Evaluation of wound healing activity of Aerial parts of *Asteracantha longifolia Nees by* various wound healing models

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Abstrac- ***Asteracantha longifolia*** **knows as Kokilaksha belonging to the family Acanthaceae is an important medicinal plant used in traditional medicine. Wound healing effect was produced by Topical Application as wall as oral administration of** ***Asteracantha longifolia*** **Aerial part Extract. Objectives: The present study aimed to evaluate wound healing activity of Aerial part of** ***Asteracantha longifolia*** **by using Incision. Excision. Dead space and burn wound models and various evaluation parameters like wound contraction. Epithelisation time. Tensile strength, Hydroxiproline estimation, dry and wet granuloma weight were determined. Result: Test drug extract (Aerial part of** ***Asteracantha longifolia)*** **shows significant increase in wound contraction, Epithelisation time. Tensile strength, Hydroxiproline Level, dry and wet granuloma weightas compare to control group and standard drug. Conclusion: Flavonoidal drugs Enhances wound healing activity and** ***Asteracantha longifolia*** **contain flavonoids which significantky enhances wound healing as compare to control and standard drug.Method: Three groups of Wistar albino rats each one has six animale and divided in control, Test drug, Standard drug treated group. In Incision, Excision and Burn wound model Ointment were applied topically and in dead space wound model suspension were administered orally All the samples were applied once daily for 16 days, starting from the day of wounding. The observations of percentage wound closure were made on 4th, 8th, 12th and 16th, post wounding days in Excision wound model. All the samples were applied once daily for 16 days, starting from the day of wounding. The sutures were removed on 8th post wounding day In Incision wound model. The granulation tissue formed on the grass piths were excised on 10th post wounding day. The weight of wet and dry granulation tissues was measured along with estimation of biochemical parameter like hydroxyproline estimated in Dead space wound model.**

Keywords: Wound healing, Flavonoids, Incision, Excision, Dead Space,Hydroxiproline

I INTRODUCTION

The skin is the largest organ of the body, with a total area of about 20 square feet. The skin protects us from microbes and the elements, helps regulate body temperature, and permits the sensations of touch, heat, and cold. A wound is break in the skin (the outer layer of the skin is called the epidermis). Wound are usually caused by cuts or scalps, symptoms at wound or injury include swelling, stiffness, tenderness, discoloration skin tightness, scabbing, itching and scar formation, two types of tissue injury: Partial Thickness Injuries Limited to the epidermic and superficial dermis with no damage to the dermal blood vessels. Healing occurs by regeneration of epithelial tissue Injury involves loss of the dermis and extends to deeper tissue layers and disrupts dermal blood vessels. Wound healing involves the synthesis of several types of tissue and scar formation[1]The process by which tissue repair takes places is called as wound healing and is comprised of a continuous sequence of inflammation and repair, in which epithelial, endothelial, inflammatory cells, platelets and fibroblasts briefly come together outside their normal domains and interact to restore resemblances of their used discipline and having done so as to resume their normal function Wound healing is a complex dynamic process. Wound environment changes with the changing health status of the individual. The knowledge of the physiology of the normal wound healing trajectory through the phases of homeostasis inflammation, granulation and maturation provides a framework for an understanding of the basic

principles of wound healing[2] Flavonoids are members of a large class of colorful plant pigments with potent antioxidant properties. They provide a broad spectrum of well-documented benefits in cases of heart disease, cancer, vision disorders, allergies, viral infections, and more - and they have no known toxicities, adverse reactions, or other side effects[3]Another recent study demonstrates that infected wounds heal more quickly if treated with flavonoids[4] Wound healing is an intricate process where the skin or other body tissue repairs itself after injury. In normal skin, the epidermis (surface layer) and dermis (deeper layer) form a protective barrier against the external environment. When the barrier is broken, an orchestrated cascade of biochemical events is quickly set into motion to repair the damage. This process is divided into predictable phases: blood clotting (hemostasis), inflammation, the growth of new tissue (proliferation), and the remodeling of tissue (maturation). Sometimes blood clotting is considered to be part of the inflammation stage instead of its own stage[5] Whether wounds are closed by primary intention, subject to delayed primary closure or left to heal by secondary intention[1], the wound healing process is a dynamic one which can be divided into three phases. It is critical to remember that wound healing is not linear and often wounds can progress both forwards and back through the phases depending upon intrinsic and extrinsic forces at work within the patient[6]

Asteracantha longifolia knows as Kokilaksha belonging to the family Acanthaceae is an important medicinal plant used in traditional medicine. From the literature review of *Asteracantha longifolia* it possess refrigerant, diuretic, anti-inflammatory, analgesic, haemopoictic, hepatoprotective ,antioxidant activity. Thus from the knowledge of different causes of wound we can correlate that all of this activity can lead to wound healing. *Asteracantha longifolia* is a spiny, stout, annual herb, common in Water logged places throughout the country. It is wildly distributed throughout India from Himalaya to Sri lanka, Nepal, Malaysia and one of the most promising alternative medicines for wound healing. few active compounds like essential oils, alkaloids, phytosterols, fatty acids, polyphenols, glycosides, flavonoids, terpenoids etc

II MATERIAL AND METHOD

Aerial parts of *Asteracantha longifolia,* was collected during the month of June to August and late September from the surrounding area of Bhopal (MP). The Aerial part was authenticated from Department of Botany, SAFIA College Bhopal (Voucher No. 281/bot.1/SAF/12) and a specimen copy submitted in the college. The crude drug was then allowed to shade drying and crushed in small pieces for extraction.

A. Preparation of extract

The powdered drug material were first defatted by the pet ether then successive extraction were performed by solvent as increasing their polarity. Aerial part of drug was extracted with 90% Ethanol in soxhlet extractor. The extracts obtained were concentrated to dryness in evaporated disc at 40 ^{0}C and stored the dried extract at 4 ^{0}C in the refrigerator until further use

B. Formulation preparation

1) Formulation of ointment

Ointment was prepared of test drug extracts by using simple ointment base BP. All the doses for the test extract were fixed from the acute toxicity studies Two types of drug formulations were prepared from the extracts. Topical application was made in the case of excision, incision and burn wound model whereas, dead space wound model receives oral treatment. For topical administration, 5% w/w of extract ointments was prepared using simple ointment base BP[7]

Table 1: Composition of Simple ointment base for control Group (100 gm)

S.No.	Constituents	Quantity
1.	Polyethylene Glycol 400	40 gm
2.	Polyethylene Glycol 600	60 gm

2) Formulation of suspension

Suspension of test drug extracts was prepared by mixing 2 gm of drug with added to 20 ml of Tragacanth mucilage. Mucilage was prepared by using formula given below. In which purified water make it 100gm

Procedure: Glycerin 18gm, water 75ml were mixed in a tarred vessel and heated. The mixture was heated to boil then add Tragacanth 6gm and Benzoic acid 0.2gm, macerated the mixture occasionally .then added enough purified water, stirred actively until uniform consistency and strained forcibly through muslin.

Table 2: Composition of Tragacanth mucilage (100 gm)

S.No.	Constituents	Quantity
1.	Glycerin	18 gm
2.	Purified water	75 ml
3.	Tragacanth	2 gm
4.	Benzoic acid	0.2 gm

C) Wound healing activity

1) Selection and procurement of animals

After taking permission for animal studies from Institutional Animals Ethics Committee (TIT/IAEC/831/P'COLOGY/2015/54,Wistar albino rats were procured and rats of either sex weighing 150-200 gm were selected, maintained at 24-28^{0}C, housed individually with free access to food and water. The animals were left for 48 hr. to acclimatize to the animal room conditions. They were fed with standard diet. To perform the experiment, the rats were divided into SIX groups (n=6). Statistical Analysis[8] results were analyzed by one-way ANOVA and a P-value less than 0.01 was considered significant.

2) Selection of model

Excision, Incision, Dead space, Burn wound model, using Wistar Albino rats was selected for assessing the wound healing activity. This model was employed to study the rate of wound contraction, time required for full epithelization, tensile strength, granuloma weight and hydroxyproline estimation. These parameters were selected because of easy availability of Albino rat and simplicity in handling them

3) Excision wound model [9]

In the excision wound model, rats were depilated by removing hairs at the dorsal thoracic region before wounding. Rats were anaesthetized by diethyl ether prior to excision. Circular wound of about 2.5 cm diameter was made on depilated dorsal thoracic region of rats under aseptic conditions and were observed throughout the study. The areas of the wounds were measured (in mm^2) immediately by placing a transparent polythene graph paper over the wound and then tracing the area of the wound on it (Approx. area 500 mm^2). This was taken as the initial wound area reading. The rats are categorized into Three groups (n=6). The animal of group I treated as control and only ointment base applied topically. The animal of group II treated as TEST I and received ointment of *A.longofolia* Extract, Group III received standard drug Povidone iodine ointment. All the samples were applied once daily for 16 days, starting from the day of wounding. The observations of percentage wound closure were made on 4th, 8th, 12th and 16th, post wounding days. The wound area of each animal was measured by using tracing paper method. The percentage of wound contraction was calculated from the days of measurements of wound area. paremeters like Wound Contraction and Epithelization time were evaluated in excision wound model.

4) Incision wound model [9]

In the incision wound model, rats depilated by removing hairs at the dorsal thoracic region before wounding. Rats were anaesthetized by diethyl ether prior to incision. Six centimeter long para vertebral incisions were made through full thickness of skin on either side of vertebral column of the rat. The wounds were closed with interrupted sutures of one centimeter apart. The rats are categorized into Three groups (n=6). The animal of group I known as control which received only ointment base, The animal of group II known as TEST I, which received ointment of *A.longofolia,* topically, Group III known as standard group and recieved standard drug Povidone Iodine**.** All the samples were applied once daily for 16 days, starting from the day of wounding. The sutures were removed on 8th post wounding day. The tensile strength of wounds was measured on 10th day following continuous water flow technique.

5) Dead space wound model [9]

In this model, a dead space wound were created by subcutaneous implantation of sterilized cylindrical grass pith (2.5 cm x 0.3 cm), under light ether anesthesia, on either side of the dorsal paravertebral surface of rat. The rats are categorized into three groups (n=6). The animal of group I treated as control and received one ml of 2 % tragacanth solution, orally. The animal of group II treated as TEST 1 and received one ml of oral suspension of *A.longofolia,* and Group III received standard drug. All the samples were given once daily for 10 days, starting from the day of

wounding. The granulation tissue formed on the grass piths were excised on 10th post wounding day. The weight of wet and dry granulation tissues was measured along with estimation of biochemical parameter like hydroxyproline estimated

6) Burn wound model [10]

Male albino rats of wistar strain (150-200gm) body weight were selected and maintained at uniform temperature and diet in well ventilated cages. Partial thickness burn wound were inflicted on overnight starved animal under light ether anesthesia using a metal rod(1.5cm in diameter) heated to 80-85^0 c and exposed for 20sec.after 24 hrs dead tissue were excised using sterile surgical blade. Wound contraction and Epithelialization period were evaluated in burn wound model.

III RESULT AND DISCUSSION

For the evaluation of wound healing activity of plant extract, Three groups were prepared for Incision, Excision and Burn wound model which was divided in control, Test I, standard drug and for Dead space wound model Three groups were prepared which was divided in control, Test I, standard drug.

1) *Wound Contraction and Epithelization time in Excision wound model*

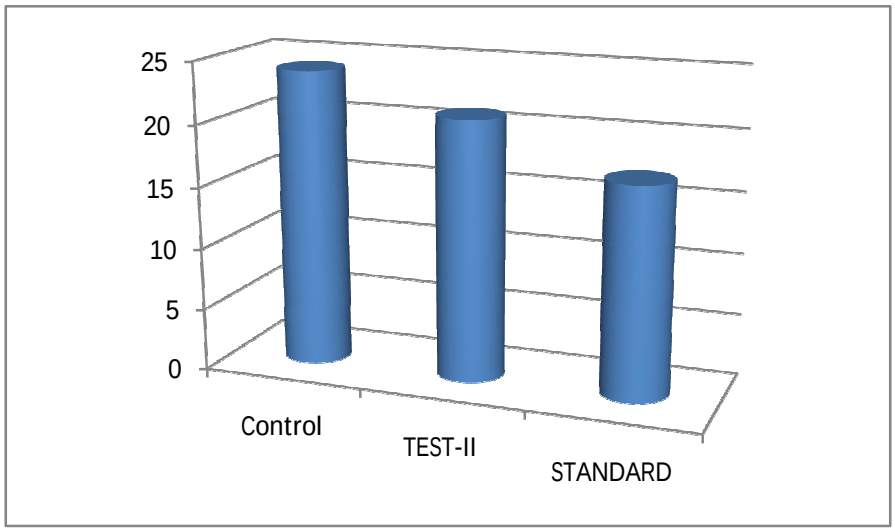
The wound contraction was calculated as percentage reduction in wound area with respect to initial wound area while the epithelization time was noted as the number of days after wounding required for scar to fall off leaving no raw wound behind. The percentage wound contraction was determined by using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

$$\% \text{ Closure} = \frac{\text{wound area on corresponding day-wound area on dazero}}{\text{Wound area on day zero}} X100$$

Effect of control, Test I, standard drug (Povidone Iodine) was observed on percentage wound contracton in Excision wound model on Initial, 4th, 8th, 12th, 16th day interval which is shown in Table No 03.It has been seen that significant wound healing took place in case of animals treated with *Asteracantha longifolia* extract which is 21 days as compare to control and standard drug which took 24 and 16 respectively for complete wound healing. The least rate of wound healing was seen in control group which received no treatment and fastest rate of wound healing was seen in standard drug group where animals received standard drug which is Povidone iodine .Epithelization period (days) in excision wound healing model also shown in Graph No 1.

	Area of wound closure (sq mm ± S.E.M)					
Groups	**Initial**	**4th day**	**8th day**	**12th day**	**16th day**	**Epithelization period (Days)**
I (CONTROL)	10.82±0.68	18.82±0.68	38.12±1.80	48.21±1.80	68.69±2.60	24
II (TEST Drug)	11.24±1,23*	24.22±1.42*	48.94±1.24*	66.92±0.13*	76.12±1.93*	21
III (Standard)	15.26±1.25*	42.24±1.07*	65.34±1.70*	90.12±1.08*	100±0.75*	16

Table 3: Mean Percentage wound contraction in Excision wound model # Initial wound area approx. 500 sq mm



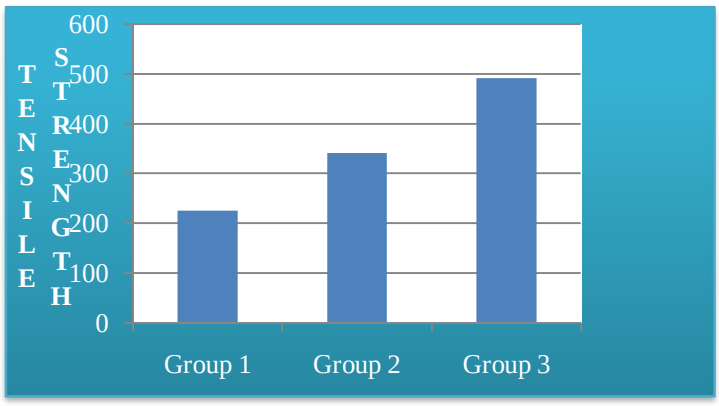
Graph1: Epithelization period (days) in Excision wound healing model

2) Measurement Tensile strength in Incision wound model

The tensile strength was calculated in incision wound model. On 10th day the rats were again anesthetized and each rat is placed on a stack of paper towel on the middle of the board Effect of control, Test I, standard drug (Povidone Iodine) was observed on Tensile strength in Incision wound model which is shown in Table No6 which indicate that animals treated *Asteracantha longifolia* extract significant increase in his Tensile Strength. The least tensile strength seen in control where animal not received any treatment and highest Tensile Strength seen in standard drug group where animals received standard drug which shown in Graph No 2 Tensile strength was measured by the Tensiometer.

Groups	**Tensile strength (in Grams)**
Control	225.16±3.51
Test I	340.39±2.40
STANDARD	490.50±2.71

Table 6:Tensile Strength in incision wound model ≠ Result expressed as Mean Area ± S.E.M. (Standard Error Mean

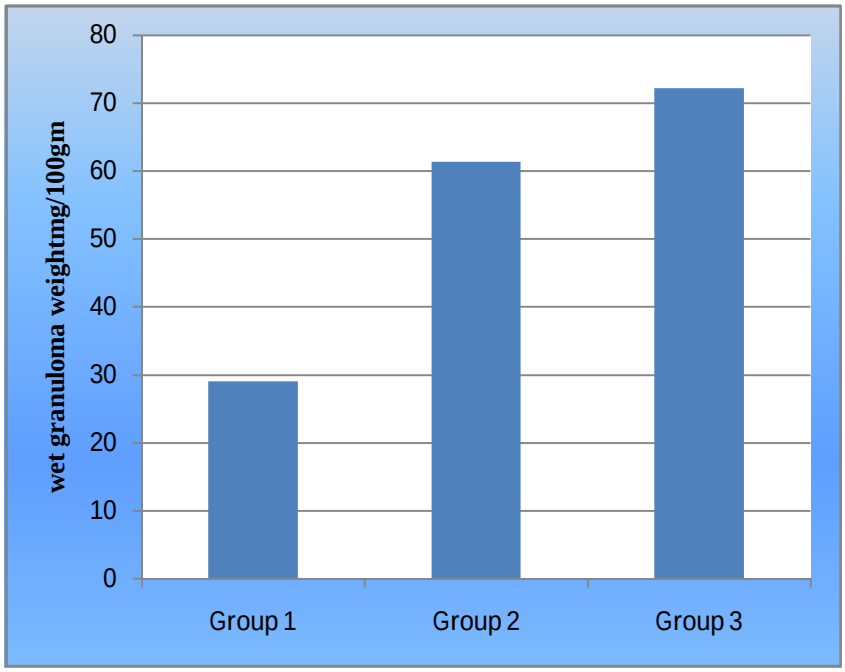


Graph 2: Tensile strength (gm) in incision wound healing model

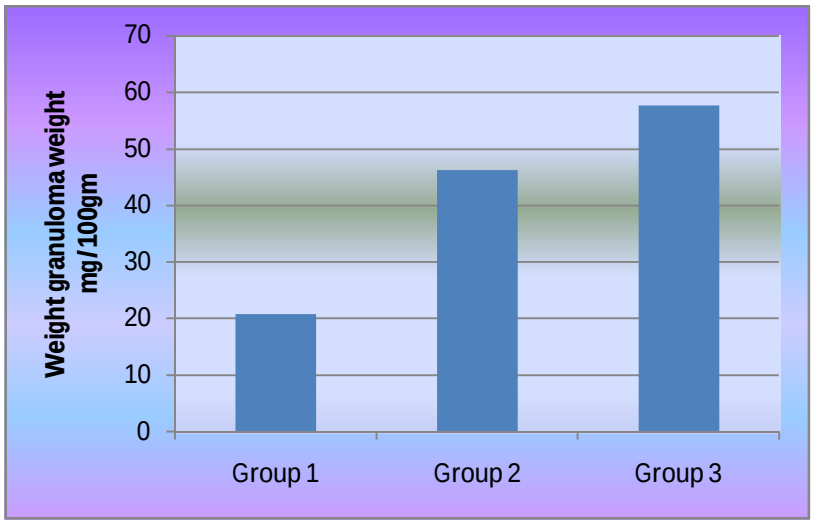
3) Wet Granuloma, Dry Granuloma Weight and Hydroxiproiline Measurement for Dead space wound model
Effect of control, Test I ,standard drug (Povidone Iodine) was observed on Dry, Wet granuloma weight and Hydroxyproline estimation in Dead space wound model which is shown in Table No 7 which indicate that animals received *Asteracantha longifolia* extract significant Wet granuloma weight (mg/100gm,Dry granuloma weight (mg/100gm), Hydroxiproline (mg/gm of tissue) Estimation. control group where animals not received any treatment shown least Wet, Dry granuloma weight and Hydroxiproline estimation and animals received standard drug (Povidone iodine) shown highest Wet, Dry granuloma weight and Hydroxiproline estimation. Graph No 3,4,5 indicate effect of extract on wet granuloma weight, dry granuloma weight and on Hydroxiproline estimation

Group(n)	Wet granuloma weight(mg/100gm	Dry granuloma weight(mg/100gm)	Hydroxiproline(mg/gm of tissue)
Control	29.12.±1.20	20.71±3.20	34.70±4.92
Test II	61.36±0.91	46.22±0.82	40.20±1..62
Standard	72.19±1.66	57.62±2.12	69.35±3.22

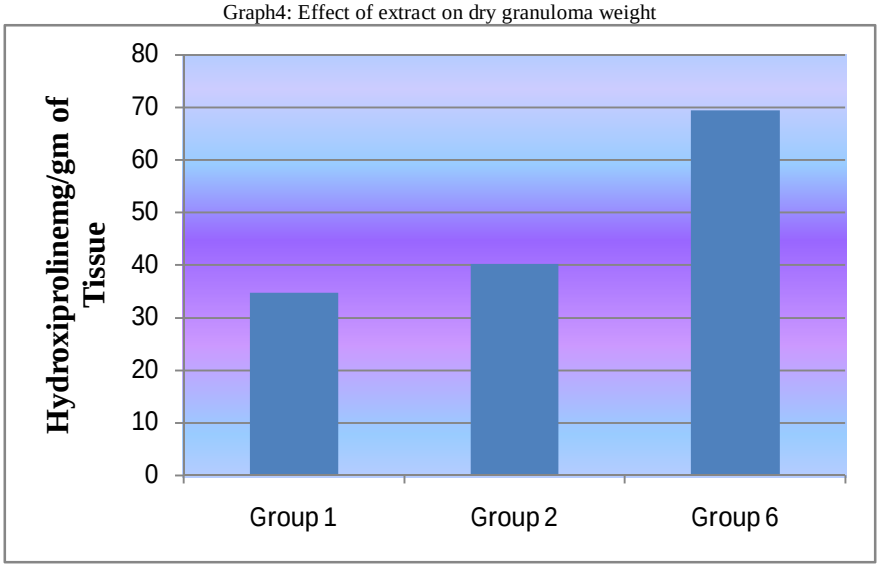
Table 7:Dry, Wet granuloma weight and Hydroxyproline estimation in Dead space wound healing model ≠



Graph3: Effect of extract on wet granuloma weight



Graph4: Effect of extract on dry granuloma weight



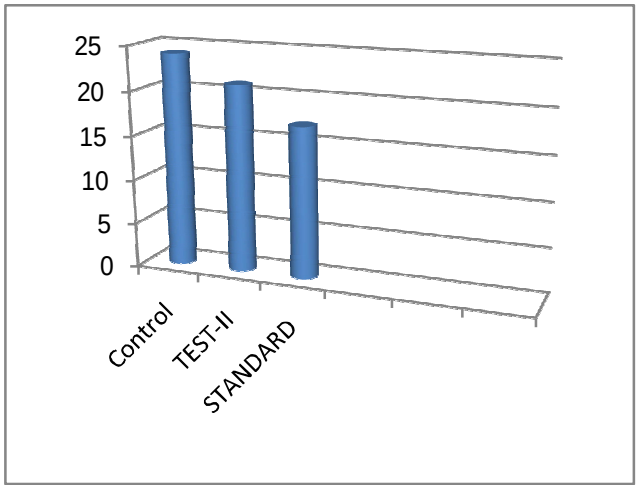
Graph5: Effect of extract on Hydroxiproline estimation

4) Wound Contraction and Epithelization time in Burn wound model

Effect of control, Test I, standard drug (Povidone Iodine) was observed on percentage wound contraction in Burn wound model on Initial,4^{th},8^{th},12^{th},16^{th} day interval which is shown in Table No 8 which indicate that significant wound contraction took place in case of animals treated with *Asteracantha longifolia* extract which is 18 days. The least rate of wound healing was seen in control group which received no treatment and took 24 days for complete healing and fastest rate of wound healing was seen in standard drug group where animals received standard drug which is silve sulphadiazain which took 17 day for complete healing**.** percentage wound contraction in burn wound model shows in Graph 6

	Area of wound closure (sq mm ± S.E.M)					
Groups	**Initial**	**4th day**	**8th day**	**12th day**	**16th day**	**Epithelization period (Days)**
I (CONTROL)	5.92±0.72	20.19±0.92	40.22±1.80	60.11±1.21	70.19±1.20	24
II (TEST-I)	10.14±1.22	30.11±1.22	42.94±1.11	60.12±0.66	69.12±1.29	21
III (silvesulphadiazain)	12.13±2.12	40.24±1.24	66.12±1.29	91.92±0.92	99.19±0.71	17

Table 8: Mean Percentage wound contraction in Excision wound model



Graph 06:Epithelisation period(days) in burn wound model

5) Wet Granuloma, Dry Granuloma Weight and Hydroxiproiline Measurement for Burn wound model Effect of control, Test I, standard drug (silvesulphadiazain) was observed on Dry, Wet granuloma weight and Hydroxyproline estimation in Burn wound model which is shown in Table No 09 which indicate that animals treated with *Asteracantha longifolia* extract showed significant increase in wet,dry granuloma weight and increase hydroxiproline estimation. The least rate of wound healing was seen in control group which received no treatment and took 24 days for complete healing and fastest rate of wound healing was seen in standard drug group where animals received standard drug which is silve sulphadiazain

Group(n)	Wet granuloma weight(mg/100gm	Dry granuloma weight(mg/100gm)	Hydroxiproline(mg/gm of tissue)
Control	27.62±1.20	18.92±2.10	31.29±2.23
Test I	59.29±0.91	41.21±2.19	27.13±2.12
Standard	74.23±2.22	64.42±2.32	59.92±2.12

Table 9:Dry, Wet granuloma weight and Hydroxyproline estimation in Burn wound model

IV CONCLUSION

The result of the study showed that Aerial parts of *Asteracantha longifolia* extract and standard drugs like Povidone Iodine in Incision, Excision and Dead space wound model and silvesulphadiazain in Burn wound model shows significant wound healing activity it is most common that standard drugs heal wound faster than Test drug but *Asteracantha longifolia* also shows significant wound healing activity. *Asteracantha longifolia* shows wound healing activity because of Flavonoids which is present in drug and it is a promising drug for wound healing.

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