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Evaluation Of Wound Healing Activity Of Ethanolic Extract Of Flower Of Calotropis Gigantea Linn

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Abstract:- The Ethanolic Extract of *Calotropis Gigantea* Linn. (Family of Apocynaceae) Flower were tested for Wound Healing Activity in Healthy Wister albino rats using Wound Healing Evaluation Parameters Skin irritation test, Measurement of Wound Contraction and Epithelialization Period, Measurement of Tensile. Strength Statistic healing activity by using Incision model and Excision model. The dose of extract ointment 2% and 5% was applied for 10 days. The result of ethanolic extract of *calotropi sgigentea linn* possess the wound healing activity as evidenced by its significant effects by Excision wound and Incision wound in Rats.

Keywords :- Wound, Providon Iodine, ointment, Herbal Medicinal

Introduction:-

Herbal Medicinal Plant:- The term medicinal plants include various types of plants used in herbalism and some of these plants have some medicinal activities. Medicinal plants are the "backbone" of traditional medicine, which suggests quite 3.3 billion people within the less developed countries consume medicinal plants on a daily basis. Medicinal plants are the rich resources of ingredients which will be utilized in the event and synthesis of medicine. Further that these plants play a crucial role within the development of human cultures round the whole world. India features a rich diversity of plant species in an in depth sort of ecosystems. Around 17.000 species of upper plants, of which approximately 8.000 species are considered medicinal which are employed by village tribal communities like the Ayurveda [1]

During the past decade, traditional systems of drugs became a topic of worldwide importance. Present evaluations suggest that, in many rising countries, an outsized section of the population depends on traditional practitioners and medicinal plants to satisfy the requirements of primary health care. Albeit modern medicines are available in these countries, herbal medicines (phytomedicines) have frequently maintained popularity for historical and cultural reasons. [2]

Raw materials of medicinal plants often used because the extraction of active ingredients that are utilized in the synthesis of various drugs.[3] As defined by WHO, health may be a state of complete physical, mental, and social wellbeing and not merely the absence of disease or infirmity. Medicinal plants can make a crucial influence on the WHO goal to make sure , by the year2000, thateachone peoples, worldwide.[4]

The development and commercialization of medicinal plant-based industries within the rising countries are reliant upon the supply of amenities and knowledge regarding upstream and downstream bioprocessing, extraction, purification, and marketing of the economic potential of medicinal plants.[5]

Plants in traditional medicines:-Four thousand years ago, the medical knowledge of the Indian subcontinent was termed as Ayurveda. Ayurveda remains an important system of medicine and drug therapy in India. Plant alkaloids are the primary active ingredients of Ayurvedic drugs. Today the pharmacologically active ingredients of many Ayurvedic medicines are being identified and their usefulness in drug therapy being determined. It is roughly estimated that of the discovered 17,000 species, nearly 3,000 species are used in medicinal field. The therapeutic action of important medicinal plants and its parts used. The indigenous systems of medicine in India are reported in under supplementary material. The pharmacological properties of some Ayurvedic crude drugs support for their therapeutic claims.[6] The World Environment has originated from French word "environ" mean surroundings includes each and everything outside the plant, which influences directly or indirectly the life of the plant. This is an integral part of the earth's ecosystem. Each component of the environment is called environmental factor. Plants grow best within certain ranges of various factors includes temperature, soil moisture, soil nutrients, light, air pollutants, humidity, soil structure and PSH. Although these factors affect all plants are frequently grown or kept in cultural particles (fertilization, irrigation, spraying with pesticides) that may affect their growth considerably.[7]

Future of Medicinal Plants:-

Medicinal plants have a promising future because there are about half million plants around the world, and most of them their medical activities have not investigate yet, and their medical activities could be decisive in the treatment of present or future studies.

Characteristics of Medicinal Plants:- Synergic medicine – The ingredients of plants all interact simultaneously, so their uses can complement or damage others or neutralize their possible negative effects. Support of official medicineIn the treatment of complex cases like cancer diseases the components of the plants proved to be very effective.

Preventive medicine – It has been proven that the component of the plants also characterize by their ability to prevent the appearance of some diseases. This will help to reduce the use of the chemical remedies which will be used when the disease is already present.

Significances of Medicinal Plants to Human Being:- Medicinal plants have played an essential role in the development of human culture, for example religions and different ceremonies. Many of the of the modern medicines are produced indirectly from medicinal plants, for example aspirin. Many food crops have medicinal effects, for example garlic. Medicinal plants are resources of new drugs. It is estimated there are more than 250,000 flower plant species. Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons. Cultivation and preservation of medicinal plants protect biological diversity, for example metabolic engineering of plants. The medicinal effects of plants are due to metabolites especially secondary compounds produced by plant species. Plant metabolites include: primary metabolites and secondary metabolites Phytotherapy is the use of plants or plant extracts for medicinal purposes (especially plants that are not part of the normal diet). Phytochemistry is the study of phytochemicals produced in plants, describing the isolation, purification, identification, and structure of the large number of secondary metabolic compounds found in plants.

Plant Primary Metabolites:- Organic compounds produced in the plant kingdom have metabolic functions essential for plant growth and development produced in every plant. Include carbohydrates, amino acids, nucleotides, fatty acids, steroids.

Plant Secondary Metabolites :- Organic compounds produced in plant kingdom Don"t have apparent functions involved in plant families, in specific groups of plant families or in specific tissues, cells or developmental stages throughout plant development. Include terpenoid, special terpenoids, special nitrogen metabolite (including, on-protein amino acids, amines, cyanogeic glycosides, glucosinolates and alkaloids), and phenolics.[8]

Wound :- Wound is defined simply as the disruption of the cellular and anatomic continuity of a tissue Wound may be produced by physical, chemical, thermal, microbial or immunological insult to the tissue.[9] Wound healing is the dynamic process take place by regeneration or repair of broken tissue.[10] Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers. It consists of four continuous, overlapping, and precisely programmed phases. Wound healing is a biological process that is initiated by trauma and often terminated by scar information. Thus healing is essentially a survival mechanism and represents an attempt to maintain normal anatomical structure and functions. [11] In a normal state wound healed by the various process, which is fundamentally a connective tissue response, initial stage of this process involves an acute inflammatory phase followed by the synthesis of collagen and extracellular macromolecules which later form a scar.[12] The process of wound healing occurs in different phases such as coagulation, Epithelization, granulation, collagen formation and tissue remolding. Animal wound healing models are important biological tools to understand the basic process of tissue repair and to develop and validate strategies for the treatment of wounds. Wound healing in human beings have many unique aspects that related to physiology, age, environmental factors, etc. but the opportunities to carry clinical experiments. To understand the mechanism and to formulate therapy for wound healing are limited. [13] The process of wound healing also gets affected by other diseases such as diabetes etc., antineoplastic drugs and antibiotics may also interfere. Although animal wound healing models are an imperfect reflection of wound healing processes in human beings and its clinical challenges, these models continue to be crucial tools for the development of new strategic and approaches for therapy of wound healing. [14]

Classification of Wound:- Wounds may be classified by several methods; their etiology, location, type of injury or presenting symptoms, wound depth, and tissue loss or clinical appearance of the wound. Wounds are classified as open and closed wound on the underlying cause of wound creation and acute and chronic wounds passed on the physiology of wound healing.

Open wounds:- In this case blood escapes the body and bleeding is visible. It is further classified as Incised wound, Laceration or tear wound, Abrasions or superficial wounds, Puncture wounds, Penetration wounds, and gunshot wounds.

Closed wounds:- escapes the circulatory In closed wounds blood system but remains in the body. It includes Contusion or bruises, heamato mass or blood tumor, Crush injury ,etc.

- **Acute wounds:-** An acute wound is a tissue injury that normally precedes through an orderly and timely reparative process those results in sustained restoration of anatomic and functional integrity. Acute wounds are usually caused by cuts or surgical incisions and complete the wound healing process within the expected time.
- **Chronic wounds:-**Chronic wounds are wounds that have failed to progress through the normal stages of healing and therefore enter a state of pathologic inflammation chronic wounds either require a prolonged time to heal or recur frequently. [15]

Two types of factor influencing the wound healing

Local factors

- Infection by tissue organization which delay healing
- Poor blood supply which shows healing
- Movement of the affected part of delay healing
- Exposure to ionizing radiation delay granulation
- Exposure to ultraviolet light facilities healing
- Foreign bodies including sutures interfere in healing

Systematic factors

- Wound healing is rapid in young and slow in aged people
- Nutritional deficiency of vitamin C and zinc delay healing
- Heamatological abnormalities also affects healing
- Diabetics are more prone to infection and hence delay healing
- Administration of glucocorticoids (anti-inflammatory) delay healing. [16]

Signs and Symptoms of Wounds: -

Wounds may present with the following signs and symptoms

- Chronic pain or completely painless
- Signs of inflammation (swelling, redness, heat, pain and loss of functions)
- Signs of infection (pus drainage, discharge, bad odor and dead tissue)
- New numbness and dullness (signs of nerve damage)Fever and/or chills (signs
- of progressively worsening infection that can be limb-threatening or even life- threatening).[17]

General process of wound repair:-Wound healing is a process by which tissue regeneration occurs. It is a simple, dynamic process of restoring integrity and tissue layer, which involves an array of inter related and concomitant events. [18] The process of wound repair differs little from one type to another and is generally independent of the form of injury. Although the different steps in the wound healing process occurs in a continuous, integrated manner, it is convenient to divide the overall process into three overlapping phases and several natural components for descriptive purpose

Tissue Repair Phases and Time Scale Inflammatory Phase (0-5 Day):-

The inflammatory response is initiated at the moment of injury. Surgical or traumatic wounds disrupt the tissue shape and architecture and cause hemorrhage. Initially, blood fills the wound and exposure of this blood to collagen in the wound leads to platelet deregulation and activation of the Hageman factor. This is turn sets into motion a number of biological amplification system including the complement in and clotting cascades and plasma in a generation. This condition serves to amplify the original injury signal and lead not only to clot formation, which unites the wound edges, but also to the accumulation of a number of mitogen and chemo attractants at the site of the wound. Production of both kinins and prostaglandins leads to vasodilatation and increased small vessel permeability in the region of the wound. This results in edema in the area of the injury. Within 6 hours, circulating immune cells start to appear in the wound. Poly morpho nuclear neutrophils (PMN) are the first blood leucocytes to enter the wound sites. Their main functions appear to be phagocytes of the bacteria, which have been introduced into the wound during injury. In the absence of infection, PMNs have a relatively short life span in the wound and their numbers decrease rapidly after the third day. The next cellular, immune component enter to the wound is macrophages. These macrophages have a much longer life span than the PMN and persist in the wound until healing is complete.

Proliferative Phase:- (3-14days):- In the absence of significant infection or contamination, the inflammatory phase is short, and after the wound has been successfully cleared of devitalized and unwanted material it gives away to the proliferative phase of healing. Granulation tissue consists of a combination of cellular elements, including fibroblasts and inflammatory cells. Fibroblasts first appear in significant numbers in the wound on the third day post-injury and achieve peak numbers on the seventh day. This rapid expansion in the fibroblast population at the wound site occurs via a combination of proliferation and migration. Fibroblasts are the primary synthetic element in the repair process and are responsible for production the of the majority of structural proteins used during tissue reconstruction. Capillary buds sprout from blood vessels adjacent to the wound and extend into the wound space. While these events are proceeding deep in the wound, restoration of epithelial integrity is taking place at the wound surface. Re-epithelization is complete in less than 48 hours in the case of approximated incised wound, but may take substantially longer time in the case of larger wounds where there is a significant tissue defect.

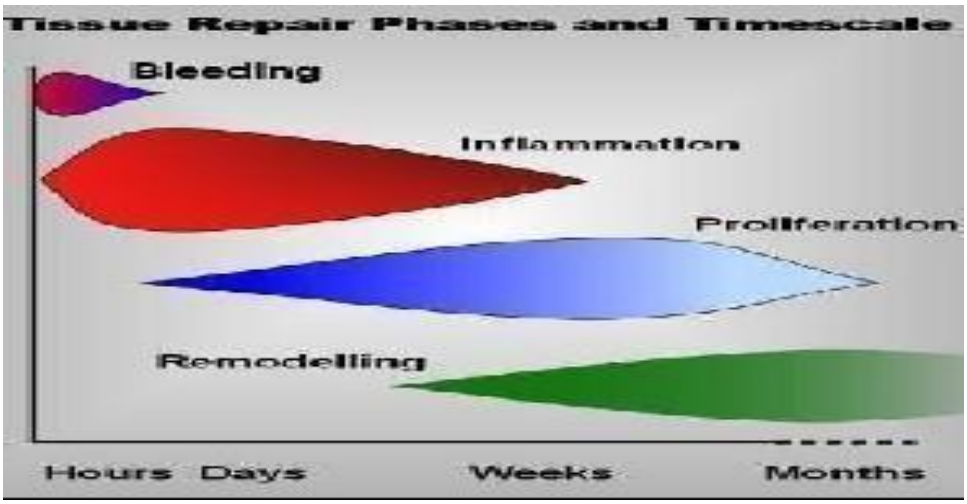


Figure No 1- Tissue Repair Phases

Maturation Phase (Day 7 to 1 year):-Collagen remodeling during the maturation phase depend on continuous collagen synthesis. Some of the growth factors that stimulate the synthesis of collagen and other connective tissue molecules also modulate the synthesis of activation of metalloproteinase, enzymes that serve to degrade these epithelial cell migrations (ECM) components. The net result of ECM synthesis versus degradation result in the remodeling of the connective tissue framework-an important feature of both chronic inflammation and wound healing. Collagen rapidly becomes the predominant constituents of the matrix. The initially randomly distributed. collagen fibers become cross linked and aggregated into fibrillar bundles, which the gradually provide the healing tissue with increasing stiffness and tensile strength. After a 5-day lag period, which corresponds to early granulation tissue formation and a matrix largely composed of fibronectin and hyaluronic acid; their remodeling during scar formation is dependent on both continued collagen synthesis and collagen enzymes. The high rate of collagen synthesis within the wound returns to normal tissue levels by 6-12 months, while the active remodeling of the scar continues for up to 1year after If left uncontrolled for a long time, or if the diabetic patient fail to adapt their lifestyles to manage the injury .[19]

Animal Models: - Animal model for Evaluation of Wound Healing Activity Generally these types of wound models are used to study the wound healing property.

- Excision wound model Incision wound
- Burn wound model
- Dead space wound model
- Ear wound models
- Superficial wound model

1. **Excision Wound Model:-**In this type of model circular wounds of about 2 cm are made on depilated dorsal thoracic region of rats under aseptic conditions and should be observed throughout the study. The area of wounds should be measured immediately by placing a transparent polythene graph paper over the wound and then tracing the area of the wound on it. This is taken as initial wound area reading. Drugs are to be applied test and standard as well and observations are to be taken on the alternate post wounding days by tracing the wound area and percentage area of wound closure is calculated.[20]
2. **Incision Wound Model: -** In this model cuts are made in the skin of the animal after giving anesthesia with anesthetic ether. Two para-vertebral long incision of 6 cm length made through the skin and cutaneous muscles at distance about 1.5 cm from the midline on each side of the depilated back of the rats. After the skin incision made, the parted skin kept together and should be stitched at 0.5 cm intervals continuously and tightly by using suture thread (No. 000) and a curved needle (No. 11). When the wounds get cured thoroughly, the sutures are to be removed on day 9 and tensile strength of thehealed wound should be measured on day 10 by continuous and constant water flow technique.[21]

3. **Burn Wound Model:-**Partial thickness burn wounds are created on overnight starved animals. Under anesthesia, pentobarbitone (30 mg/kg, i.p.), hot molten wax at 800C is poured into a cylinder of 300 mm2 circular opening placed on the shaven back of the animal until wax get solidified. Solidification of wax normally takes 10-12 minutes. Cylinder is now removed that leave the demarked partial thickness circular burn model.[22,23]
4. **Dead Space Wound Model:-**In this type of model the physical changes in the granuloma tissue. The subcutaneous dead space wounds are to be created in the region of axilla and groin by making a pouch through asmall nick in the skin. The cylindrical grass piths measuring 2.5 cm in length and 0.3 cm in the diameter are introduced in to the pouch. Each animal receive 2 grass piths in different locations. Implantations of grass pith induce granuloma formation. The wounds are sutured and mopped with an alcoholic swab. Granulomas surrounding the grass piths were excised and slit open. The tensile strength of tissue piece (obtained by trimming the rectangular strip of granular tissue) measuring about 15 mm in length and 8 mm width was determined on 10th post wounding day by adopting continuous water flow technique. [24,25,26]
5. **Ear Wound Models:-**In cases where human healing occurs entirely by re epithelialisation and granulation formation without contraction, the ear wound model may be more suitable because it heals without contraction and has a vascular cartilage wound bed.[27]

Diagnosis of wound infection:- Understanding the risk factors, and the signs and symptoms of wound infection is imperative for health professionals. The presumptive diagnosis of wound infection is principally based on the clinician‟s assessment of the individual (host), the wound and periwound tissue, and host responses such as systemic inflammatory response or sepsis. Comprehensive assessment for wound infection aids early detection and timely treatment.

Risk of Infection:- Characteristics of the individual, their wound and the wound environment can contribute to the development of infection in a wound. The type of wound (i.e. acute or chronic) contributes to infection risk, and a variety of additional factors associated with the operative procedure increase the risk for infection in surgical wounds. In most cases, development of wound infection is multifactorial and occurs when cumulative risk factors overwhelm the host's defense system.[28,29]

Characteristics of the wound:-

- Acute wounds
- Contaminated or dirty wounds
- Trauma with delayed treatment
- Pre-existing infection or sepsis
- Spillage from gastro-intestinal tract
- Penetrating wounds over 4 hours Inappropriate hair removal
- Operative factors (e.g. long surgical procedure, hypothermia, blood transfusion)[30]
- Chronic wounds
- Degree of chronicity/duration of wound
- Large wound area
- Deep wound
- Anatomically located near a site of potential contamination (e.g. perineum or sacrum)[31]

Treatment of Wounds:- The best treatment is prevention since medical treatment for wounds provides limited help. If a wound occurs, treatment can include:-

- Keeping all wounds clean and properly dressed
- Antibiotics (for infected wounds or as a preventive measure for wounds at risk of getting infected) the healthy tissue to heal and regenerate.
- Referral to a podiatrist or a wound care center (for patients with calluses, corns, hammertoes, bunions, toenail problems ,or chronic non-healing ulcers)
- Limb amputation (to save as much of a limb as possible when there is a serious infection)

- Daily inspection and cleaning of your extremities as they are more prone to ulcers and injuries. Carefully trimming the nails with a safe nail trimmer refer to an expert if the patient requires extra care or if there are skin lesions.
- Always wear dry, clean socks to help protect your feet, and never walk barefoot (avoid tight socks that may reduce the blood circulation to the feet). [32]

Drugs used for wound healing:-

- **Pentoxifylline,** a methylxanthine that improves perfusion of peripheral vascular beds, is useful in patients with ulcers secondary to peripheral vascular disease. It improves capillary Micro circulation by decreasing blood viscosity and reducing platelet aggregation. It may also inhibit tumor necrosis factor an inflammatory cytokine involved in non-healing wounds. Although mainly indicated for ulcers secondary to peripheral vascular disease, pentoxifylline is useful in patients with venous leg ulcer who cannot tolerate compression or in whom compression is ineffective. It may also be beneficial in rare but complex ulcers such as sickle cell ulcers, livedoid vasculitis and necrobiosis lipoidicloprost, a prostacyclin analogue, is an established treatment for intermittent claudication, severe limb ischaemia, and prevention of imminent gangrene, and to reduce the pain and clinical symptoms associated with Raynaud‟s disease. Intravenous iloprost is useful in promoting healing of arterial ulcers and vasculitic ulcers secondary to connective tissue diseases such as rheumatoid arthritis and scleroderma.
- **Antimicrobials** including iodine based preparations and silver releasing agents are used to treat infected wounds (there may be a dose dependent effect). Antimicrobial agents target bacteria at several level (cell membrane, cytoplasmic organelle, and nucleic acid), thus mini mizing bacterial resistance. They can be used either on their own or in conjunction with systemic antibiotics. The many silver releasing agents, in dressing form, aim to deliver sustained doses of silver to the wound. In addition to the micro bicidal effect of silver on common wound contaminants, silver may also be effective against methicillin resistant Staphylococcus aureus (MRSA).
- **Glyceryl trinitrate,** a nitric oxide donor, is effective in the management of chronic anal fissures when applied topically as 0.2% ointment. Nitric oxide causes vasodilatation, and uncontrolled studies have suggested a potential role for glyceryl trinitrate in treating chronic wounds of ischaemicaetiology, including vasculiticulcers.Headache, sometimes troublesome, is the most commonly encountered side effect with glyceryltrinitrate: lower concentrations may avoid this side effect. Calcium antagonists such as diltiazem and nifedipine are useful in treating vasculitic ulcers secondary to Raynaud‟s disease and connective tissue diseases. In Raynaud‟s disease, they restore blood flow to the digits and thus are useful in treating ulcers and the prevention of necrosis in the extremitie Systemic corticosteroids are useful in treating ulcers secondary to connective tissue diseases, including rheumatoid arthritis, scleroderma, and other vasculitic disorders. They promote healing by attenuating the excessive inflammatory response. Long term use of corticosteroids, however, may have a detrimental effect on healing. Patients taking long term, high dose steroids should be offered bone protection with bisphosphonates.
- **Zinc** an antioxidant, used in a paste bandage may be useful in treating infected leg ulcers. Oral zinc sulphate treatment may be beneficial in patients with chronic ulcers who have low serum zinc levels.
- **Phenytoin** applied topically, promotes wound healing by inhibiting the enzyme collagenase. It is effective in some low grade pressure ulcers and trophic ulcers due to leprosy. The possibility of systemic absorption and toxicity has limited its use.

- **Retinoids** (derived from vitamin A) have an impact on wound healing through their effects on angiogenesis, collagen synthesis, and epithilization. Vitamin A is necessary for normal epidermal maintenance. Although the value of retinoids in chronic wounds is unclear, topical tretinoin (0.050.1%) has been shown to accelerate re-epithilization of dermabraded and chemically peeled wounds in humans, and partial and full thickness wounds in animal models.

- **Analgesics** are needed for many ulcers. They may range from simple analgesics to opiates in individuals whose the pain is severe. Pain from ulcers associated with neuropathy may benefit from treatment with certain tricyclic antidepressants (such as amitriptyline) or antiepileptic drugs (such as gabapentin). Intractable pain may necessitate intervention by specialist pain management teams.[33]

Natural wound healing products:-

- **Aloe Vera** commonly known as Kumari is a perennial herb belonging to liliaceae family. It has short stem and shallow root system with large fleshy, rosettes sessile leaves. It can be seen as wild herb in dried parts of India. Today aloe vera gel is an active ingredient in hundreds of skin lotions, sun blocks and Cosmetics. Aloe Vera is an excellent remedy for minor burns, cuts and sunburns. Both juice and aqueous extract from the leaves shows significant healing properties. It is also reported that it not only speeds up healing but also prevents injured surface from getting infected. Aloe Vera was studied for burn wounds by routine dressing by A. Vera extract every 3rd day in chemically produced burn on healing subjects. The wound healing time and bacteriological control was significantly in Aloe group. The working mechanism of Aloe Vera for wound healing is reported to be enhancing collages turnover rate and increased level of lysyl oxidase (responsible for cross linking of newly synthesized collage.. Beside wound healing effect, it is reported to have ulcer healing property (when taken internally) and protective action on skin.[34]
- **Gingko biloba** It belongs to the family Gingkoaceae and commonly known as Kew tree. It is widely planted in Korea and China. Propagation type includes seeds and vegetative methods.6 Gingko biloba has found to have significant activity against both dead space and excision wound models in male rats. A 50 mg/kg of dose has significantly promoted the breaking strength and hydroxyproline content of granulation issue in dead space wounds and in case of excision wound model, it is found to shorten the epithelization period . It is also reported that the activity of G.B. is due to its high amino acid content which absorbs rapidly in blood stream and in combination with vitamins; they provide essential nutrients to the wound area to promote healing. Beyond wound healing, it is used as an anti-inflammatory and antiallergic agent in ancient Chinese medicine.[35]
- **Centella asiatica** is a small trailing herb bearing white to reddish flowers which normally grows widely in the wet places. Commonly it is known as Brahmi and it is propagated by seeds and vegetable propagation. Clinical studies of the formulation (ointment, cream & gels) of aqueous extracts of Centella asiatica reports that, when it is applied topically thrice daily for 24 days on open wound site. The treated wound epithelized faster and the rate of wound contraction was higher as compared to control wound. Gel formulation produce better results as compared to other two formulations . It is reported that the active constituents responsible for the activities of Centella asiatica are found to be asiaticosides and madicassoides[36]
- **Nelumba nucifera** belonging to family Nymphacaceae is called as Kamal in Hindi and Lotus in English. It is perineal aquatic herb embedded in mud with large flower. It is commonly cultivated in ponds and swamps by using rhizomes for propagation. Nelumba nucifera is very common among natural and traditional healers. They collect leaves and rhizomes, dry them and burn to produce ash which acts as wound healer. But now it is reported that the methonolic extract of rhizomes of Nelumba nucifera in the formulation of ointment is effective in different types of wound model in rats. The effect were studied on excision wound model, incision wound model and dead space wound model by using two different concentrations i.e. 5 % w/w & 10 % w/w ointment. The ointment in both the concentration responded significantly in all the wound models. Both the extract ointment shows the significant effect in respect with wound contracting activity, wound closer time, tensile strength, regeneration of tissue at the wound site and lysyl oxidase activity. The effects produced are comparable to that of standard drug. [37]
- **Tulsi**This extract is derived from the plant of Ocimum sanctum belonging to family Labiatae. It has been widely grown throughout the world and commonly cultivated in gardens. Traditionally Ocimum sanctum is used in malarial fevers, gastric disorders andin hepatic infections. Ocimum sanctum leaves are alsoused in bronchitis, ringworm and other cutaneous diseases and earache. The leaves are used as a nerve tonic and to sharpen memory. Ocimum sanctum leaves are abundant in tannins like gallic acid, chlorogenic acid etc and also contain alkaloids, glycosides, and saponins along with the volatile oil. The major active constituent of Holy basil leaves includeurosolic acid. It contains 70% eugenol, carvenol and eugenol-methyl-ether. [38]
- **Eucalyptus** is also called Dinkum Oil. This oil is obtained by steam distillation of fresh leaves of Eucalyptus globules belonging to family Myrtaceae. It is indigenous to Australia and Tasmania. It is cultivated in United States, Spain, Portugal, and in India. It contains cineole, also known as eucalyptol. It also contains pinene, camphene, and phellandrene, citronellal, geranyl acetate. In skin care it can be used for burns, blisters, herpes, cuts, wounds, skin infections and insect bites. It can furthermore boost the immune system and is helpful in cases of chicken pox, colds, flu and measles Oil is used as a counter irritant, an antiseptic, and expectorant. It is used to relieve cough and in chronic bronchitis in the form of inhalation. It is ingredient of several liniment s and ointments. Solution of eucalyptus oil is used as nasal drop. [39]

- **Bael** is also called a Bael fruits, Indian bael. It consists of unripe or ripe fruits of the plant knowns as Aegelmarmelos belonging to family Rutaceae. It is indigenous to India and found in Mynmar and Sri Lanka. The pulp is red in colour with mucilaginous and astringent taste. The chief constitute of drug is marmelosin which is furocoumarin. The drug also contains carbohydrates, protein, volatile oil and tanines. The pulp also contains vitamin C and vitamineA. two alkaloids Omethylhalfordional and isopentylhalfordinol have been isolated from fruits. It is used as digestive, appetizer and also used in the treatment of diarrhea and dysentery. It is also a tonic and it has a wound healing properties. [40]
- **Myrobalan** (Harde) also called Haritaki, chebulic myrobalan. It consists of dried, ripe, and fully matured fruits of Terminalia Chebula belonging to family Combretaceae. It is found in subHimalayan tracks from Ravi to West Bengal, Asam and all forest in India. It is found growing at an altitude of 1800 m. it is not cultivated and fruits are collected from wild grown forest plants. It is a tree, 15 to 25 m in height, and 1.5 to 2.5 m in diameter. It has yellowishwhite flowers in the terminal spike. It contains hydrolysable tannins which upon hydrolysis yield chebulic acid and dgalloyl glucose.it also contains chebulagic, chebulinic, ellagic and gallic acids. It is used mainly as a astringent, laxatives, stomachic and tonic, anthelmintic. Fruit plup used to cure bleeding. It is an ingredient of ayurvedic preparation Triphala‟. It is also used in piles and external ulcers. [41
- **Neem** Alcoholic extract of neem is useful in eczema, ringworm and scabies. Neem leaf extracts and oil from seeds has proven anti-microbial effect. This keeps any wound or lesion free from secondary infections by microorganisms. Clinical studies have also evealed that neem inhibits inflammation as effectively as cortisone acetate; this effect further accelerates wound healing. Neem oil contains margosic acid, glycerides of fatty acids, butyric acid and trace valeric acid. Alcoholic extract of neem is useful in eczema, ringworm and scabies. Neem leaf extracts and oil from seeds has proven antimicrobialeffect. This keeps any wound or lesion tree from secondary infections by microorganisms. Clinical studies have also revealed that neem inhibits inflammation as effectively as cortisone acetate; this effect further accelerates wound healing. [42]
- **Turmeric** is also called Indian saffron, curcuma. It consists of dried as well as fresh rhizomes of the plant known as curcuma longa belonging to family zingiberaceae. It contains not less than 4% of volatile oil. India account for as much as 90% of the total output of the world. Curcuma longa is the main species of commerce and is cultivated for its rhizomes in India, China and in Sri Lanka. India is the major grower with almost 80,000 hectors under the crop producing 1, 44,000 tons per annum. The plants are grown for 7 to 9 months after which the rhizomes are harvested, cooked, dried and then processed for powder, oleoresin and curcumin. The extraction of powder is carried out by using solvents, water or both. It contains about 5% of volatile oil, resin. Starch grains and curcuminoids which is the chief constitutes of curcumin, Volatile oil, content sesquiterpenes such as α and β pinene, α- phellandrene,camphor, zingiberene. It is used as a condiment or spices, and colouring agent, especially for ointments and creams. It is used for the detection of boric acid. Traditionally it has been proved as antiinflamatory, anticancer, antiseptic. [43]

Effect of some commonly used drugs on wound healing [44]

Class And Name Of Drugs	Effects
NSAIDs Ibuprofen	Affects inflammatory phase by inhibitingcyclo-oxygenase Production ; reduces tensile strength of wound
Corticosteroids (prednisolone)	Affects haemostatic phase by decreasing platelet adhesion; affects inflammatory phase by affecting phagocytosis; affects remodelling phase by reducing fibroblasts activity and inhibiting collagen synthesis
Antiplatelets (aspirin)	Affects haemostatic phase by inhibiting platelet aggregation; inhibits inflammation mediated by arachidonic acid metabolites
Anticoagulants Heparin	Affects haemostatic phase by its effect of fibrin formation; can leadto thrombus formation by causing thrombocytopenia (white clot syndrome)

Warfarin	Affects haemostatic phase by its effect on fibrin formation; can cause tissue necrosis and gangrene by release of athermanous plaque emboli in form of micro cholesterol crystals (blue toe syndrome)
Vasoconstrictors (nicotine, cocaine, adrenaline)	Affects proliferative phase by inhibiting neovascularization and decreasing granulation tissue formation; impairs microcirculation and increases rejection and ulcer necrosis

Table No 1:- Effect of Drugs

1.3] *CALOTROPIS GIGENTEA LINN*:-

Calotropis Gigentea is a well-known medicinal herb commonly known as has been used in Unani, Ayurveda, and Siddha system of medicine for years. ApocynaceaeJuss. Commonly called as the dogbane family, comprises 357 genera and about 5100 species of flowering plants including herbaceous or shrubby climbers. Calotropis is a succulent and xerophytic shrub or small laticiferous tree up to 2.5 m, commonly known a "milkweed" or "Crown flower" The stem usually simple and branched at the base, woody covered with a corky bark, leaves simple, opposite, sub- sessile, white and purple-colored flowers and not scented. Inflorescence is a dense, multiflowered, umbellate cyme, highly cross-pollinated through insects such as monarch butterflies, simple, follicle fruit. Following figure shows the purple and white colored flowers and follicle fruit of Calotropis found in Sri Lanka. Calotropis species are most diverse in tropical and subtropical parts of Asia and South East Asia (Bangladesh, Cambodia, Burma, China, India, Indonesia, Malaysia, Pakistan, Philippines, Sri Lanka and Thailand) and extend into temperate areas. Calotropis is a versatile tree used for different purposes .Calotropis Gigentea species are evergreen perennial shrub reaching 2.4-3 m high; barkyellowish white, furrowed, rough, corky; branches stout, terete, less or more covered with fine appraised cottony pubescence. Leaves are opposite-decussate, sessile, elliptic oblong or obviate-oblong, acute, thick and pale in green, clothed beneath and less or more above with fine cottony tomentum, about 10-20 by 3.8-10 cm, base narrow, chordate. Flowers are regular, bisexual, purple or light greenish yellow with faint odor, 3.8-5 cm dia. In umbellate lateral cymes; periodicals are much longer than the flowers, the, pedicels are overspread with cottony wool, buds ovoid, calyx divided to the base, consist 5 white sepals 4mm, ovate, acute, cottony, corolla 2 cm long, lobes of the corona 1.3cm long, broadly in 5mm at the middle, smaller than the column, slightly thickened margin, the apex rounded with two obtuse auricles just below it. Follicles are 9-10 cm in length,wide, abundant, plump ventricose, green. Green color spongy fruits consist of light brown seeds 6 x 5 mm. Ovate, flattened, arrow margined, minutely tomentose, brown; coma 2.5-3.2 cm long and the hairs at the one end. The roots are deep plump taproot with less lateral roots near surface.[45]

Figure No.2 - Flower of *Calotropis Gigantean* Linn

Figure No.3 - Plant of *Calotropis Gigantean* Linn

Plant profile Taxonomical [46]

Scientific classification	
Kingdom	Plantae
Order	Gentianales
Family	Apocynaceae
Subfamily	Asclepiadaceae
Genus	Calotropis
Species	C.Gigentea

Table No. 2: Taxonomical classification *calotropisgigentealinn*

Vernacular Names

English	Aak, Madar, Shive,Aak,SwetaAak
Hindi	Milkweed , Crown flower
French	Mercure Vegetal
Sankrit	Rak

Table No.3: Vernacular names of *calotropisgigentealinn*

Geographical Sources:- India, China, Bangladesh, Pakistan, Srilanka

Part used of plant:-Root, Leaves , Stem, Bark

Chemical Constituents:- Sterols, Resin, Alkaloids, Glycosides, Carbohydrates, Protin, Quinones, Anthraquinon, phenol, Tannins, Flavenoids

Medicinal uses:- Asthma, Abortifacient, Anti-cancer, Anthelmintic, CNS activity, Epilepsy, Eczema Expectorant, Fever, Leprosy, Migraine. Finally the result of these things useful of ethanolextract of Calotropis gigantean. The plant is purgative, anthelmintic, alexipharmic, cures leprosy, leucoderma, ulcers, tumors, piles, diseases of the spleen, the liver, and the abdomen; the juice is anthelmintic and leucoderma, tumors, ascites, diseases of the abdomen. The leaves are applied to paralyzed parts, painful joints, swellings; heal wounds. The tincture from the leaves used as antiperiodic in cases of intermittent fevers. Inflammations, tumors, rat-bite, good in ascites. The milk is bitter, heating, purgative; Laxative; cures piles. The root bark is diaphoretic; cures asthma and syphilis. The flower is sweet, bitter, anthelmintic, analgesic.[47]

Materials and Method:-

Experimental Animls:- were used Healthy Wister albino rats weighing between 150-250 gram in the present studies were procured from Bomb sniffer nachan dog trainer Indore (M.P.) India. The animals were fed with standard pellets diet and water. All the animals were acclimatized for acclimatized for a week before use and maintained at standard husbandry conditions, Room temperature27±3oC Relative humidity 65±10%, 12:12 hrs light/dark cycle and fed with standard diet and water during the study . All the protocols and the experiments were conducted in strict compliance according to ethical principles and guidelines provided by the committee for the purpose of Control and Supervision of Experiments on animals (CPCSEA). Animal experiment was performed in Charak Institute of Pharmacy, Mandleswar with under guideline, CPCSEA.

List of Instrument and chemicals:-

Drugs:- Providone Iodine Ointment USP 5% w/w (Cipla Ltd.), Mumbai -400013 India

Chemicals:- Ethanol (95% v/v) used in the present study were of general grade and were procured from Sun-Chem. Chemicals Pvt. Ltd Satna, M.P.

Names of Glassware	Volume of Glassware	Quantity of Glassware
Beaker	500ml	3
Glass rod	20mm	1
Measuring Cylinder	100ml	2
Pipette	10ml	1
Round bottom flask	250ml	2
Soxhlet Apparatus	2000ml	2
Test tube	10ml	15
Volumetric flask	100ml	5

Table No.4- List of Glasswares

Name of Instruments	Manufacture
Soxhlet Apparatus	Profit Indian An Iso 9001company
Heating Mental	Lab Hosp Corporation Mumbai
Weight Balance	Weigh Pad Series Digital Scale
Hot plate	Lab Hosp Corporation Mumbai
Refrigerator	Videocon

Table No 5:- List of Instruments

Evaluation of Wound

Statistic healing activity by using following models

Incision model and Excision model

Incision wound model:- The incision wound model was studied. Under light ether anesthesia the animal was secured to operation table in its natural position. One paravertebral straight incision of 6 cm was made on either side of the vertebral column with the help of scalpel blade. Wounds were cleaned with 70% alcohol soaked with cotton swabs. They were kept in separate cages. The extract ointment 2% and 5% was applied for 10 days. The sutures. were removed after 10 days, on tenth day the tensile strength was measure continuous constant water supply technique.

Excision wound model:- Excision wounds were used for the study of rate of contraction of wound and epithelization. Animals were anaesthetized with slight vapour inhalation of di-ethyl ether and the right side of each rat was shaved. Excision wounds sized 314 mm 2 and 2 mm depth were made by cutting out layer of skin from the shaven area. The entire wound was left open. The treatment was done topically in all the cases. The extract 2%,5% ointment was applied +for 16 days. Wound areas were measured on days 1, 4,8 and 16 for all groups, using a transparency sheet and a permanent marker.

Aanalysis :- The data were expressed as mean ± standard error mean (SEM). The significance of differences among the group was assessed using one way analysis of variance (ANOVA) by prism software.

The test was followed by Dunnett‟s, $p<0.05$ were considered as significant.

Experimental Work and Results:-

Collection of Plant :-The plant of *calotropisgigentea LINN* were collected from the local nimdregion [M.P]

Authentication of Plant:- The plant was identified and authenticated Calotropis n. by Dr. S. K. Mahajan, Ex. Professor of Botany, Govt. P. G. College, Khargone, M.P.

Preparation of Extract:- The fresh flower *calotropisgigentealinn*were collected from local nimad region, Madhya Pradesh, India. This plant sample was authenticated by a botanist. Samples were washed in water and dry at room tempreture . After the sample were ground into cors powdered using a blender. 100gms. of powdered of *calotropisgigentealinn*was dissolved in 500ml of solvent like ethanol and extracted using a Soxhlet apparatus. Distillation was carried out for individual samples separately with the solvents and the filtrate was collected and it was collected and it was concentrated by evaporating the solvent to get a final stock.

PLANT NAME	PARTS USED	METHOD	ETHANOL (95%)
Calotropis Gigentea	Flower	Soxhlet assembly	15.86

Table No.6:- Extractive values of different extracts of flower *Calotropis Gigentea Linn*

Figure No. 4 -Soxhlet apparatus

Preparation of Formulation:- Two types of ointment formulation 2% and 5%(w/w),were prepared from the extract where 5 and 10 gram of the extract were incorporated into 100 g. of simple ointment base (BP), Respectively Povidone iodine ointment (5%w/w) was used as a standard drug for comparing the wound healing potential of the extract.

100 g. Simple Ointment base (BP) contain :- Wool fat 5 g.,hard paraffin 5,cetostearyl alcohol 5g.,white soft paraffin 85g Types of preparation absorption ointment base procedure: Hard paraffin and cetostearyl alcohol on water -bath .wool fat and white soft paraffin are mixed and stirred until all the ingredients are melted and from homogeneous mixture,remove from the water bath and cool.

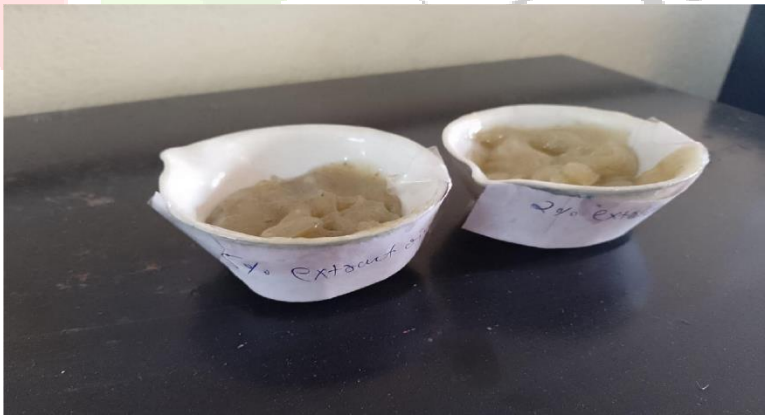


Figure No 5 - Extract ointment prepration

Preliminary Phytochemical Screening

Preliminary Phytochemical screening ethanol extracts of Flower of *calotropisgigentealinn*.

1] Test for Alkaloids

Wagner's test: A fraction of 1 ml extract was treated with few drops of Wagner's reagent And observed for the formation of reddish brown colour precipitate.

Hager's test: A few ml of extract was treated with Hager's reagent and observed for the formation oprominent yellow precipitate.

Mayer's test: A fraction of 1ml extract was treated with 1ml Mayer's test reagent and observed for the formation of cream coloured precipitate.

2] Test for Tannins

Acetic Acid Test: The extract was treated with acetic acid solutions and observed for the formation of red colour solution.

Dilute HNO3 Test: The extract was treated with dil. HNO3. The extract turns from reddish to yellow colour which indicates the presence of tannins.

3] Test for Phenols

Ferric chloride test: The fraction of extract was treated with 5% ferric chloride and observed for the formation of deep blue or black colour

Liebermann's test: The extract was heated with sodium nitrite, added H2SO4 solution diluted with water and excess of dilute NaOH was added and observed for the formation of deep red or green or blue colour.

4]Test for Flavonoids

NaOH test: A small amount of extract was treated with aqueous NaOH and HCl, observed for the formation of yellow orange colour.

H2SO4 test: A fraction of the extract was treated with concentrated H2SO4 and observed for the formation of orange colour.

5] Test for Terpenoids

Salkowski test:2ml extract was treated with 1ml of chloroform and few drop con. H_2SO_4 were also added and formation of raddish brown precipitate indicated the positive result.

6] Test for Saponins

Foam Test: The extract or dry powder was vigourosly shaken with water and not observed for the formation of persistent foam.

7] Test for Anthraquinones

Borntrager's test: About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia and observed for the formation of pink or deep red colouration of aqueous layer.

8] Test of Protein

Ninhydrin test: The extract was treated with aqueous ninhydrin and observed for the presence of blue colour, indicating the presence of amino acid or purple colour indicating the presence of protein.

Biuret test: To 1ml of chloroform, 2ml extract was added and few drops of conc. H_2SO_4 were also added .The formation of reddish brown precipitate indicating the presence of protein.

9] Test for Quinones:- A small amount of extract was treated with concentrated HCl an observed for the formation of yellow colour precipitate

Sr. No.	Phytochemical Constituents	Ethanol (95%)
1.	TEST FOR ALKALOIDS	
	Mayer's test	++
	Wagner's test	++
	Hager's test	++
2.	TEST FOR TANNINS	
	Acetic Acid Test	+
	Dilute HNO3 Test	+
3.	TEST FOR FLAVONOIDS	
	NaOH test	+
	H2SO4 test	+
4.	TEST FOR PHENOLS	
	Ferric chloride test	+
5	TEST FOR TERPENOIDS	
	Salkowski test	+
6.	TEST FOR SAPONINS	
	FoamTest	-
7.	TEST FOR ANTHRAQUINONES	
	Borntrager's test	+
8.	TEST FOR PROTEINS	
	Biuret test	++
9.	TEST FOR QUINONES	
	++	
+ Present ++ Moderately Present Absent -		

Table No. 7:- Result of Preliminary phytochemical screening of ethanol extracts of flower of *Calotropis gigantea linn*

Determination of wound healing evaluation parameters:-

Measurement of Wound Contraction and Epithelialization Period. In the excision wound model, wound area was measured by tracing the wound with the help of transparent sheet using millimeter based graph paper on days 0, 4, 8, 12, and 16 for all groups. Wound contraction was measured every 4th day until complete wound healing and represented as percentage of healing wound area . Percentage of wound contraction was calculated taking the initial size of the wound as 100% using the following formula:

$$\% \text{ wound contraction} = \frac{(\text{Intial wound area} - \text{Specific day wound area})}{\text{Intial wound area}} \times 100.$$

Epithelialization period was calculated as the number of days required for falling off the dead tissue remnants of the wound without any residual raw wound

Measurement of Tensile Strength.

The tensile strength:- The tensile strength of a healing skin wound indicates the degree of wound healing. It represents how much the healed tissue resists to breaking under tension and may identify the quality of healing tissue. On the 10th day, all the animals were anesthetized by light diethyl ether.the sutures were removed, and the healed tissue was excised from all animals. Tensile strength of excised tissue was measured with the help of tensiometer.

Primary skin irritation test: The skin irritation test was done by the method described with some minor modifications. The hairs from the rat dorsal part was removed by hair removing cream (Veet). A 2-cm2 dorsal area was shaved and cleaned with surgical spirit. The animal did not show any toxic effect when 2%,5% w/w extract ointment was applied to a shaved area of rats. Hence the prepared extract ointment was considered safe for topical application.

Wound Healing Studies:-

Excision model and Incision model

1. Excision Model

Test compound:-The Ethanolic extract of flower of *calotropisgigentealinn*and standard drug providon iodine solution 5 % w/w were used.

Chemicals and Reagent:-simple ointment base ointment (BP)

Experimental Animal :-

Rat(150-250gm) used in the present study. The animals were fed with pellet diet and water add libitum. All the animals were acclimatized for a week before use.

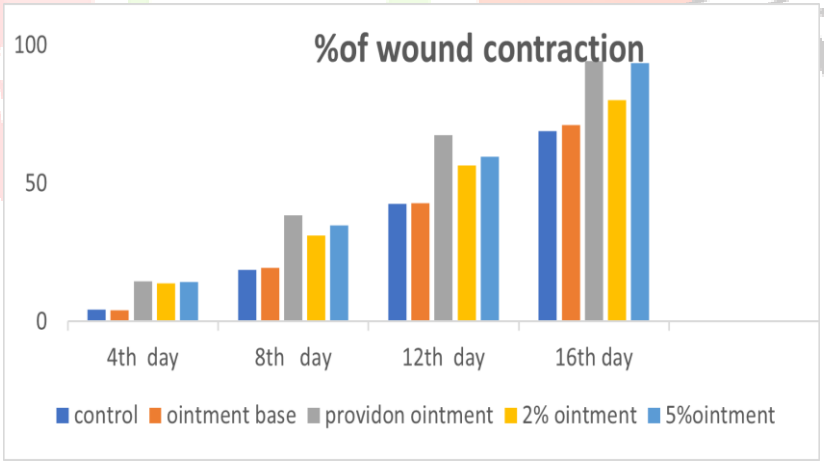
Procedure:-Excision Wound Model:-

Excision wound were used for the study of rate of contration of wound and epithelization. five groups of animals each containis rats were shaved on the dorsum portion using depilatory cream (veet) and anesthetized using light di-ethylether. An impression was made on shaved dorsal region and area of the wound to be created was marked. A full thickness excision wound with a circular area of 314 mm2 was created along the marking using toothed forceps, a surgical blade, and pointed scissors. Rats were left undressed to the open environment. The simple ointment base, formulated extract ointment, and standard drug were applied once daily from the day of the operation until the complete healing. In Journal of Pharmaceutics this model, wound contraction and epithelialization period were evaluated. Wound contraction was measured as percent contraction every 4th day after wound formation.

Treatment	% of wound contraction				
	4th day	8th day	12th day	16th day	Epithelialization period (days)
Group I (untreated)	4.39±0.82	18.62±0.69	42.62±2.56	68.87 ±0.91	19.16 ± 0.7
Group II (ointment base treated)	4.21 ± 0.19	19.48 ±0.93	42.76±1.36	71.2 ±0.93	19.66 ±0.66
Group III (Standard)	14.55±0.87**	38.39 ±0.46*	67.38 ± 2.01**	94.3 ±0.43**	17.33 ± 0.4*
Group IV (2 % w/w ointment)	13.83 ± 1.0*	31.11±0.83*	56.46 ±0.79*	80.21 ±0.27	18.33 ±0.22*
Group V (5 % w/w	14.33 ±0.94*	34.82 ±0.67*	59.64 ± 1.18*	93.58 ±0.76*	17.66 ±0.7*

Table No.8: Excision wound model induced in rats

All values are represented as mean ± SEM, n = 6 animals in each group. Data were analyzed by one-way ANOVA, followed by prism software. Multiple Comparisons Test. significant difference as compared to untreated group (group I); significant difference as compared to ointment base treated group (group II);significant difference as compared to standard group (group III), and **P < 0.01, ***P < 0.001.



Graph No. 1 :- Excision Wound Model Induced

DIFFERENTIATION OF DOSES

Group (I) = (untreated)

Group (II) = (ointment base treated

Group (III) = (standard) Providon iodine ointment

Group (IV) = (2%w/w ointment)

Group (V) = (5% w/w ointment)

- **Incision Wound Model:-**

Test compound:-The Ethanolic extract of flower of Calotropis gigentea.

Drug :- Providon iodine ointment

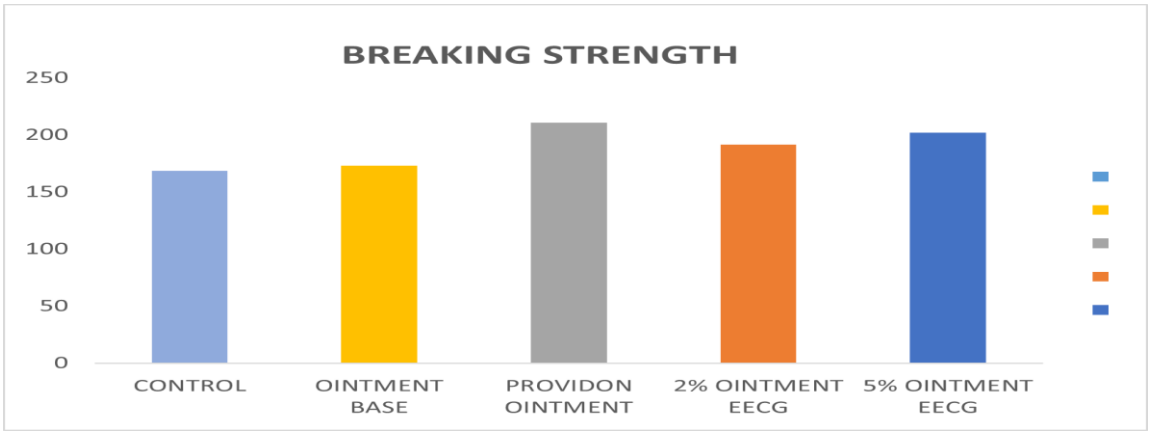
Experimental Animal :- Wister albino Rats (150-200gm) used in the present study. The animals were fed with pellet diet and water add libitum. All the animals were acclimatized for a week before use.

Procedure:-In incision wound model, all the animals of each group were anaesthetized under light ether anesthesia. Two full thickness par vertebral long incisions were made through the skin at the distance of about 1 cm from midline on the each side of the depilated back of rat .After the incision was made the both edges of skin kept together and stitched with black silk surgical thread (no.000) and a curved needle (no.11) was used for stitching . The sutures were removed on the 7th day. wound breaking strength was measured.

S.no.	TREATMENT	BREAKING STRENGTH(g)
1.	Control	168.35± 3.53
2.	Ointment base	172.95 ± 1.24
3.	Providon ointment	210.76 ± 6.65**
4.	2%Ointment	191.35 ± 6.43*
5.	5% Ointment	201.83 ± 4.98 *

Table No 9:- Incision wound model in rat

All values are represented as mean ± SEM, n= 6 animals in each group. Data were analyzed by one-way ANOVA, followed by prism software Multiple Comparisons Test.:significant difference as compared to untreated group (group I): significant difference as compared to ointment base treated group (group II); significant difference as compared to standard group (group III), and **< 0.05, ***< 0.01, And ****< 0.001.



Graph No. 2 :- Incision Wound Model Induced In Rat

DIFFERENTIATION OF DOSES

Group (I) :- Untreated

Group (II) :- Ointment base

Group (III) :- Standard Drug (ProvidonIodine Ointment)

Group (IV) :- Ethanolic Extract 2%Ointment

Group (V) :-(5% w/w ointment)

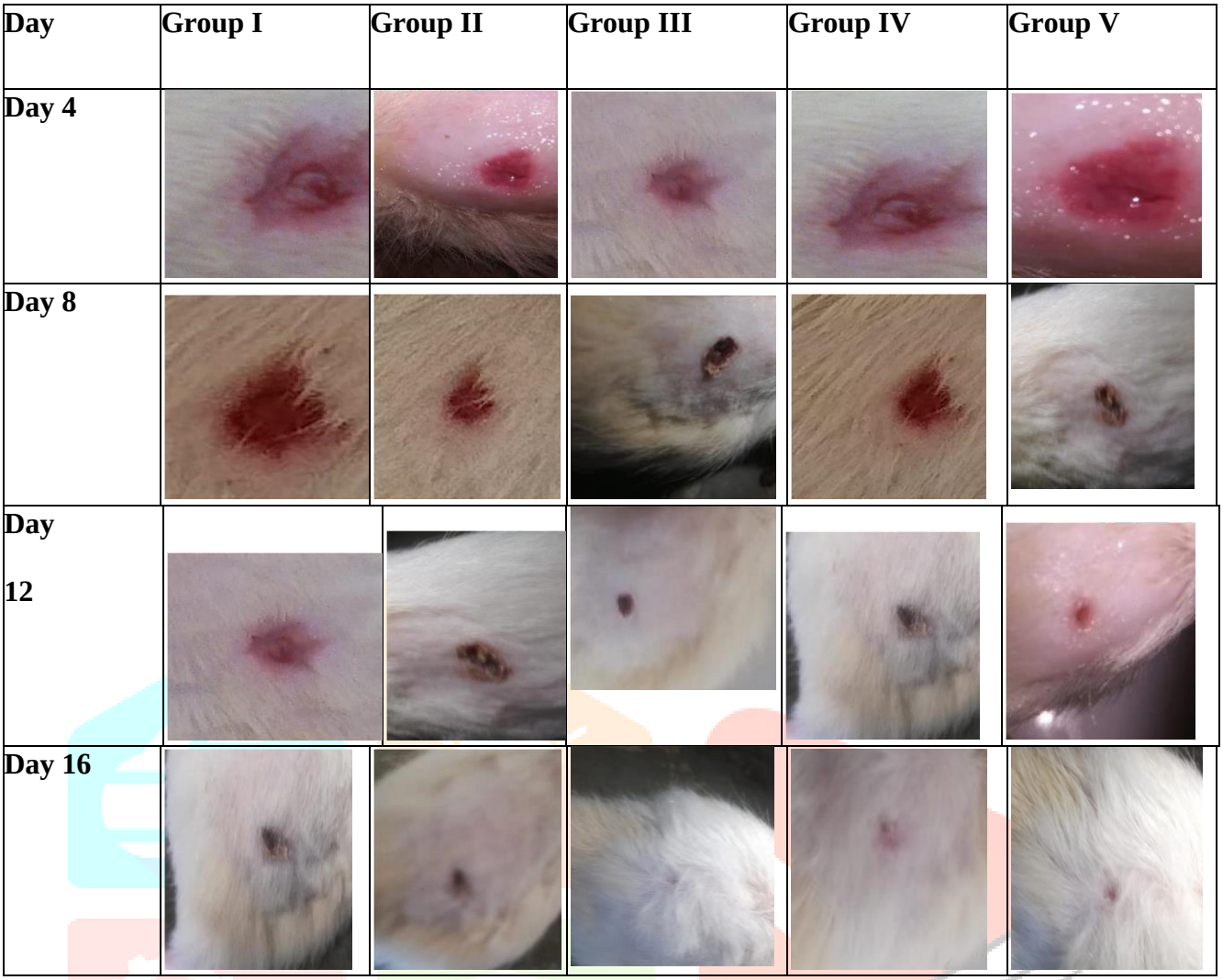


Figure 6: Photographs of wound repair at different time interval in excision wound model in rats

Discussion:-Extractive value:-The powder of flower (100gm) was taken for extraction and the extraction proceeding with two solvent using ethanol processes. From among the extracts Ethanolic Extract afforded maximum yield (15,86%)

Phytochemical screening:- Investigation on the preliminary phytochemicals screening of ethanolic extract showed the presence of Resin ,Protin ,Quinones ,Anthraquinon ,Carbohydrates, Alkaloids, Glycoside, Steroids, and Phenols. By ethanol extract using standardized procedure and also subjected to wound healing activity exhibited significant wound healing activity with respect to control.

Wound healing activity:- The present study showed that the ethanolic extract of *calotropisgigentea linn* possess the wound healing activity as evidenced by its significant effects by Excision wound and Incision wound in Rats.

Excision wound model:- Exposure of animals to various extract of was evaluated for *calotropisgigentealinn*wound healing activity. Excision wound model was chosen science this is effective, cheap ,simple less time consuming and required no preliminary training to the rat and do not cause much discomfort to the animals while handling . In order to provide a scientific explanation for use of we have investigated the *calotropisgigentealinn* biological effects of its extracts ointment, mainly the ones related to wound process. The present data clearly showed that the extract of dried flower of *calotropisgigentealinn*has wound healing activity the highly significant response of some extracts .The result obtained from the excision wound model, Effect of EECG on Percentage Wound Contraction and Epithelialization Period. During the course of treatment the extract was found to show its preliminary effect from day 4 up to day 16 .

Incision wound model:- The result obtained from the incision wound model, Effect of EECG on Tensile Strength of the Wound. An ideal wound healing agent must have the property of increasing the viability of collagen fibrils around the wound area that increases the tensile strength of the wound that was assessed by evaluating the tensile strength of the healed wound using tensiometer. The EECG was found to possess significant concentration dependent action in increasing the tensile strength as compared to control and ointment base treated group.

Summary:-A wound is any injury to the body that typically involved laceration or breaking of membrane and usually damage and the underline tissue. Proper healing of wound is essential for the restoration of disrupted anatomical stability and disturbed function status of the skin in this project. The present investigation of *calotropisgigentealinn*flower extract was studied for wound healing activity. The results may be summarized as follows: *calotropisgigentealinn*was found in large parts of India. *calotropisgigentealinn*is obtained from local named region (M.P).

First the plant flower was dried at the room temperature after that size reduction to a coarse powder by using the grinder. Above 100 gm of dried flower powder . In preliminary phytochemical screening Ethanolic extract showed following result. The Ethanolic extract showed presence of Tannin ,Terpenoids, & Flavonoids, alkaloid ,phenols, protein,quinones, Athraquinones. The relative order for different groups in accordance to collagen stability or wound strength was at standard 5% povidone iodine > extract 5% > extract 2% > ointment base treated > control

Conclusion:- In conclusion, the results of the present study revealed that the ethanolic extract ointment of EECG contains the phytoconstituents that promote natural healing process and it could be effectively used as a wound healing agent. EECG ointment efficiently stimulates the wound strength and increases the rate of epithelialization, tensile strength, around the wound area. Further studies are in-process to isolate the active compound responsible for wound healing and efforts shall be taken to develop the commercial preparation for wound healing

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