



WOUND HEALING ACTIVITY OF ETHANOLIC EXTRACT OF ALLIUM SATIVUM ON ALLOXAN INDUCED DIABETIC RATS FAMILY (LILIACEAE)

Mohammad.Zuber, Voskula.Rajesh, Karra.Anusha,Chitteti.Rakesh Reddy,Ajimera.Tirupathi

Mallareddy college of pharmacy, Dundigal, Rangareddy, Andhra Pradesh, India.

ABSTRACT

Natural products have numerous medicinal applications and play important roles in the biology of the organisms that accumulate them. Flavonoids are one large group of natural products with a diverse number of functions in plants and in human health. The isolates of the bulbs of *Allium sativum* (Family:liliaceae) was screened for wound-healing activity on the Swiss albino rats by Excision wound model and Incision wound model respectively. The studies on excision wound model reveals significant wound healing activity of the extract, which is comparable with the reference control framycetin. The isolates of *Allium sativum* show significant activity on all wound models.

Keywords: Wound healing activity Anti-diabetic, alloxan, Flavonoids, *Allium sativum*, Diabetic.

INTRODUCTION

Diabetes:

Diabetes is a defect in the body's ability to convert glucose (sugar) to energy. Glucose is the main source of fuel for our body. When food is digested it is changed into fats, protein, or carbohydrates. Foods that affect blood sugars are called carbohydrates. Carbohydrates, when digested, change to glucose. Examples of some carbohydrates are: bread, rice, pasta, potatoes, corn, fruit, and milk products. Individuals with diabetes should eat carbohydrates but must do so in moderation. Glucose is then transferred to the blood and is used by the cells for energy. In order for glucose to be transferred from the blood into the cells, the hormone - insulin is needed. Insulin is produced by the beta cells in the pancreas (the organ that produces insulin). In individuals with diabetes, this process is impaired. Diabetes develops when the pancreas fails to produce sufficient quantities of insulin.

- Type 1 diabetes or the insulin produced is defective and cannot move glucose into the cells
- Type 2 diabetes either insulin is not produced in sufficient quantities or the insulin produced is defective and cannot move the glucose into the cells.

There are two main types of diabetes^(1,3,5,6):

Type 1 diabetes occurs most frequently in children and young adults, although it can occur at any age. Type 1 diabetes accounts for 5-10% of all diabetes in the United States. There does appear to be a genetic component to Type 1 diabetes, but the cause has yet to be identified.

Type 2 diabetes is much more common and accounts for 90-95% of all diabetes. Type 2 diabetes primarily affects adults, however recently Type 2 has begun developing in children. There is a strong correlation between Type 2 diabetes, physical inactivity and obesity.

symptoms of diabetes⁽⁶⁾:

If you have more than one of these symptoms you may want to ask your doctor to test your blood sugar.

- ❖ Blurred vision
- ❖ Unusual thirst
- ❖ Frequent urination
- ❖ Slow-healing cuts
- ❖ Unexplained tiredness

- ❖ Rapid weight loss (Type 1 diabetes)
- ❖ Erectile dysfunction
- ❖ Numbness or tingling in hands or feet

Symptoms may occur rapidly with Type 1 diabetes; however, with Type 2 diabetes the onset is more insidious and may not be noticed.

Treatment for diabetes⁽⁷⁾:

As yet, there is no “cure” for either type of diabetes, although there are many ways of keeping diabetes under control. Diabetes treatments are designed to help the body to control the sugar levels in the blood. Studies have shown that good control of blood sugar is the key to avoiding diabetic complications.

- ❖ Type 1 diabetes requires insulin. Injected insulin replaces the insulin missing in the body. You will need to learn how to balance your insulin with your food intake and your physical activity. It is important that you work with a diabetes educator and are under the care of a diabetes team, who can assist you in managing your diabetes
- ❖ Type 2 diabetes treatment will vary dependent on your blood sugar levels. Many patients are counseled to change their lifestyle and lose weight. It is important to work with a diabetes educator and dietitian. Treatment begins with changing certain food choices and beginning an exercise program. Diabetes is a progressive disease, and the treatment may change over time, requiring oral medication; if you are already taking medication, you may need an increased dose or multiple medications, and eventually, you may need to start on insulin.

Literature Review:

Literature on sativum has been found from various sources.

Sources of Literature:

- ❖ Invitrostudies roleof garlic (Allium sativum) in various diseases an overview.
- ❖ Londhev.p,gavasaneA.T,NipateS.S,BandwaneD.D,Chandhari P.D.

Invitrostudies:

- ❖ Effects of aged garlic extract as wound healing:A new frontier in wound management july 2009,vol.32,pages191-203
- ❖ Sohailejaz,Irinachakarova,Jae woo cho,Seungyemlee,Shoabashraf,chaewoong lim.
- ❖ Effect of clonidine on blood glucose levels in euglycemic and alloxan induced diabetic rats. S.Manjunath,santosh .N,Kagali, Priyadarshani,M.Deodurg(2009) INTERNATIONAL JOURNAL OF PHARMACOLOGY 41(5):218-220

Websites:

- ❖ www.pubmed.com
- ❖ www.sciencedir

Objective of Work:

On the basis of literature survey it has been found that most of the tribal people are using *Allium sativum* mainly for wound healing, activity apart from in other conditions. It was also found that little work has been reported regarding its pharmacology and phytochemistry. So in the present study emphasis will be laid on the pharmacological screening of the plant with special reference to the above mentioned activities. The present experimental investigation will be an attempt to give scientific justification to the acclaimed activities.

Rationale of the work:

Garlic contain large sulfur compound is alliin (*S*-allylcysteine sulfoxide). When garlic is chopped, crushed or bruised the alliin converts to the active ingredient, allicin. Garlic exerts antimicrobial activity against many species of bacteria, virus, parasites, protozoan and fungi). Allicin more effective as antimicrobial agent.

The patients with diabetes mellitus are more prone with wounds and dermatological disorders. so we are selected this study.

Plan of Work:

Phytochemical Evaluations:

- ❖ Collection ,identification and authentication of *sativum*L..bulbs
- ❖ Extraction procedure with ethanol, petroleum ether.
- ❖ Preliminary phytochemical screening of above extracts.
- ❖ Induction of diabetes by alloxan monohydrate.

Pharmacological Evaluation:

- ❖ Wound healing activity of diabetic rats
- ❖ Excision wound model
- ❖ Incision wound model

EXPERIMENTAL

Phytochemical Investigations:

Collection of Allium Sativum Linn Bulbs:

Bulbs of Allium sativum Linn.were collected from the market ,Hanamkonda. It is commonly known as garlic.

Extraction⁽²⁰⁾:

150grams of mised garlic is taken then take 500ml of ethanol and place garlic in it.shakeoccasionally for 48 hours.Then rotary evaporator,theethanolic extract is filter using a cotton wool.Then the small quantities of benzene was added to it.Then heated to purify and then filter using wool gauze.Then extract is kept in a tightly closed container and stored at 4*c in the refrigerator for further use.

Preliminary Phytochemical Screening:

Preliminary tests were carried out for the presence or absence of phytoconstituents like Glycosides, Flavanoids, Saponins ,Alkaloids,Carbohydrates, Sterols, Proteins, Phenolic compounds and Reducing compounds. A description of methods adopted for performing the tests are summarized below.

Test for Alkaloids :

- A. **Mayer's Test:** The Extract to be tested is treated with few drops of dilute 2N HCL and 0.5 ml Mayer's reagent .White precipitate was obtained which confirm the presence of alkaloids.
- B. **Wagner's Test:** The extract is treated with few drops of 2N HCL and 0.5 ml Wagner's reagent. Brown flocculent precipitate was obtained which confirm the presence of alkaloids.
- C. **Dragendroff's Test:** The extract is treated with few drops of dilute 2N HCL and 0.5 ml Dragendroff's reagent. Brown precipitate was obtained which confirm the presence of alkaloids.

Test for Carbohydrates:

Molisch's test:

It was performed for the presence of carbohydrates. 1 ml of 10%alcoholic solution of α -naphthol was added to the extract and mixed. Then 1ml of concentrated sulphuric acid was carefully poured along the sides of the test tube violet ring formed at the junction which is considered positive test for carbohydrates.

Test for reducing Sugar:

- A. **Fehling's test:** 5ml of solution of extract was heated with equal volumes of Fehling's solution A & B. Transition of color from blue through green to reddish orange confirms the presence of reducing sugars.
- B. **Benedict's test:** 5 ml of solution of the extract was heated with 5 ml of Benedict's reagent .A green, yellow or orange red precipitate was considered as a positive test for reducing sugars.

Test for Glycosides:

- A. The dried extract was dissolved in glacial acetic acid and few drops of ferric chloride were added followed by concentrated sulphuric acid. A bluish green precipitate was considered to be a positive test for glycosides.

Test for Saponins:

- A. **Foam's test:** A small amount of dry extract was boiled with water and allowed to cool. It was then shaken vigorously for a minute. The formation of persistent honey comb like froth was not observed, as a negative test for saponins.

Test for Steroids:

- A. **Liebermann-Burchard test:** A small portion of extract was dissolved in chloroform and 2ml of Liebermann- burchard reagent was added. Appearance of bluish green was considered as positive test for sterols and pink or violet coloration was considered as positive test for Steroids.
- B. **Salkowski test:** A small portion of extract was dissolved in chloroform and treated with an equal volume of concentrated sulphuric acid. A Red to purple color formation was considered as a positive test for steroids.

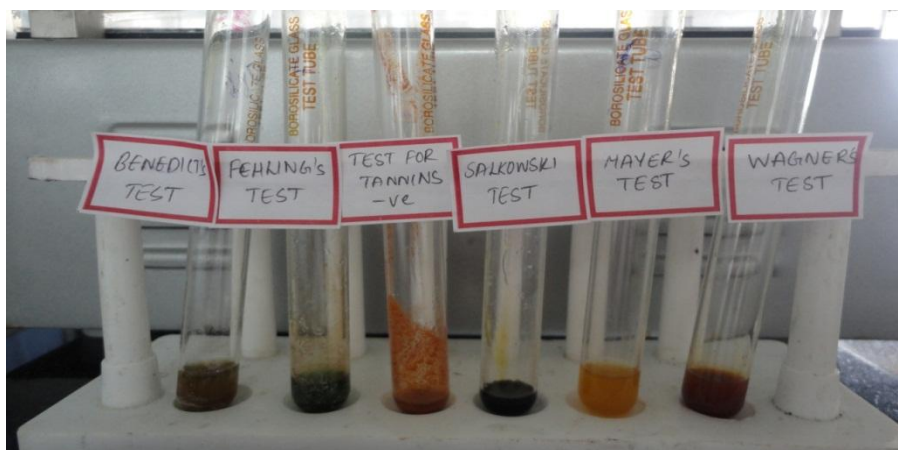
Test for Tannins:

- A. A small portion of extract was treated with 5%ferric chloride solution. Appearance of green to blue color was taken as a positive test for tannins.
- B. Small portion of extract was treated with lead acetate. Appearance of creamy precipitate was considered as a positive test for tannins.

Phytochemical Analysis of ALLIUM SATIVUM Bulbs:

| Type of phytoconstituent | Methanol extract |
|--------------------------|------------------|
| Alkaloids | + |
| Carbohydrates | + |
| Flavanoids | + |
| Glycosides | + |
| Saponins | + |
| Steroids | + |
| Tannin | - |

Positive: present, Negative: absent



Test for Alkaloid's



Test for Carbohydrates

Pharmacological Investigation:

Wound Healing Activity:

A wound is a disruption in the continuity of cells—anything that causes cells that would normally be connected to become separated. Wound healing is the restoration of that continuity. Skin wound healing starts immediately after injury and consists of four phases: hemostasis, Inflammation, Proliferation and maturation. These phases proceed with complicated but well-organised interaction between various tissues and cells.

Phases of Wound Healing:

I. Hemostasis

- ❖ Vasoconstriction
- ❖ Platelet aggregation
- ❖ Thromboplastin makes clot

II. Inflammation

- ❖ Vasodilation
- ❖ Phagocytosis

III. Proliferative Phase (Proliferation, granulation and contraction)

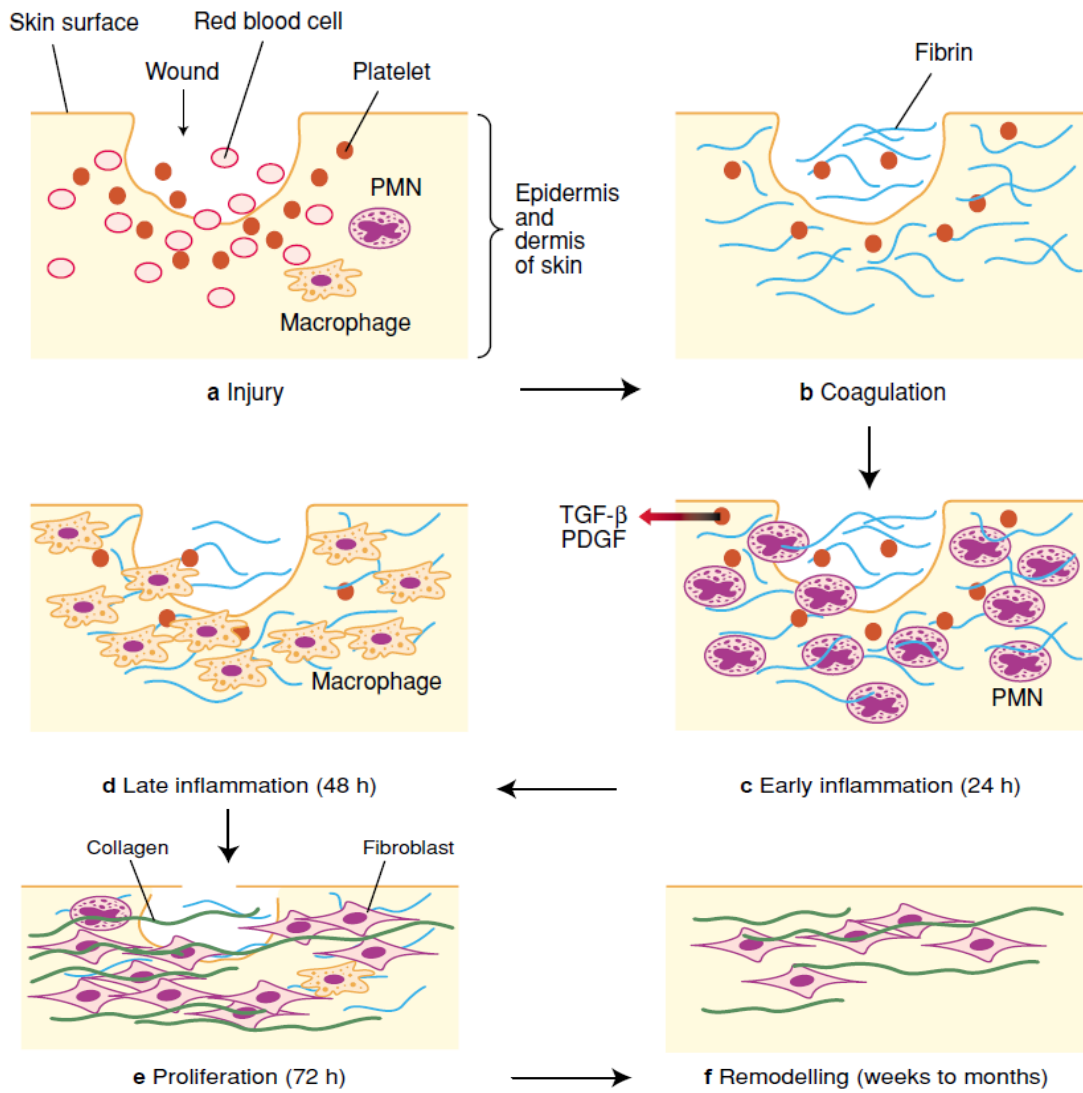
- ❖ Fibroblasts lay bed of collagen
- ❖ Fills defect and produces new capillaries
- ❖ wound edges pull together to reduce defect

IV. Remodeling Phase

- ❖ New collagen forms which increases tensile strength to wounds
- ❖ Scar tissue is only 80 percent as strong as original tissue

Phases of Healing:

| Phases of Healing | Days of post Injury | Cells involved in phase |
|-------------------|---------------------|--|
| Hemostasis | Immediate | Platelets |
| Inflammation | Day 1 - 4 | Neutrophils |
| Proliferation | Day 4- 21 | Macrophages |
| Granulation | | Lymphocytes Angiocytes Neutrocytes |
| Contracture | | Fibroblasts Keratinocytes |
| Remodelling | Day 21- 2 yrs | Fibrocytes |



The phases of cutaneous wound healing

MATERIAL AND METHODS

Chemicals:

Framycetin (Sanofi Aventis),
Wool fat,
Hard Paraffin,
Cetostearyl alcohol and White Soft Paraffin,
Alloxan monohydrate.

Animals:

Swiss albino rat weighing 180-250gm of either sex were used in the study. Animals were procured from Laboratory Animal House of St.peter's institute of pharmaceutical sciences. All animal experiments strictly complied with the approval of institutional animal ethical committee. The animals were kept in polyacrylic cages and maintained under standard housing conditions of temperature (24-27°C) and humidity (60-65%) with 12 h light-12 h dark cycle. They were acclimatized for seven days. Food was provided in the form of dry pellets and water ad libitum.

The prior approval for conducting the experiments in rats was obtained from our Institutional Animal Ethical Committee.

Preparation of ointment by fusion method:

(a) Preparation of simple ointment

| | |
|---------------------|------|
| Wool fat | 2 g |
| Hard Paraffin | 2 g |
| Cetostearyl alcohol | 2 g |
| White Soft Paraffin | 34 g |

Each ingredient was mixed and heated gently with stirring then cooled. The base was then packed in a wide mouth container.

(a) Preparation of 10% ointment: 4 g methanol extract of *Allium sativum* L. was added slowly to the above melted ingredients and stirred thoroughly until the mass cools down and a homogeneous product is formed. The ointment was then packed in a wide mouth container.

Treatment Protocols:

The animals were numbered, weighed and then divided into four groups with five animals in each as follows:

Group I : Serve as control.

Group II : 1%, w/w, framycetin ointment applied.

Group III : simple ointment.

Group IV : 10%, w/w, garlic ointment is applied.

Methods:

Induction of diabetic in rats⁽²¹⁾:

Rats were made diabetic by a single I.P.injection of 150mg/kg of alloxan monohydrate dissolved in saline to overnight fasted animals.It is followed by 0.5ml of 25% destrose after 2hours of alloxan and 5%destrose solution ad libitium for next 24hours.

After 72hours of alloxan,blood samples were withdraw from rat tail vein and blood glucose levels were estimated in all animals. Animals with normal blood glucose level ≥ 200 mg/dl (diabetic) were seleted for study.

1. Excision wound model:

Hairs were removed from the dorsal thoracic central region of anaesthetised rat.The rat were depilated on the back. One excision wound was inflicted by cutting away a 300 mm² full thickness of skin from a predetermined area; the wound was left undressed to the open environment. Then the ointments were applied (as stated above) everyday to the specific groups till the wound is completely healed. This model was used to monitor wound contraction and wound closure time. Wound contraction was calculated as percent reduction in wound area.

$$\% \text{ Wound contraction} = \frac{\text{Healed area}}{\text{total area}} * 100$$

The progressive changes in wound area were monitored planimetrically by tracing the wound margin on graph paper every alternate day. Epithelialisation time was noted as a number of days after wounding

required for the scar to fall off leaving no raw wound behind. From the healed wound, a specimen sample of tissue is isolated from each group of rats for histopathological examination.



Excision wound model

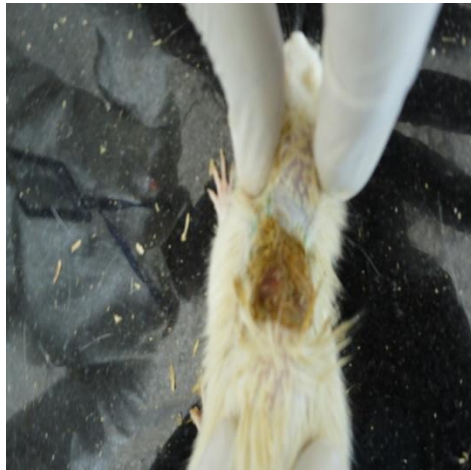
2. Incision wound model:

Rat in each group were anaesthetised and two paravertebral-long incisions are made through the skin and cutaneous muscles at a distance of about 1.0 cm from the midline on each side of the depilated back of the rat. Full aseptic measures were not taken and no local or systemic antimicrobials were used throughout the experiment. After the incision was made, the parted skin was kept together and stitched with black silk thread at 0.5-cm intervals. The wound was left undressed. Extract ointment along with simple ointment and standard ointment were administered once daily for 9 days. When wounds were cured thoroughly the sutures were removed on the ninth day and tensile strength was measured with a tensiometer.

Determination of tensile strength:

The sample drugs along with the standard and control were applied throughout the period, once daily for 9 days. The sutures were removed on the tenth day and the rat were again anaesthetised. Small piece of healed wound was cut out such that the healed incision wound comes exactly in the middle. Four small curved needles (No: 14) were pierced through the healed skin, two on either side. The one side two needles were tied to a rod and the other side two needles were tied to a plastic bottle, which hang freely in the air (the either side of needles were placed equidistant from the healed incision wound). Then slowly water was added to a bottle until the wound began to open. The amount of water in the bottle was weight and considered as an

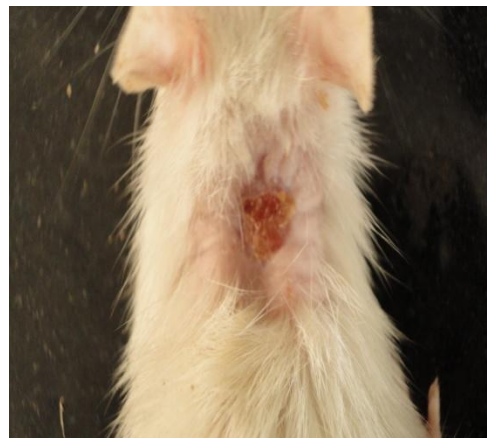
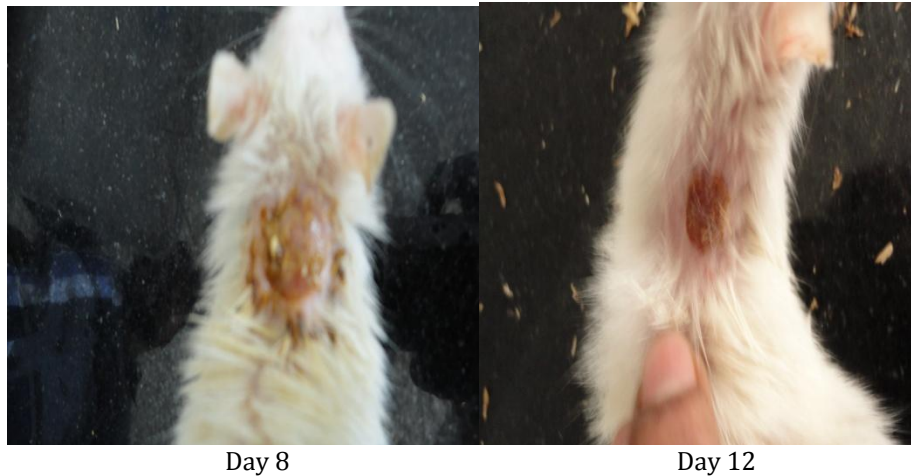
indirect measure of tensile strength of the wound. The mean determination of tensile strength on the two paravertebral incisions on both sides of the animals are taken as the measures of the tensile strength of the wound for an individual animal. The tensile strength of the extract- treated wounds are compared with control. The tensile strength increment indicates better wound healing stimulation by the applied drug.



Day 3



Day 5



Day 16

Statistical analysis:

The values were calculated as mean \pm S.E.M. The values obtained in control and test were found to be statistically significant at a level of $P < 0.05$ by using ANOVA.

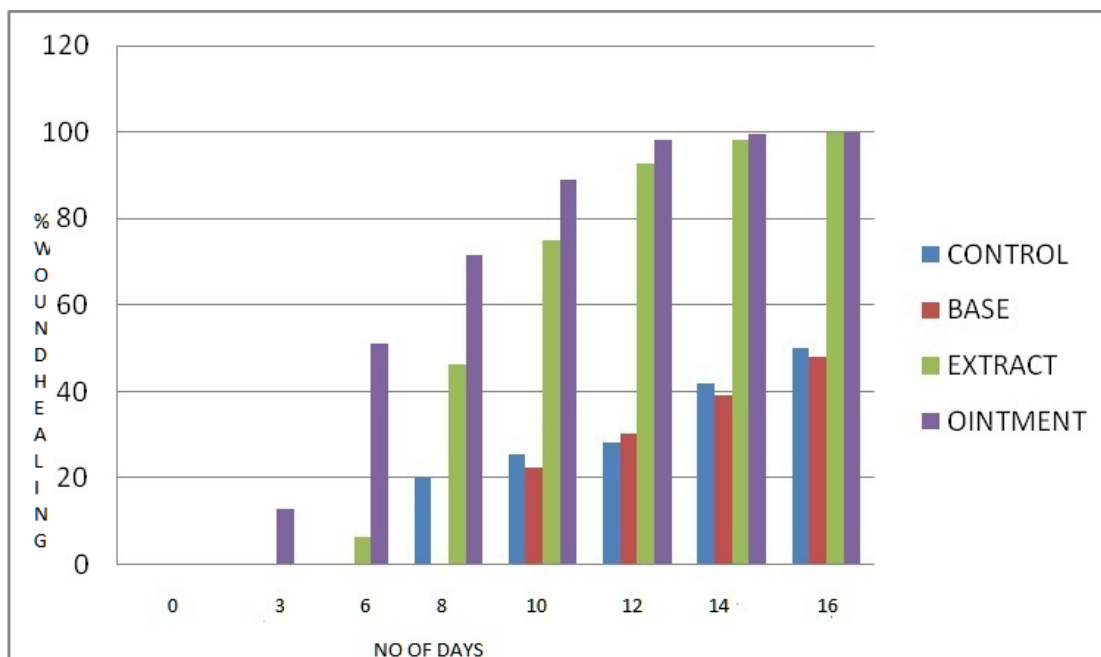
RESULTS

The results of excision wound model are shown. The garlic extract exhibited significant wound healing activity as compared to control in excision wound model. It is observed that the wound contracting ability of the 10% (w/w) extract ointment treated groups showed significant wound healing from the sixth day onwards. The wound closure time was lesser, as well as the percentage of wound contraction was more with the 10% (w/w) extract ointment treated group. The epithelization of wound with 10%(w/w) extract ointment treated group was found to be earlier as compared to control. In the 10% (w/w) extract ointment

treated rat the wounds were completely healed (epithelization period) in 16 ± 2 days whereas in the control animals it took more than 20 ± 2 days.

Evaluation of garlic and framycetin ointment on wound healing by exision wound method in rat

| Post wounding days | Wound area (mm ²) (mean \pm SEM) and percentage of wound contraction | | | |
|-----------------------|--|------------------------------|------------------------------|------------------------------|
| | CONTROL | FRAMYCETIN | BASE | GARLIC EXTRACT |
| 0 | 706.5 \pm 0.52 (0.00) | 706.5 \pm (0.00) | 706.5 \pm 0.35S (0.00) | 706.5 \pm 0.43 (0.00) |
| 3 | 706.5 \pm 0.0.38 (0.00) | 615.44 \pm 0.35 (12.88) | 706.5 \pm 0.38 (0.00) | 706.5 \pm 0.49 (0.00) |
| 6 | 706.5 \pm 0.42 (0.00) | 346.18 \pm 0.29 (51.00) | 706.5 \pm 0.36 (0.00) | 660.18 \pm 0.39 (6.55) |
| 8 | 706.5 \pm 0.35 (19.00) | 200.96 \pm 0.42 (71.55) | 706.5 \pm 0.47 (0.00) | 254.34 \pm 0.37 (46.22) |
| 10 | 176.625 \pm 0.25 (22.8) | 78.5 \pm 0.45 (88.81) | 153.86 \pm 0.43 (21.77) | 176.63 \pm 0.39 (74.95) |
| 12 | 226.865 \pm 0.36 (32.00) | 12.56 \pm 0.46 (98.22) | 215.34 \pm 0.42 (30.88) | 50.24 \pm 0.36 (92.88) |
| 14 | 314.26 \pm 0.45 (44.00) | 3.14 \pm 0.38 (99.55) | 254.34 \pm 0.33 (36.00) | 12.56 \pm 0.38 (98.22) |
| 16 | 348.62 \pm 0.32 (50.22) | 00 (100) | 315.26 \pm 0.37 (48.00) | 00 (100) |



DISCUSSION

The present investigation describes some unique features of the bulbs extract from the plant *Allium sativum* with respect to its potential wound healing capacity in infected rats. Plant products are potential wound healing agents, and largely preferred because of their widespread availability, non-toxicity, absence of unwanted side effects, and effectiveness as crude preparations.

Earlier it was reported that *Centella asiatica* and *Terminalia chebula* are effective in wound healing in rats. Various activities were conducted in this study to evaluate the potential of *A. sativum* as a wound healing agent. One such activity is the phytochemical screening test.

The phytochemical results reveal the presence of flavo, alkaloids, reducing sugars and steroids in the Ethanolic extract. The constituents of the bulb extract, such as terpenoids and alkaloids, may play a major role in the wound healing process observed in this study, however, further phytochemical studies are needed to isolate the active compound(s) responsible for these pharmacological activities.

The topical application of drugs is an efficient therapy method of destroying microbial populations because the availability of the drug at the infected wound site leads to enhanced wound healing activity. The virulence capacity of microorganisms, amount of inoculums, and host immune response are important factors that can cause massive damage during infection. Normally, common wound pathogens such as *S. aureus*, *C. albicans*, and *P. aeruginosa*.

After injury, revascularization of the wound bed and redevelopment of the extracellular matrix are achieved through cell proliferation and the production of granulation tissue. Wound contraction, a part of the proliferative phase of wound healing, occurs through the centripetal movement of the tissues surrounding

the wound, which is mediated by myofibroblasts. The increased wound contraction in the treated group may be due to the enhanced activity of fibroblasts and successful elimination of yeast by the chitraka root extract. The slow rate of wound closure in the control group might be attributed to the presence of microorganisms and their metabolites, which inhibit wound contraction and deteriorates the wound healing activity .A significant increase in collagen content due to enhanced migration of fibroblasts and epithelial cells to the wound site was observed during the wound healing process in the treated group.

A close examination of granulation tissue sections revealed that tissue regeneration was much quicker in the treated group compared to that in control wounds .

The increased cellular infiltration observed from hematoxylin and eosin staining in both groups may be due to the presence of pathogens, but the antimicrobial property of *A.sativum*massively reduced the bacterial population,thereby indirectly reducing the inflammatory cells on the wound site. Early dermal and epidermal regeneration in the treated group confirmed that the ointment containing the *A.sativum*extract had a positive effect toward cellular proliferation, granulation tissue formation, and epithelialization. Incomplete epithelialization with less extracellular matrix synthesis was observed in control rats, . Clumps of degenerating neutrophils, necrotic changes, and the persistence of inflammatory exudates in the upper dermis with loss of epidermis were also observed up to day 8.The treated rats showed marked epithelialization, a moderate amount of extracellular matrix synthesis,and new blood vessel formation.

CONCLUSION

The results obtained in the present study clearly indicate that the Ethanol extract of leaves of *Allium sativum*are having significant wound healing activity in rats. Flavonoids, saponins, alkaloids and phenolics are known to be having active antibiotic principles. The wound healing effect of Ethanolic extracts of bulb of *Allium sativum*may be due to the presence of more than one active principles mentioned above.

Further pharmacological and biochemical investigation will clearly elucidate the mechanism of action and will be help full in projecting this plant as an therapeutic target in wound healing and other diseases.

REFERENCES

1. Effects of Diet and Exercise in Preventing NIDDM in People With Impaired Glucose Tolerance: The Da Qing IGT and Diabetes Study. MD,Guang-Wei Li, MD, Ying-Hua Hu, MDJi-Xing Wang, MD, Wen-Ying Yang, MD, Zuo-Xin An, MD, Ze-Xi Hu, MD,
2. Assessing Patient Beliefs about Self-Monitoring of Blood Glucose: Development of a New Self-Report ScaleAuthors:
3. WH, Fisher L, Parkin CG, Jelsovsky Z, Wagner RS Citation: Poster presented at ADA 71st Scientific Sessions (2011).
4. Psychological insulin resistance: The patient perspective. Authors: Polonsky, Citation: The Diabetes Educator, Vol. 33 (Supplement 7), 241S-244S (2007).
5. A community-based program to encourage patients' attention to their own diabetes care: Pilot development and evaluation.Authors: Polonsky WH, Zee J, Ah Yee M, Crosson MA, Jackson. Citation: The Diabetes Educator, 31, 691- 699 (2005).
6. Assessing psychosocial distress in diabetes: Development of the Diabetes Distress Scale.Authors: Polonsky WH, Fisher L, Earles J, Dudl RJ, Lees J, Mullan J. Citation: Diabetes Care, 28, 626-631 (2005)
7. Emotional and quality of life aspects of diabetes management.Authors: Polonsky WH. Citation: Current Diabetes Reports, 2, 153-159 (2002).
8. Blood glucose awareness training (BGAT-2): Long-term benefits.Authors: Cox D, Gonder-Frederick L, Polonsky W, Schlundt D, KovatchevB.Citation: Diabetes Care, 24, 637-642 (2001).
9. Diabetes-related emotional distress in Dutch and U.S. diabetic patients: Cross-cultural validity of the Problem Areas in Diabetes Scale.Authors: Snoek FJ, Pouwer F, Welch G, PolonskyWH.Citation: Diabetes Care, 23, 1305-1309 (2000).
10. The Problem Areas in Diabetes Scale: An evaluation of its clinical utility.Authors: Welch G, Jacobson AM, PolonskyWH.Citation: Diabetes Care, 20, 760-766 (1997).
11. Wound healing activity of garlic on diabetic rat. Indian journal of experimental biology (INDIAN J EXP BIOL)
12. Phytochemical analysis and anti-diabetic activity of CadabafruticosaR.Br.S. Arokiyaraj1, R. Radha, S. MartinKamboj VP, Dhawan PM (1982) Research on plants for fertility regulation in India J, Ethnopharmacol 6 (2): 191-226.