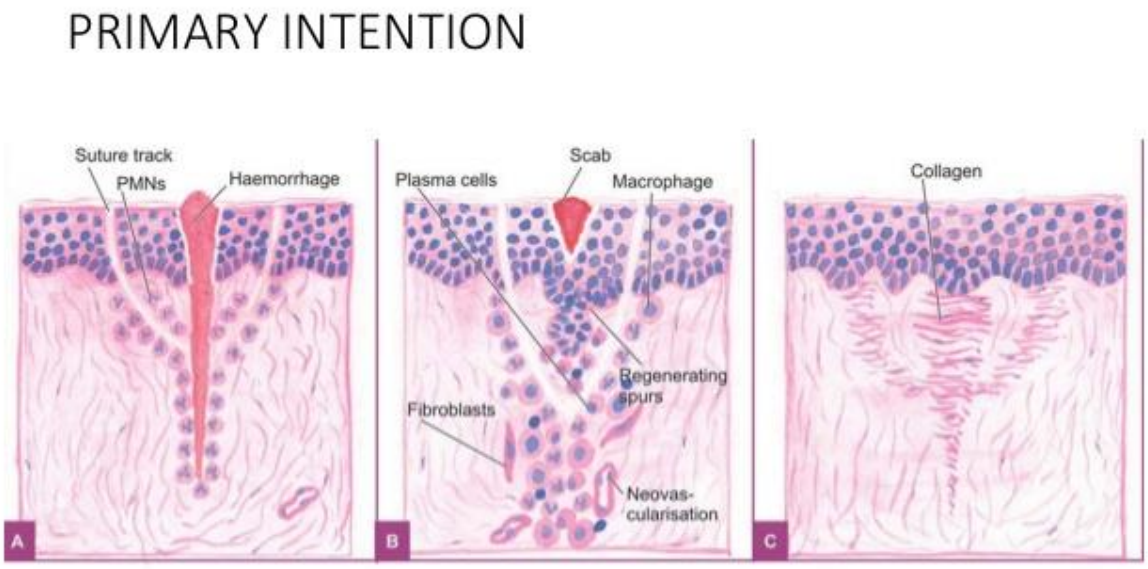
FACTORS INFLUENCING WOUND HEALING : • **Local factors**:-(1)*Infection*- one of the most important factor which delays the process of healing. (2)*poor blood supply :*-To the wounds slows healing e. g. Injuries to face heal quickly due to rich blood supply while injury to leg with varicose ulcers having poor 6blood supply heals slowly .(3) *Foreign bodies* :- Including sutures interfere with healing and cause intense inflammatory reaction and infection. (4)*Movement :*- delays wound healing .(5)Exposure to *ionising radiation delays* granulation tissue formation .(6)Exposure to *ultraviolet light* facilities healing. (6)*Type,size and location* of injury determines whether healing takes place by resolution or Organisation.

•**Systemic factors:** (1)*Age* : Wound healing is rapid in young and somewhat slow in aged and debilitated people due to poor blood supply to the injured area in the latter .(2) *Nutrition :*- Deficiency of constituents like protein, vitamin C, vitamin A and zinc delays the wound healing. (3)*systemic infection* delays wound healing (4)*Uncontrolled diabetes* are more prone to develop infections and hence delay in healing. (5)*Hematologic abnormalities* like defect of neutrophil functions (chemotaxis and phagocytosis) neutropenia, and bleeding disorders slow the process of wound healing[4].

Wound healing can be accomplished by one of the following two ways : • Healing by first intention (primary union) . •Healing by second intervention (secondary union) .

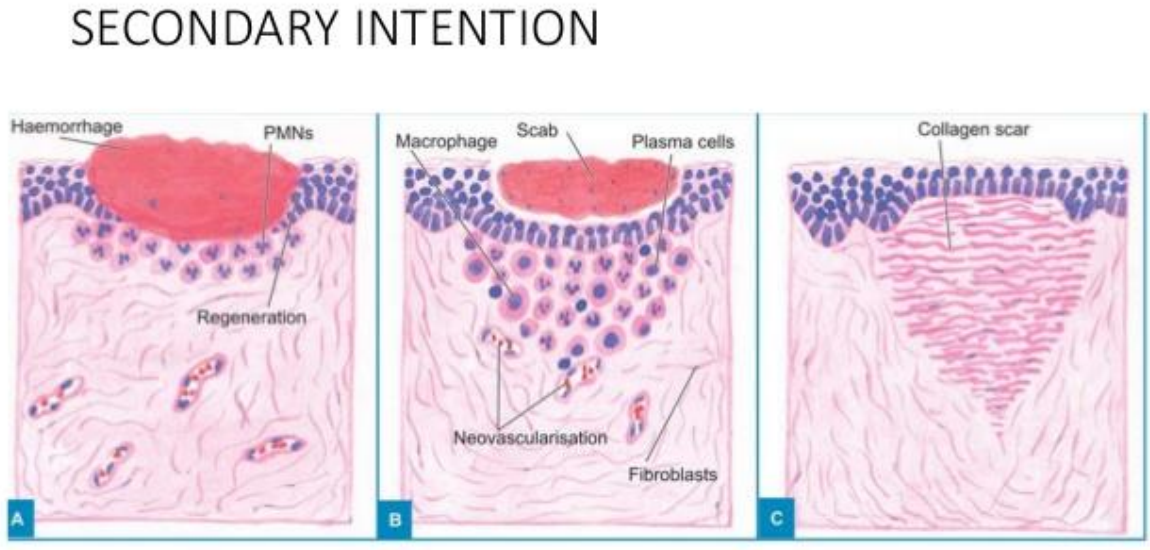
•**PRIMARY UNION** : This is defined as healing of wound which has the following characteristic : Clean and uninfected, surgically incised, without much loss of tissue and cells ,edges of wounds are approximated by surgical sutures ,the outcome of the primary union will be Neat linear scar.

The sequence of events in primary union are: • Initial haemorrhage. • Acute inflammatory



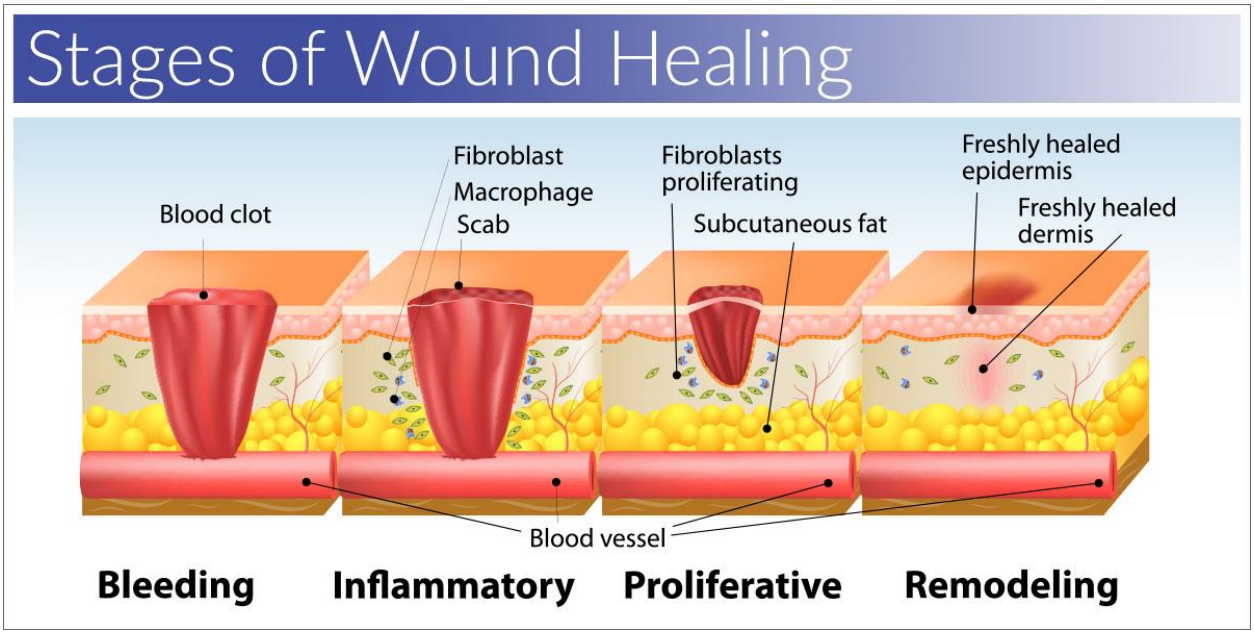
response•Epithelial changes• Organisation • suture tracks.

SECONDARY UNION : This is defined as healing of wound having the following characteristic : Unclean type of wound ,having excessive loss of tissues and cells , the wound is not approximated by surgical sutures but is left open , Outcome of the secondary union is slow and results in large at times ugly scar. The sequence of events in Secondary union are :•Initial hemorrhage •



Inflammatory phase • Epithelial changes • Granulation tissue • wound contraction • presence of infection [4].

•STAGES OF WOUND HEADING : It consist of Four stages : • Hemostasis phase •Inflammatory phase •Proliferative phase And Maturation phase.



Hemostasis phase : It is the first phase of healing begins at onset of injury and the objective is to stop bleeding. In this phase, the body activates it's emergency repair system, the blood clotting system and forms a dam to block the drainage. During this process, platelets comes in contact with collagen, resulting in activation and aggregation. An enzyme called thrombin is at the center and it initiate the formation of fibrin tissues, which strengthen the platelet clump into a stable clot.

Inflammatory phase : This phase focus on destroying bacteria and removing debris, essentially preparing the wound bed for growth of new tissues. Neutrophils enters the wound to destroy the bacteria and remove the debris. These cells often reach their peak population in 24 to 48 hours after injury, reducing greatly in number after 3 days, As the WBC leave, specialized cells called macrophages arrive to continue clearing debris. These cells also secrete growth factor and protein that immune system cell to the wound to facilitate tissue repair. This phase last for 4 to 6 days and associated with edema, erythema, heat and pain .

Proliferative phase : This phase features 3 stages (1) Filling of wound (2) Contraction of wound margin (3) Epithelization . During the First stage, shiny, deep red granulation tissues fills the wound bed with connective tissue and new blood vessels are formed. During contraction, wound margins contracts and pull towards center of wound.. In the third stage, Epithelial cells arise from wound bed or margins and begins to migrate across the wound bed until the wound is covered with epithelium. Proliferative phase often last from 4 to 24 days.

Maturation phase : During this phase ,the new tissue slowly gains strength and flexibility.

Here, collagen fibers reorganize the tissue remodels and mature and there is an overall increase in the tensile strength .The maturation phase varies from wound to wound, often lasting from 21 days to 2 years [5,6].

In homeopathy, *Arnica montana* is used as a wound healing medication and for treatment of hematomas. It is especially suited to cases when an injury, however remote seems to have caused the present trouble. After traumatic injuries, overuse of an organ, strain. *Arnica* is disposed to cerebral congestion. Acts best in plethoric, feebly indebilitated . Traumatism of grief, remorse or sudden realization of financial loss. A muscular tonic. Marked effect on blood .Affects the venous system inducing stasis. Relaxed blood vessels, black and blue spots .**sore, lame, bruised Feeling.** Tendency to tissue degeneration, septic condition, abscess that do not mature[7,8]. Since homeopathic remedies are highly diluted, they contain only miniscule amount of the original substance they are made from. As a result they don't have the toxic effects common to many conventional medicine. Arnica will check the hemorrhage of mechanical violence [7].

In all acute diseases brought on by mechanical injuries [9,10].Mechanical injuries even though received years back [11,12].Especially adapted to those who remain long impressed by even slight mechanical injuries. Sore, lame, bruised feeling all throughout the body, as if beaten; traumatic affection of muscles. Mechanical injuries especially with stupor from concussion, involuntary feces and urine .After injuries with blunt instrument [12,13].

NEED FOR STUDY

The basic mechanism in the healing process and then regulating the processes for faster healing or to avoid negative outcomes such as infection or scarring are fundamental to wound research. Although skin wound healing has been studied for decades, the molecular mechanism behind the process are not completely clear and most of the molecular level of understanding has been derived from various animal models. Species anatomical and physiological difference affect healing mechanism varyingly,thus different biological processes are species specific [14].

Skin, the largest organ of human body, protects the visceral organs from infection by microbes and injury. Wound healing mechanism is obligatory to regain the lost tissue and maintain tissue homoeostasis. Steps such as inflammation, angiogenesis ,granulation tissue formation re epithelisation and extracellular matrix reconstruction are taking place [15]. Upon injury to the skin cells such as fibroblast, keratinocyte, macrophages, Other immune cells rapidly proliferate and migrate towards the wound and initiate complex healing process. Here migration of cell towards wound is one of the key phases of wound healing process and in general is governed by various stimulating factors of tissue micro environment [16].

In vitro cell based scratch assay is appropriate and in experimental for understanding of wound healing efficacy of new Therapeutic agents [17].Natural extracts has been playing a fundamental

role in acceleration of wound healing process. However, scientific evidence of their efficacy is limited. So to identify the bioactive compounds of medically important herbal plant extracts and their mechanism of action has always having tremendous importance in medical research [18].. Identification and assessment of therapeutic potential of natural products derived from medicinal plants has led to the discovery of innovative and economical drugs to treat several diseases, including chronic wounds [19].

ARNICA MONTANA is used since centuries in homeopathic system of medicine. The *Arnica montana* is a plant belonging to Compositae family that grows on the hills of east and central Europe. Around one hundred and fifty therapeutically active substance are present in *Arnica montana* plant. Several active compounds are identified in its leaves, flowers and roots,such as alcohol, tannin, flavonoids and sesquirterpenic lactones ,especially helenalin.

The plant extracts have been supported to possess, antioxidant, anti-inflammatory, anti fungal and immuno modulatory activity. The incidental ingestion of the plant can cause vasodilatation, blood stasis, hemorrhage, edema and pain. These effects are the main topics described in *Arnica montana* Materia Medica[20].Because of this traumatic pain and edema absorption are the main indication for the Clinical and Experimental use of Homeopathic preparation of *Arnica montana* [21,22,23]. *Homeopathy*, is a holistic therapy cures the body and mind according to Hahnemann's law of similars[24].It is expressed as *Like Cures like* .

The current study is aimed at investigating the wound healing capacity of *Arnica montana* 1X by using *In vitro* scratch assay as a primary model, where proliferative and migratory capabilities of test compounds could be monitored through microscopic studies.

Homeopathy shows promising prospects in wound healing and this study is to prove the action of homeopathic medicine in promoting cell closure and proliferation of the cells; and in visualising the same by viewing their growth and closure microscopically by performing an invitro study of the action of *Arnica* 1X in L929 cell lines.

AIM :

To study the wound healing capacity of *Arnica* 1X in L929 cell lines.

OBJECTIVES :

•To show *Arnica* 1X is playing efficacious role in wound healing.

•To check the inflammatory response shown by L929 cells treated with *Arnica* 1X.

•To study the mechanism of stem repair by infusion of *Arnica* 1X.

REVIEW OF LITERATURE :

1. In the study done by Priyanka Kriplani and team titled ***Arnica montana L.* – a plant of healing,** they says that, "It has been scrutinized that extensive research has been carried out to explore the therapeutic potential of flowers of the plant. Therefore, investigations should be carried out to explore the therapeutic potential of other parts of the plant for better therapeutic utilization." The objective of their study was to evaluate the botany, phytochemistry and ethanopharmocolgy along with special emphasis given on pharmacological activity of *Arnica montana. Arnica montana* possess significant anti inflammatory, anti bacterial, anti fungal, anti oxidant and immunomodulatory activity .*Arnica montana* is reported to relieve symptoms of disease relating to restricted blood flow to nerve endings and limbs of patients and reflex sympathetic dystrophy syndrome which includes fibromyalgia, diabetic neuropathy. *Arnica* is reported to possess the anti-hemorrhagic activity in women of age group 25-30 years ,which reduce the post partum blood loss, which is a significant cause of perinatal morbidity and worldwide mortality. The pharmacological and phytochemical studies of the plant has revealed that plant possess numerous activities[25].

2. In the study titled **Effectiveness and Safety of *Arnica montana* in Post-Surgical Setting, Pain and Inflammation** which was published in the American Journal of Therapeutics, they say that "*Arnica montana* has been widely used as a homeopathic remedy for the treatment of several inflammatory conditions in pain management and postoperative settings. This study gives an overview of the therapeutic use of *Arnica montana* in the above-mentioned fields also focusing on its mechanisms of action learned from animal models and *in vitro* studies. *Arnica montana* is more effective than placebo when used for the treatment of several conditions including post-traumatic and postoperative pain, edema, and ecchymosis. However, its dosages and preparations used have produced substantial differences in the clinical outcome. Cumulative evidence suggests that *Arnica montana* may represent a valid alternative to non-steroidal anti-inflammatory drugs, at least when treating some specific conditions."[26].

3. In the study titled **Arnica and stinging nettle for treating burns – A self – experiment** published in Science Direct.Combudoron,composed of extracts from arnica and stinging nettle is used for treatment of partial thickness burns and insect bites in Europe. This study is conducted by them in order to find the efficacy of this combudoron in partial thickness burn. They finally

concluded that "All 8 experimental burns were similar from size and depth at baseline. Eschars of the verum-treated burns fell off earlier than the placebo-treated burns (verum liquid: after 14 and 19 days compared to 17 and 27 days with placebo liquid. Verum gel: after 16 and 22 days compared to 18 and 28 days with placebo gel). Eschars of the liquid treated burns fell off earlier than of the gel treated burns. Pain scores were not applicable because they were low and differences between the lesions could not be discriminated on the back."[27].

4. In the study titled **Cumulative therapeutic effect of petrochemicals in *Arnica montana* flower extracts alleviated collagen induced arthritis. Inhibition of both pro inflammatory mediators and oxidative stress.**The study was aimed to investigate the therapeutic effect and mechanism of *Arnica montana* flower methanol extract(AMME) against both inflammation and oxidative stress in a collagen induced arthritis (CIA) rat model. They concluded the study that "Oral administration of AMME was found to reduce clinical signs and improve the histological and radiological status of hind limb joints. AMME – treated rats had lower expression level of Nitric oxide, tumor necrosis factor α, Interleukin(IL-16, IL-6 and IL-12) and titer of anti -type 11 collagen antibody compared with untreated CIA rats. Further more, by inhibiting these mediators, AMME also contributed. Towards the reversal of disturbed antioxidant levels and peroxidative damage"[28].

5. This study titled **The effects of Arnica Montana on Bleeding Time: A Randomized Clinical Trial**, it is concluded that Arnica did not make any significant effect in effecting the bleeding time statistically or even clinically[29].

6. Another study titled **A Randomized, Controlled Comparison between Arnica and Steroids in the Management of Postrhinoplasty Ecchymosis and Edema** . The study compared the efficacy of both *Arnica and steroids* after rhinoplasty.They suggests that both arnica and corticosteroids may be effective in reducing edema during the early postoperative period. Arnica does not appear to provide any benefit with regard to extent and intensity of ecchymosis. The delay in resolution of ecchymosis for patients receiving corticosteroids may outweigh the benefit of reducing edema during the early postoperative period [30].

7. Another study titled **Efficacy of Arnica Montana D4 for healing of wounds after hallux valgus surgery compared to Diclofenac.** The study was done to check the efficacy of *Arnica* D4

10 pillules (taken orally, 3 times per day) and diclofenac sodium, 50mg (taken orally, 3 times per day) was investigated for equivalence in 88 patients after *hallux valgus* surgery. And the study concludes by saying that after foot operations, Arnica D4 can be used instead of diclofenac to reduce wound irritation[31].

8. The study titled, **A homoeopathic remedy from arnica, marigold St. John's wort and comfrey accelerates in vitro wound scratch closure of NIH 3T3 fibroblasts** .The objective of the study was to investigate the effect of commercial low potency, Similisan®Arnica plus spray on wound closure in a controlled, blind trail *in vitro*. Commercial homeopathic remedy, Similisian ® Arnica plus spray consist of *Arnica, marigold, St John's wort* and comfrey .It is used to treat injuries such as sprains, bruises, contusions, hematomas, muscle soreness, or pain following operations and bone fractures. The study *Says* that the low potency homeopathic remedy (0712–2) exerted *in vitro* wound closure potential in NIH 3T3 fibroblasts. This effect resulted from stimulation of fibroblasts motility rather than of their mitosis[32].

9. In the study titled, **Arnica montana Stimulates Extracellular Matrix Gene Expression in a Macrophage Cell Line Differentiated to Wound-Healing Phenotype** concludes by saying that the results of this work indicate that Arnica m. acts on macrophages by modulating a number of genes and by increasing cell motility. RNA-seq analysis allowed the identification of several genes which are particularly sensitive to ultra-low doses and high dilutions of this plant extract. Molecular analysis of gene expression suggests that a primary action of this medicinal plant is the stimulation of tissue matrix synthesis. These findings provide new insights into wound associated molecular events and specifically point to macrophage fibronectin production as a potential therapeutic target of Arnica m. for the treatment of wound repair[33].

10. Another study titled **Effectiveness of phonophoresis with Arnica Montana onto acute inflammatory process in rat skeletal muscles; An experimental study** they say that the study aimed at verifying the effects of phonophoresis associated with *Arnica montana* on the acute phase of an inflammatory muscle lesion. Forty Wistar male rats (300 ± 50 g), of which the Tibialis Anterior muscle was surgically lesioned, were divided into four groups (n = 10 each): control group received no treatment; the ultrasound group (US) was treated in pulsed mode with 1-MHz frequency, 0.5 W/cm^2 intensity (spatial and temporal average – SATA), duty cycle of 1:2 (2 ms on, 4 ms off, 50%), time of application 3 min per session, one session per day, for 3 days; the

phonophoresis or ultrasound plus arnica (US+A) group was treated with arnica with the same US parameters plus arnica gel; and the arnica group (A) was submitted to massage with arnica gel, also for 3 min, once a day, for 3 days. Treatment started 24 h after the surgical lesion. On the 4th day after lesion creation, animals were sacrificed and sections of the lesioned, inflamed muscle were removed for quantitative (mononuclear and polymorphonuclear cell count) and qualitative histological analysis. Collected data from the 4 groups were statistically analyzed and the significance level set at $p < 0.05$. Results show higher mononuclear cell density in all three treated groups with no significant difference between them, but values were significantly different ($p < 0.0001$) when compared to control group's. As to polymorphonuclear cell density, significant differences were found between control group ($p = 0.0134$) and US, US+A and A groups; the arnica group presented lesser density of polymorphonuclear cells when compared ($p = 0.0134$) to the other groups. No significant difference was found between US and US+A groups. While the massage with arnica gel proved to be an effective anti-inflammatory on acute muscle lesion in topic use, these results point to ineffectiveness of *Arnica montana* phonophoresis, US having seemingly checked or minimized its anti-inflammatory effect[34].

11. The study titled **Arnica for bruising and swelling** concludes that arnica is a popular homeopathic remedy in both Europe and the United States for the treatment of acne, bruises, sprains, and muscle aches and as a general topical counterirritant. The dried flower head is the most widely used part of the plant in commercially available[35].

12. The study titled the effect of homeopathic remedies **Arnica montana and Bellis perenis on mild postpartum bleeding** -A randomized, double blind placebo controlled study -preliminary results explains the importance of the homoeopathic remedies like *Arnica montana and Bellis perenis* in reducing post partum blood loss as compared with placebo. [36].

13. The study titled **Homoeopathic *Arnica* therapy receiving knee surgery results of Three randomized double blind trails** concludes by saying that in all three trails: that is Arthroscopy (ART) ,Artificial knee joint implant (AKJ)and Cruciate ligament reconstruction (CLR). The patients who received Homoeopathic Arnica showed a trend towards less postoperative swelling compared to patients receivied placebo. It is also highlighted the significant difference in favour of Homoeopathic *Arnica* in the CLR trails [37].

14. The study titled **Inflammatory process modulation by Homoeopathic Arnica montana 6CH,** The role of individual variation says that there is no selective modulation of leukocyte subsets migration by *Arnica montana* 6CH treatment but only vascular regulations, regarding lymphatic absorption, CD54 expression and histamine degranulation and there is a clear interference of the individual or kinetic variation in vascular events after treatment with *Arnica montana* 6CH.[38].

METHODOLOGY:

The **L929 cell lines** were initially procured from National Center for cell science (NCCS), Pune, India. These cells where maintained in Dulbecos modified Eagle medium (DMEM) (Gibco,invitrogen). The L929 cells where cultured in $25cm^2$ tissue culture flask with DMEM supplemented with 10%FBS, L-glutamine ,sodium bicarbonate and antibiotic solution. The antibiotic solution contains Penicillin (100U/ml), Streptomycin(100µg/ml), Amorphoteracin (2.5µg/ml).Cultured cell lines were kept at 37ºC in a humidified 5%CO2 incubator (NBS Eppendorf, Germany). The wound closure percentage is calculated by doing an ***invitro wound scratch assay.***

In the **scratch wound healing assay** exponentially growing L929 cells were first trypsinized and then were seeded at a density of 2,00,000 cells per well into 12 well plate ,and these were kept for 24 hours incubation (~90% confluence). One set of L929 cells are taken as a ***control set*** *and* the other is treated with the sample ***Arnica* 1X.** *Arnica 1X* is prepared using distilled ultra pure water. *Then* the scratch wounds were made by a sterile 1ml pippete tip through a pre -marked line. The Debris which is formed is being removed. After the removal of the resulting debris from five linear scratches , the cell monolayer was subsequently rinsed three times with PBS (Phosphate buffered saline) . This is being followed by incubation with the sample (ARNICA 1X) of concentration of 100µg/ml for 0 hour, 24 hours ,48 hours and 72 hours . The wound areas were displayed by taking images just above the interchanges between scratched wound areas and pre – marked lines. The effect of the sample (ARNICA 1X) on wound closure was determined

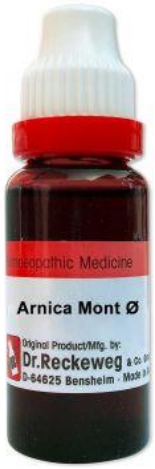
microscopically (4X magnification, Olympus CKX41) after incubation. The effect of the sample **Arnica 1X** on wound closure was measured in terms of area using MRI -image J analysis software.

Formula for calculation of wound closure percentage [39**]**

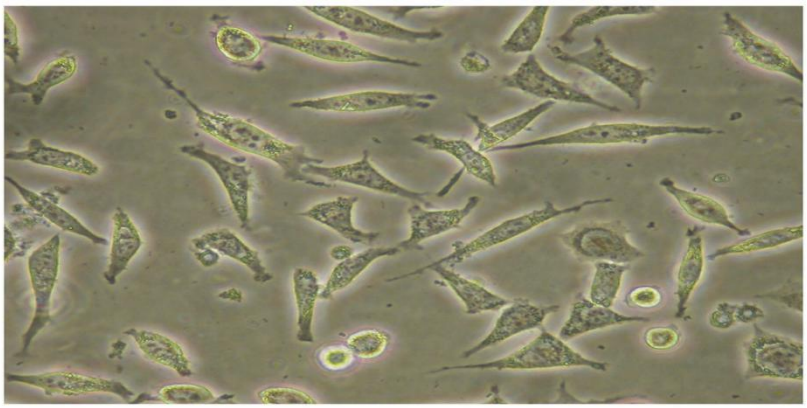
$$\text{Wound closure \%} = \frac{\text{Measurement at 0 hour -Measurement at 24 hours.}}{\text{Measurement at 0 hour.}}$$

These procedures were done in the life science tie up of our institution :- ***Biogenix Research center, Poojapura, Thiruvananthapuram .***

ARNICA MONTANA :



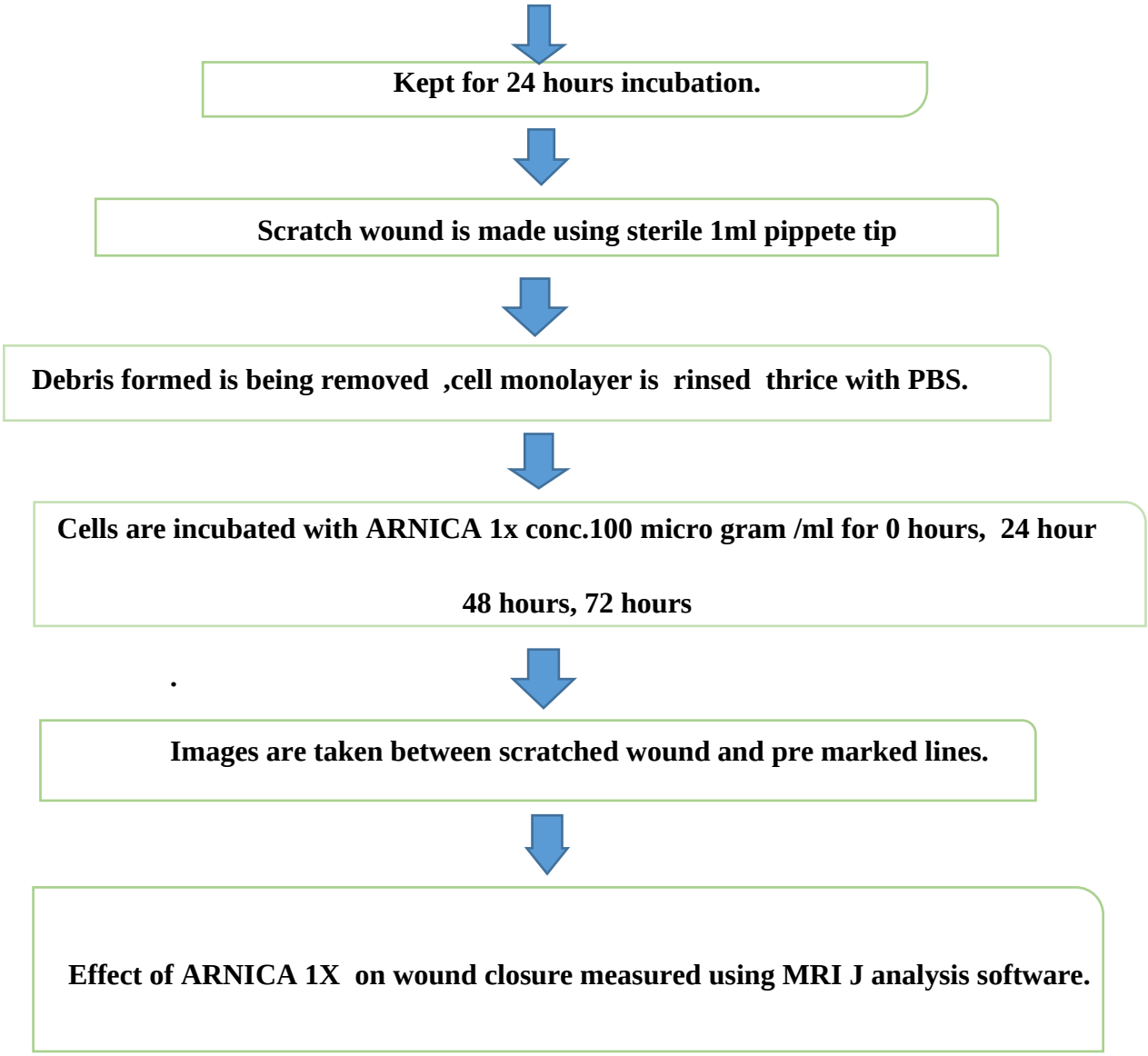
L929 CELL LINES



FLOWCHART: AN OVERVIEW ON THE METHODOLOGY ADOPTED

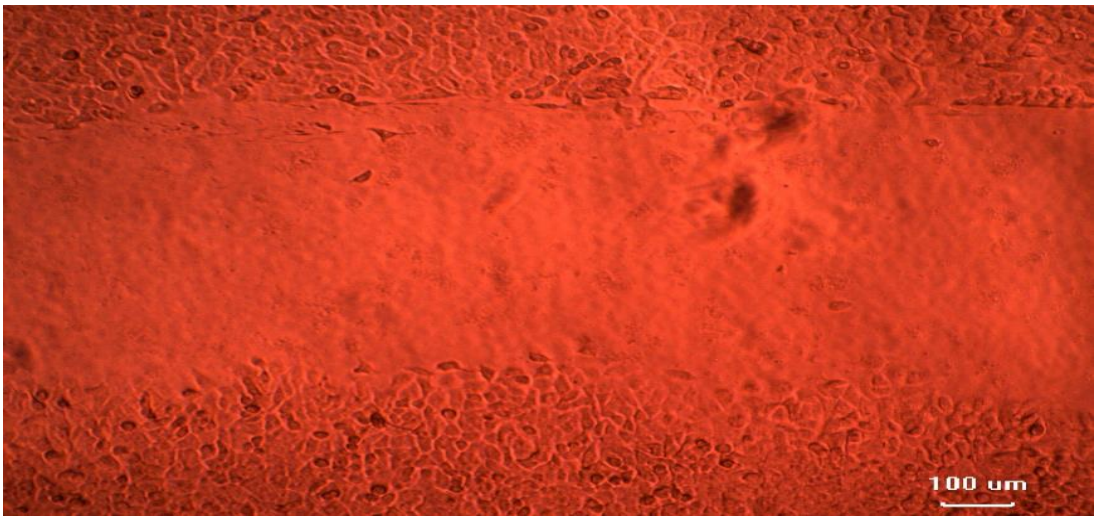
L929 cell lines are maintained in DMEM

Exponentially L929 cells are trypsinized and seeded in 12 well plate .

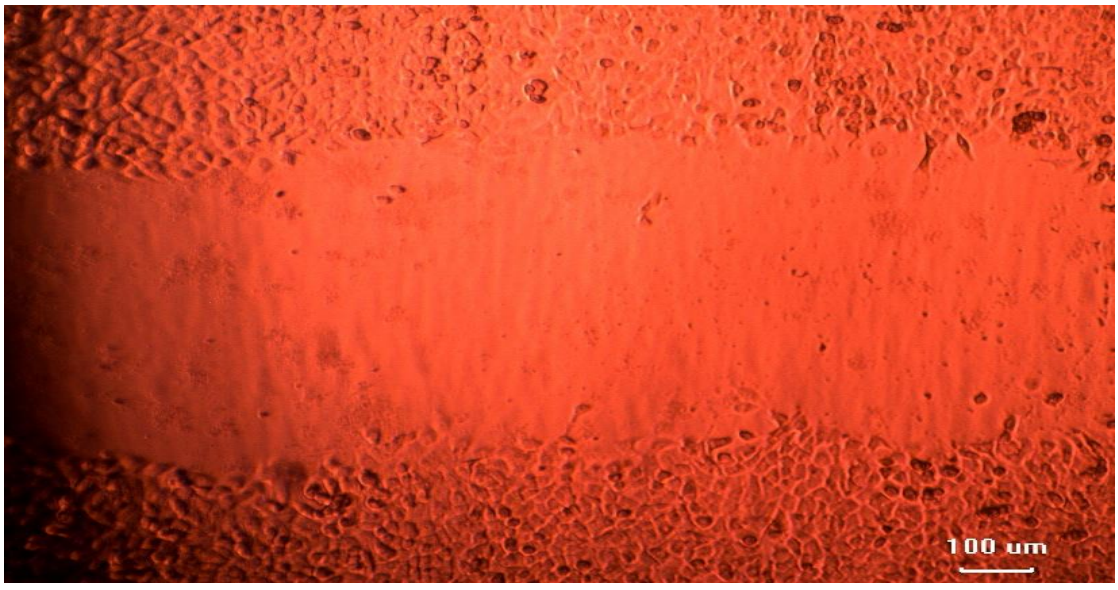


OBSERVATIONS

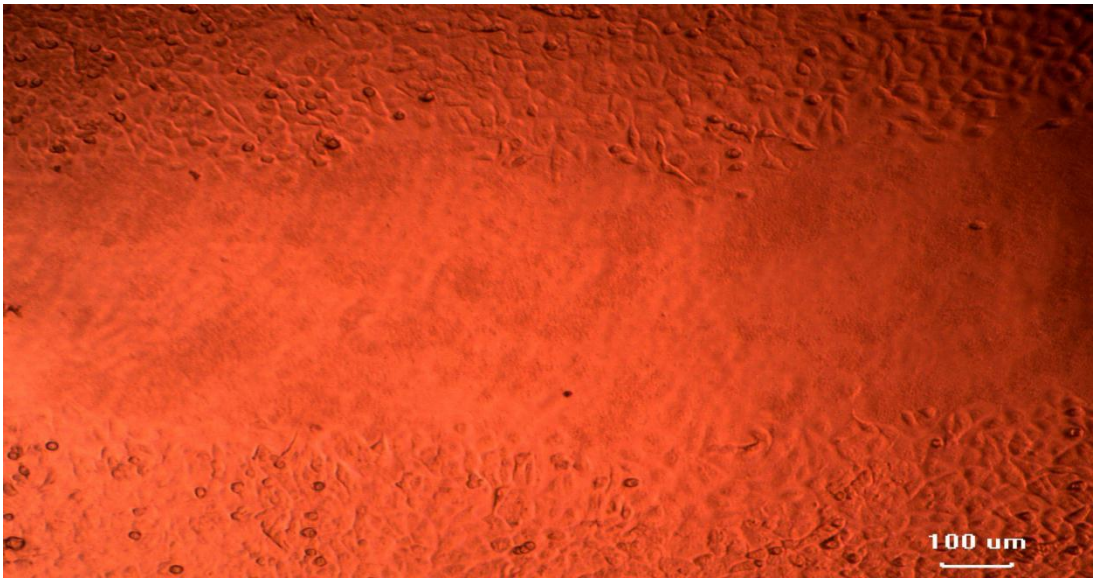
CONTROL AT 0 HRS



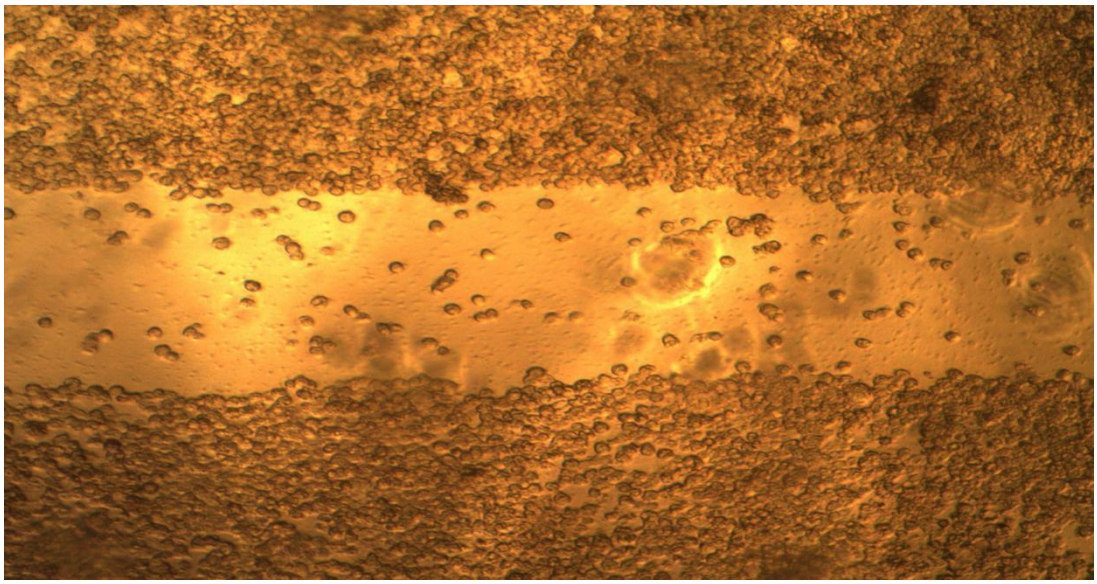
SAMPLE AT 0 HRS



CONTROL AT 24HRS



SAMPLE AT 24 HRS

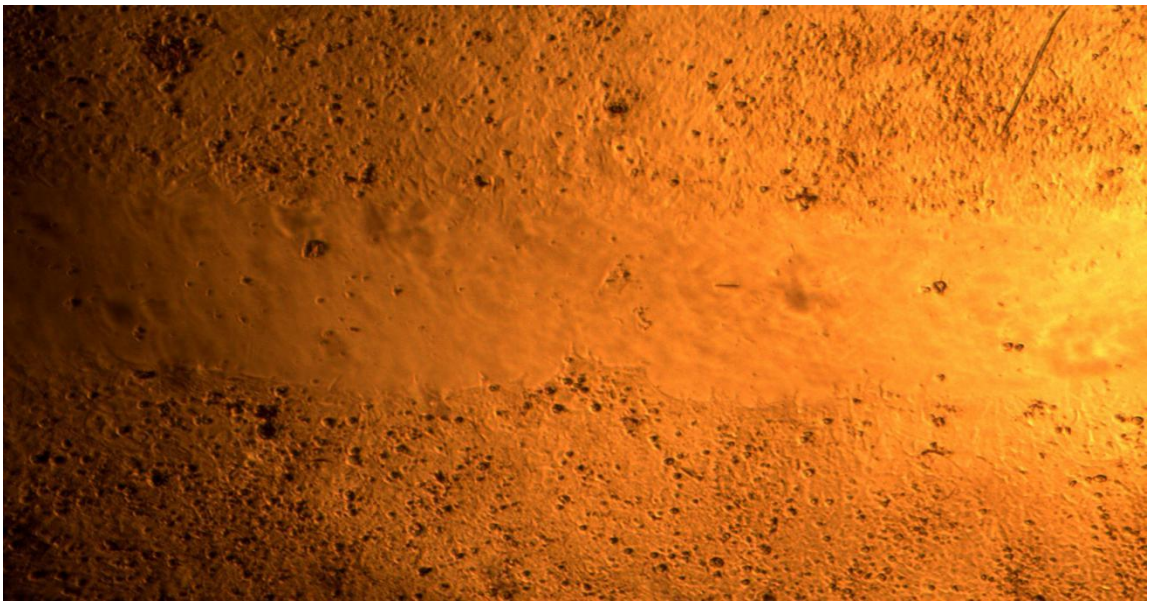


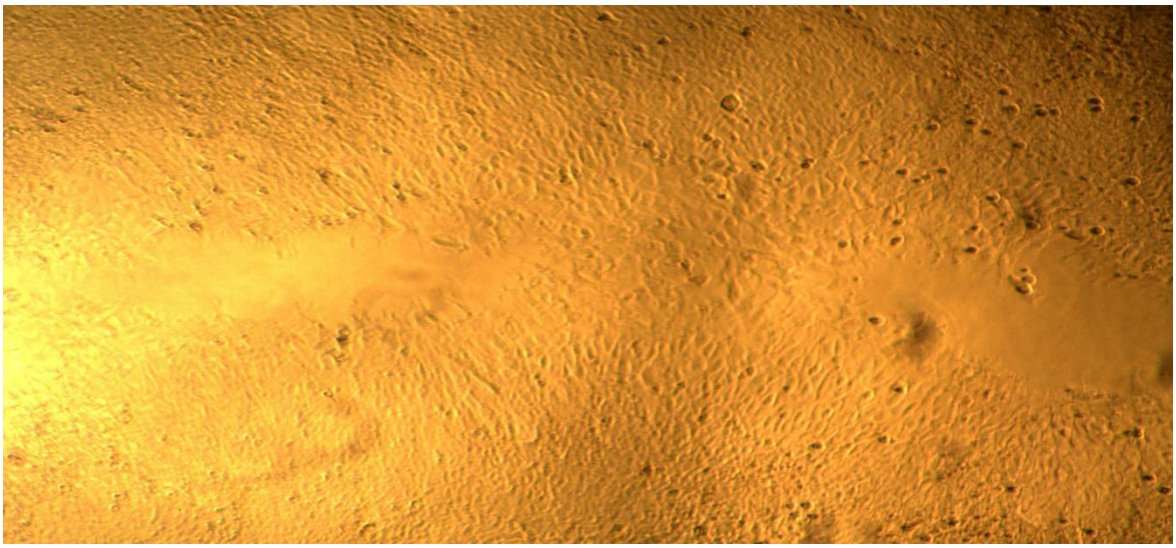
CONTROL AT 48 HRS

SAMPLE AT 48 HRS

CONTROL AT 72 HRS

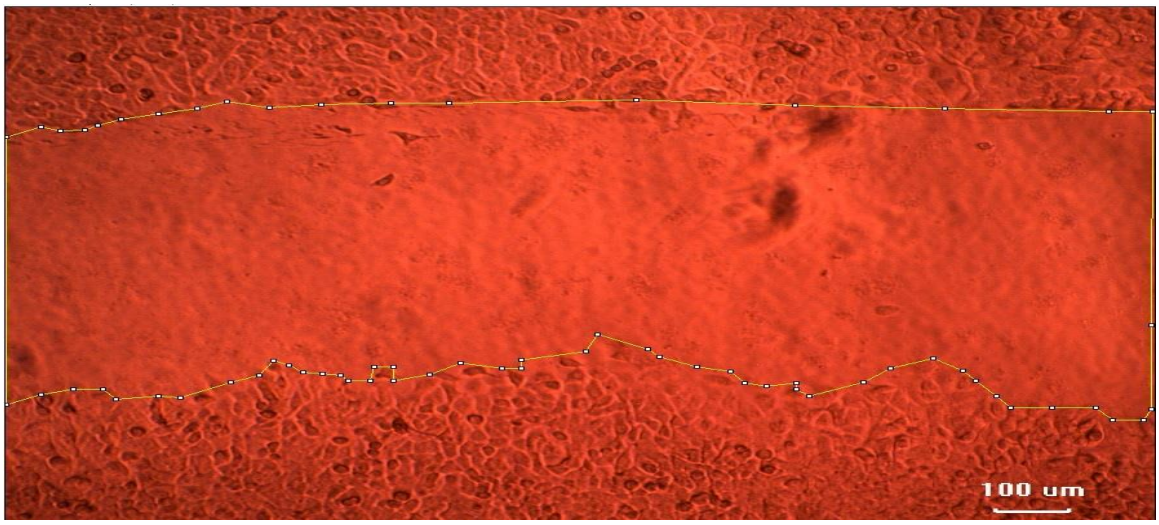
SAMPLE AT 72 HRS



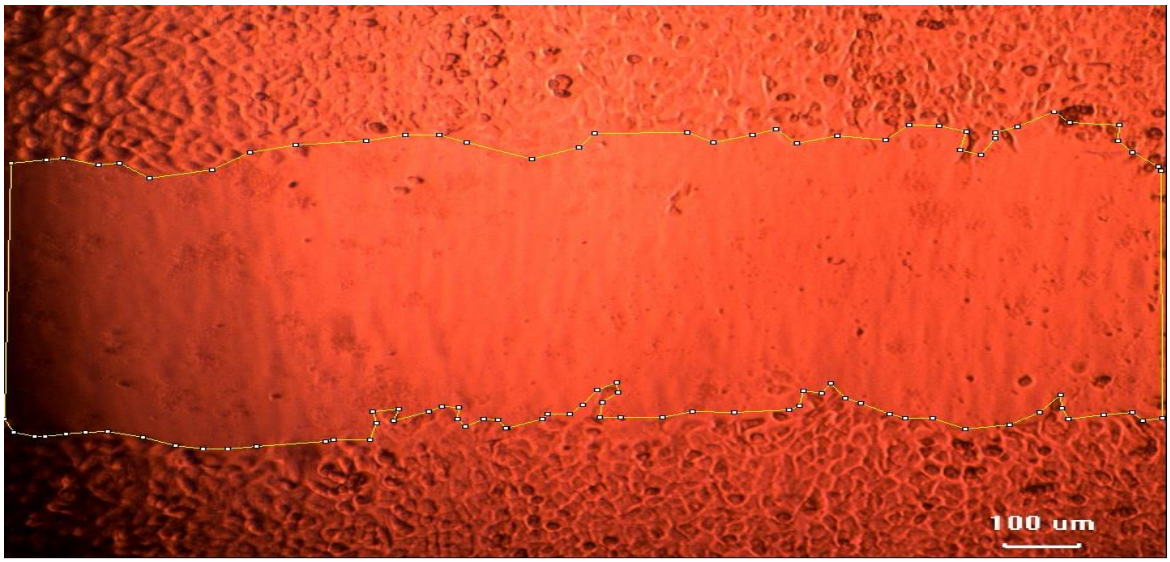


SCORED CONTROL AND SAMPLE

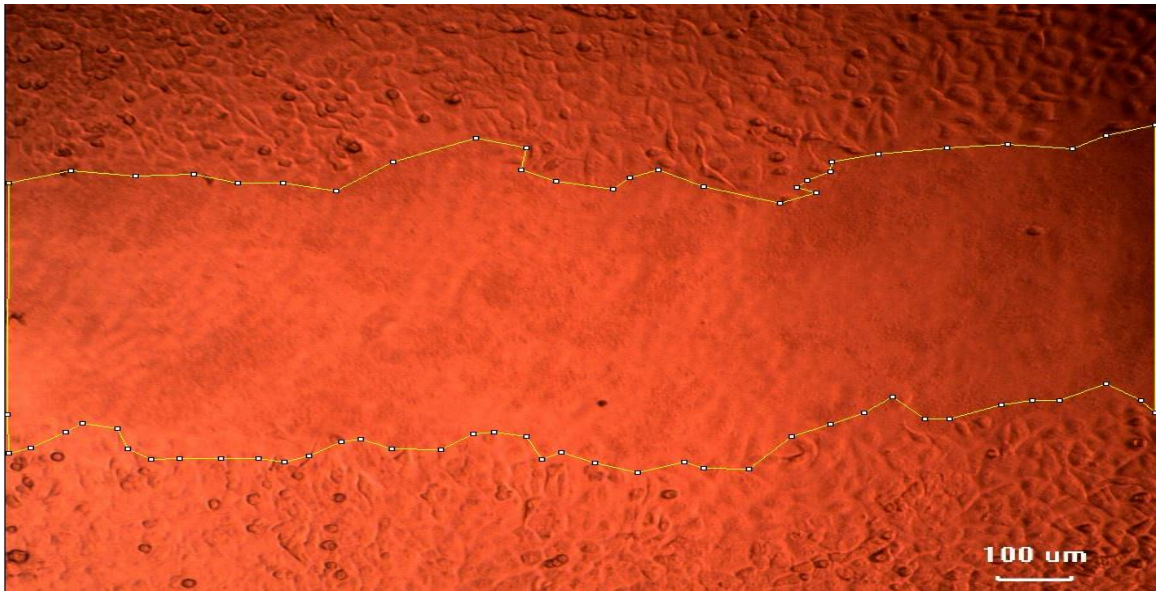
SCORED CONTROL AT 0 HOUR : The scratch is made using 1ml sterile pippete tip and represented by the dotted lines.



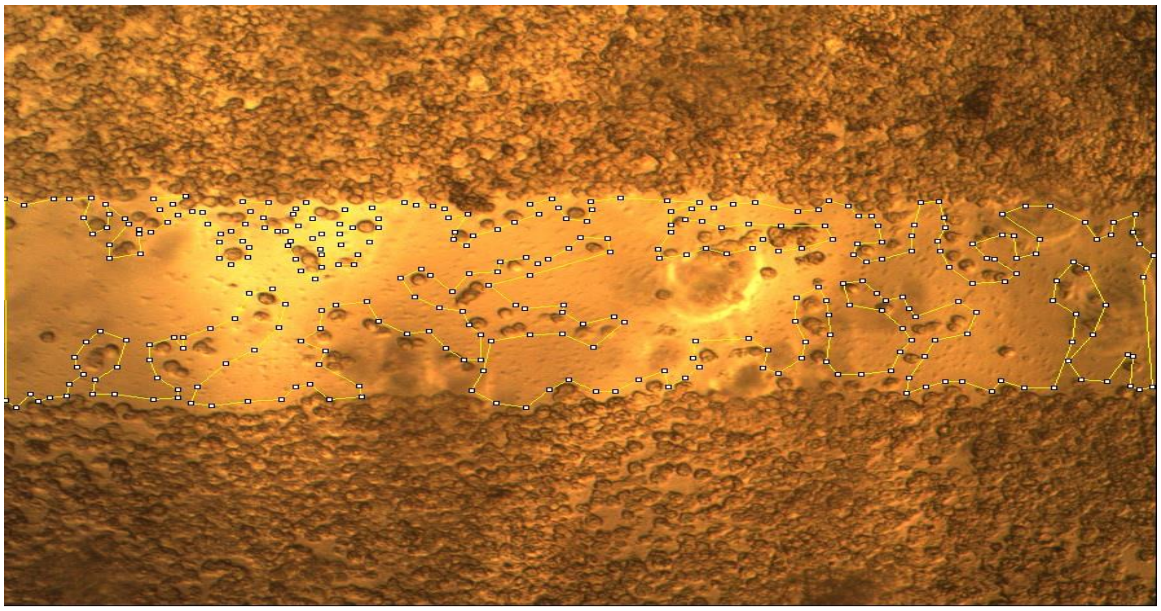
SCORED SAMPLE AT 0 HOUR : The scratch is made and treated with *Arnica* 1X , the scratch Is represented by dotted lines.



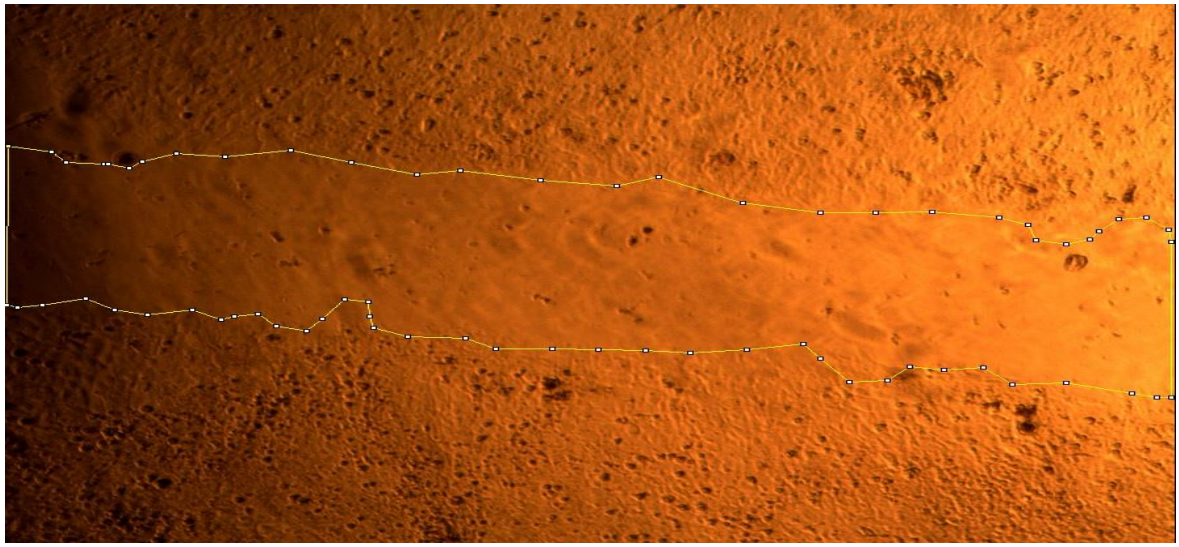
SCORED CONTROL AT 24 HOURS : At 24 hours the wound is showing a small progress in healing ,the scratch which was created is showing some reduction in size.



SCORED SAMPLE AT 24 HOURS : wound closure is taking place in this sample more rapidly In comparison with control set at 24 hours.



SCORED CONTROL AT 48 HOURS : progression of healing is slow, not much change is being observed

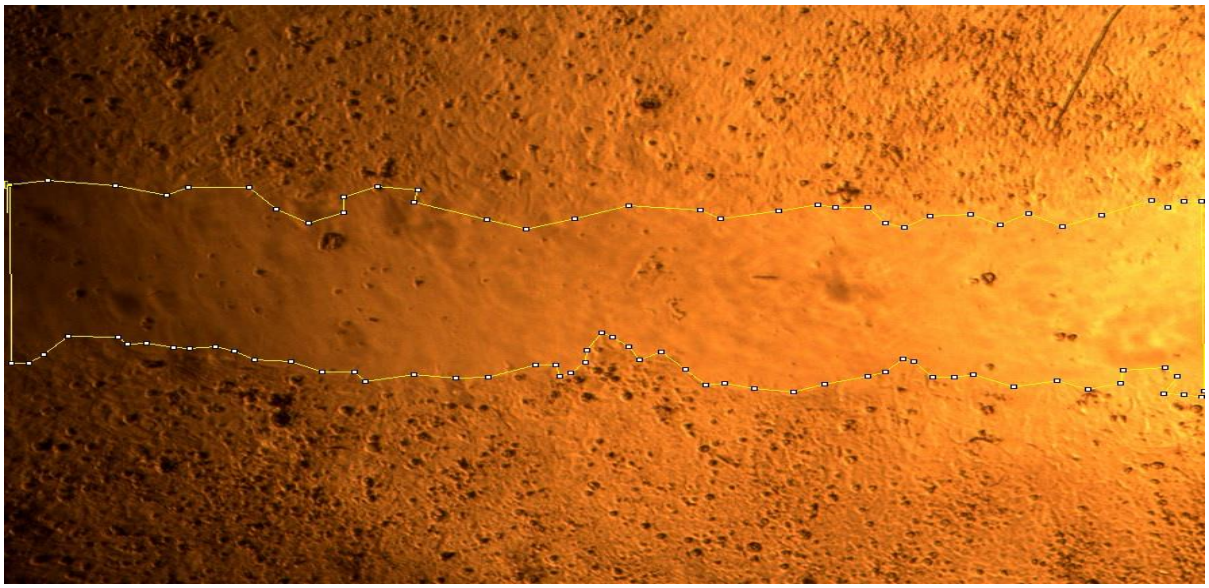


SCORED SAMPLE AT 48 HOURS : The proliferation of the wound is rapid in comparison with the

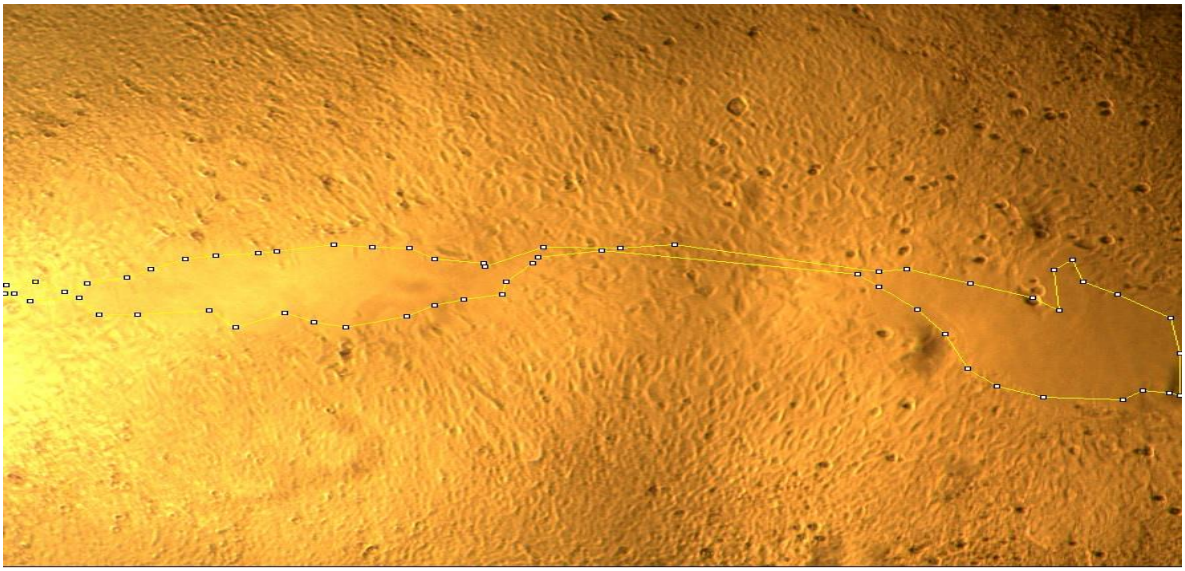
Control set at 48 hours. The scratch is showing reduction in size which is outlined by the dotted lines.

SCORED CONTROL AT 72 HOURS : The scratch which was created is slow in healing and not much

progression of healing is observed.

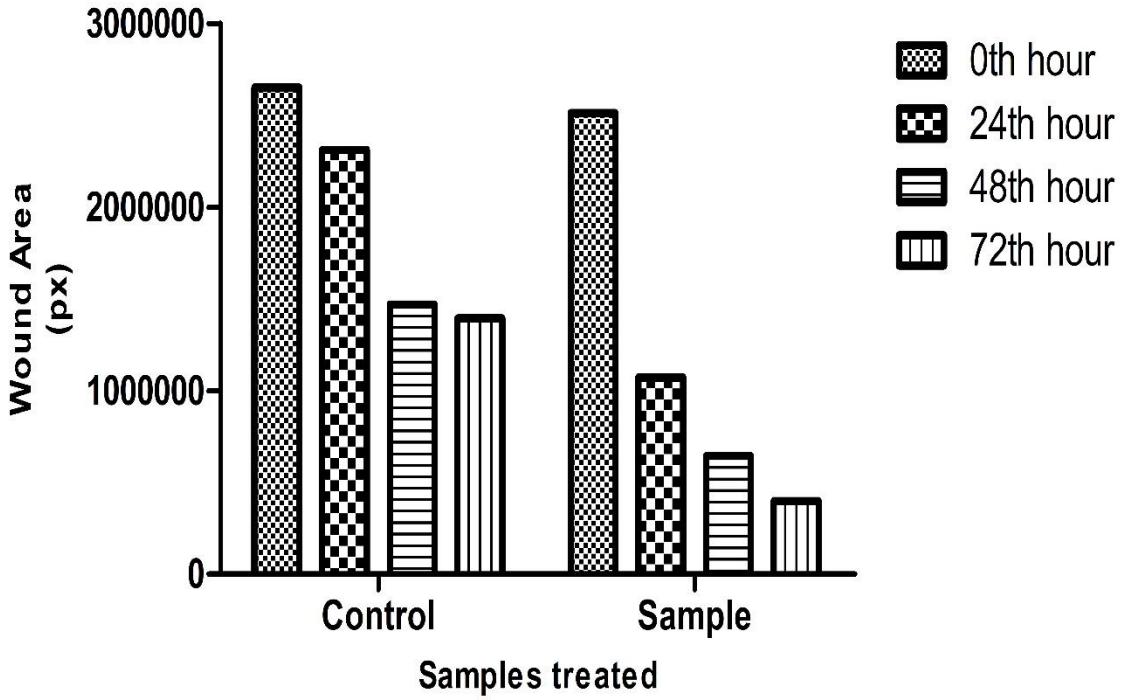


SCORED SAMPLE AT 72 HOURS : Almost complete closure of the scratch which was created is been observed **.** Narrowing of wound is represented by dotted lines



GRAPHICAL REPRESENTATION :

Time	CONTROL (Wound area in px) Approx values	SAMPLE (Wound area in px) Approx values
0^{th} Hour	2700000	2670000
24^{th} Hour	2400000	1055000
48^{th} Hour	1400000	520000
72th Hour	1350000	400000



DISSCUSSION :

As per the methodology, the scoring of wound closure was done in both Control set and Sample set at 0 hour, 24 hours, 48 hours and 72 hours. The images of wound closure are measured in terms of using MRI scan analysis J software . The comparison of both Control set and Sample set were done at 0 hour, 24 hours, 48 hours and 72 hours successively as shown above .The

wound closure is represented using dotted lines. And it is evident that the scoring sample at 72 hours is showing rapid healing When compared with the Control set at 72 hours. Here, **ARNICA 1X** is been used in the sample set. From these observations, the wound healing capacity of *Arnica montana* 1X is been observed and it is clearing the objectives of the study.

An article which was based on in vitro study of wound healing done by *Katyakyini muniady , Siva pragasam gothai,S.Suresh Kumar Govidasamy chandramohan* on the topic **"In vitro wound healing potential of stem extract of the Alternanthera sessilis .** It was demonstrated that the ethanolic extract of the stem part of A. Sessilis was effective in enriching wound closure progress in Normal and diabetic fibroblastic cells and Keratinocytes.**"**[40]

LIMITATIONS :

Due to the COVID -19 pandemic situation , the import and export of the cells were not possible. This wound healing study was planned to be conducted in HDF cell lines but due to the lack of availability of this cells. The research was conducted in L929 cell lines.

RECOMMENDATIONS :

I would like to do this invitro wound healing research study using higher potencies of Arnica montana and also in different cell lines.

CONCLUSION :

The result of the study confirms that the Homeopathic Arnica montana is effective in increasing the wound healing capacity . It is reinstate that proving of Homeopathic Pioneers to be true and useful. From this study, it is evident that Arnica 1 X can be used in healing of acute wound. The

bioactive components of Arnica helps in wound healing. The study was helpful to find out the efficacy of homeopathic medications in promoting cell proliferation which helps in wound healing . It highlights the action of homeopathic remedies in acute sphere of Injuries.

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