

EVALUATION OF WOUND HEALING ACTIVITY OF ABELMOSCHUS ESCULENTUS (LINN) IN ALBINO WISTAR RATS**Mohd Baleeghuddin Farooqui^{1*}, Dr. Shaik Mohammed Khasim², Mohd Rafiq³, Ashfaq Ali Khan⁴**^{1,4}M. Pharmacy Student (Pharmacology), Shadan College of Pharmacy, Peerancheru, Hyderabad.²Director, Shadan College of Pharmacy, Peerancheru, Hyderabad.³Associate Professor, Shadan College of Pharmacy, Peerancheru, Hyderabad.***Corresponding Author: Mohd Baleeghuddin Farooqui**

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ABSTRACT

Synthetic drugs which may suppress or relieve some diseases faster which are usually having harmful side effects therefore attention around worldwide has been shifting towards finding new herbal chemicals for the development of new drugs which are nontoxic. From the earliest times, man has started soothing and understanding the natural forces and risk factors affecting the patterns of illness and death in society. Wound was probably the first medical problem faced by the human race. A wound is the breakage of normal alignment of body cells with or without destroying them, which may impair or stop their function. The symptoms of wound or injury include swelling, stiffness, tenderness, discoloration skin tightness, itching and scar formation. Normal wound healing takes about 3 weeks, but some wounds can take months or even years to heal. Therefore it is necessary to take care for wound healing in initial state using safe medications. In the present study, an attempt has been made using aqueous extract of herbal based plant known as *Abelmoschus esculentus* (200mg/kg/day) in Wistar albino rat against excision and incision wound model following standard procedures in the form of ointment base. In excision model, aqueous extract treated animals exhibit $19.37 \pm 0.15\%$ reduction in wound area when compared to control which was $29.51 \pm 0.69\%$ and whereas standard exhibit $17.73 \pm 0.26\%$. The extract treated wounds are found to epithelize faster as compared to control. In incision wound model significant ($p < 0.001$) increase in breaking strength $305.18 \pm 2.36^{**}$ was observed where as in control group it was 116.91 ± 2.73 and in standard it was $325.45 \pm 4.77^{**}$. The Framycetin sulphate ointment (FSC) 1% w/w was used as standard. The results of the present study clearly suggested that *Abelmoschus esculentus* possesses potent wound healing activity, which could be a good choice of remedy for wound healing and it can be strongly recommend in different wound healing models like burn wound, dead space wound, injury by X-ray radiation and ultraviolet light etc.

KEYWORDS: *Abelmoschus esculentus*, Wound healing, Excision wound, Incision wound, Framycetin sulphate ointment.

INTRODUCTION

Wound healing is a normal biological process in human body that includes three overlapping phases. Inflammation, tissue formation and remodeling. It involves soluble mediators, blood cells, parenchymal cells and extracellular matrix.^[1]

Traditionally plant extracts are being used for wound healing in many parts of the world but many has been not proven scientifically. Plant extracts are less toxic with minimal side effects when compared to synthetic drug.

Abelmoschus esculentus is one of the Indian medicinal plant belongs to the family of Malvaceae.^[2] It is widely distributed in the tropical parts of India and Srilanka. Based on the traditional value of *Abelmoschus esculentus* still many activities are yet to be proven scientifically.

This study was carried out to evaluate the wound healing potential of aqueous extract of fruit *Abelmoschus esculentus* (200mg/kg/day) in Wistar albino rat using excision and incision wound model in the form of ointment base.

MATERIAL AND METHODS**Collection of Plant Material**

The fresh fruit of *Abelmoschus esculentus* was collected from the local fields of Hyderabad and washed with tap water to remove the dust and soil. The fruit was dried under shade, powdered and made to pass through sieve No.40 and stored in closed vessel. The plant specimen was identified and authenticated by L. Rasingam, Scientist In-charge, Botanical Survey of India, Deccan Regional Centre, Plot No.366/1, Pillar No.162, Attapur (V), Hyderguda (P.O), Hyderabad – 500048. Telangana State. India.

A voucher specimen no.BSI/DRC/2017-18/Tech/932.

EXTRACTION OF PLANT MATERIAL^[3]

Preparation of Aqueous Extract

1. About 500gms of dried marc was taken in a 1000 ml of beaker and macerated with 500ml of distilled water to which 5ml of chloroform was added as a preservative and kept it for seven days with occasional shaking daily in a closed vessel.
2. The supernatant was decanted and the marc was pressed then the pooled extract was concentrated on water bath at 50°C to get a dry solid mass. The percentage yield was calculated and tabulated. (Table 1).

PRELIMINARY SCREENING^[4-10]

The preliminary phytochemical screening of aqueous extract of *Abelmoschus esculentus*. Was carried out according to the standard procedures.

PHYTOCHEMICAL

PREPARATION OF OINTMENT BY FUSION METHOD FOR TOPICAL APPLICATION

(a) Preparation of simple ointment

Wool fat - 2 gm; Hard Paraffin-2 gm; Cetostearyl alcohol -2 gm; White Soft Paraffin-34 gm. Each ingredient was mixed and heated gently with stirring then cooled. The base was then packed in a wide mouth container.

(b) Preparation of 10% ointment

2 gm aqueous extract of *Abelmoschus esculentus* 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.^[11-12]

EXPERIMENTAL ANIMALS^[13]

Albino Wistar rats of either sex weighing 100-150 gm were used for the study in different models.

Animal house was well maintained under hygienic conditions. The animals were housed in standard environmental conditions of temperature (31 ±1°C), humidity (60± 0.2%) and a 12 h light and 12 h dark cycle.

They were provided with rodent diet and tap water ad libitum. Cleaning and sanitation work were done on alternate days.

Paddy husk was provided as bedding materials, which was changed every day. The cages were maintained clean and all experiments were conducted according to the guidelines laboratory animal care.

ACUTE DERMAL TOXICITY – FIXED DOSE PROCEDURE^[14]

The acute dermal toxicity study was carried out in adult female albino rats by fixed dose method of OECD Guideline No.434. Extract of the plant *Abelmoschus*

esculentus was applied topically at dose level of 2000 mg/kg body weight.

GROUPING OF ANIMALS

Animals were divided into three groups, each group consisting of 6 rats.

Selection of dose

For the assessment of cutaneous wound healing activity, dose level was chosen in such a way that, dose was approximately one tenth of the maximum dose during acute toxicity studies (200 mg/kg/day).

Group I: Control group (did not receive any treatment)

Group II: Received application of standard drug ointment i.e. Framycetin Topical (1% w/w)

Group III: Received application of aqueous extract of *Abelmoschus esculentus* (200mg/kg/day).

WOUND HEALING ACTIVITY

Excision and incision wound models were used to evaluate the wound-healing activity of aqueous extracts of *Abelmoschus esculentus*.

The study was approved by the Institutional Animal Ethical Committee of Shadan medical College, Moinabad road, Peerancheru, RR District, Telangana - India, registered under CPCSEA, India.

EXCISION WOUND HEALING MODEL

The rats were inflicted with excision wounds under light ether anaesthesia. A circular wound of about 2.5 cm diameter was made on depilated dorsal thoracic region of rats.^[15]

The animals were divided into three groups, each group containing six animals.

Group-I: It was considered as control and received simple ointment base (B.P),

Group-II: It served as reference standard and received framycetin sulfate cream 1% w/w.

Group-III: It received the test formulation.

The ointments were applied topically once in a day, till the epithelization was complete starting from the day of experiment.

The parameters studied were wound closure and epithelization time. The formulation was applied until complete wound healing.

The percentage of wound closure and the period of epithelization were calculated. The period of epithelization was calculated as the number of days required for wound healing. (Results were tabulated in Table 2).

INCISION WOUND HEALING MODEL

In incision wound model, 6 cm long paravertebral incisions were made through full thickness of the skin on either side of the vertebral column of the rat.^[16]

The wounds were closed with interrupted sutures of 1 cm apart. The animals were divided into three groups, each group containing six animals. The categorization and treatment of experimental animals was similar to that of excision wound model. The ointment containing the test formulation was applied topically once in a day.

The sutures were removed on 8th post wound day and the tensile strength of the healed wound was measured on 10th day following continuous water flow technique.¹⁷ (Results were tabulated in Table 3).

Statistical analysis: The data is expressed as mean \pm SEM and subjected to students 't' test and the level of significance was set at $p < 0.001$.

RESULT AND DISCUSSION

Table 1: Percentage yield extract of *Abelmoschus esculentus*

S.No	Extract	Nature of extract	Colour	Weight (GM)	Percentage yield (%)
01	Aqueous	SEMI-SOLID	Dark brown	28.47	2.8%

After aqueous extraction of *Abelmoschus esculentus* is taken and the percentage yield was reflected in the above table 01 in which the nature of extract was in the form of semi solid with dark brown color. Further the extract was subjected to preliminary phytochemical studies.

PRELIMINARY PHYTOCHEMICAL SCREENING

Preliminary phytochemical study showed the presence of tannins, steroids, flavonoids, saponins, alkaloids, anthraquinones, phenols, terpenoids and cardiac glycosoids.

ACUTE DERMAL TOXICITY STUDY

Before the study of wound healing activity, Acute Dermal toxicity studies of the aqueous extract has carried out.

The test extract did not cause any sign or any mortality when applied to the skin up to a maximum dose of 2000 mg/kg body weight.

Table 2: Effect of Aqueous extract of *Abelmoschus esculentus* on excision wound.

Treatment groups	Percentage wound contraction on				Epithelization Period (days)
	4th day	8th day	12th day	16th day	
Simple ointment base (B.P) (Control)	16.48 \pm 0.31	28.33 \pm 1.31	42.09 \pm 1.18	48.75 \pm 0.70	29.51 \pm 0.69
Framycetin sulfate cream 1 % w/w (Reference standard)	34.07 \pm 1.61	56.71 \pm 1.93	85.39 \pm 0.75	96.83 \pm 0.27	17.73 \pm 0.26
Test formulation (Aqueous Extract)	26.79 \pm 2.73	49.57 \pm 2.20	83.95 \pm 1.89	- (100)	19.37 \pm 0.15

Values are mean \pm S.E.M of 6 animals in each group. Numbers in parenthesis indicates percentage of wound contraction. ** $p < 0.001$ vs respective control by students 't' test.

In excision wound model study, the topical application of aqueous extract of *Abelmoschus esculentus* showed significantly greater wound healing activity when compared to control animals.

Table 3: Effect of aqueous extract of *Abelmoschus esculentus* on wound healing in incision wound.

Group	Treatment	Tensile strength in g \pm SEM
1	Simple ointment base (BP) control	116.91 \pm 2.73
2	Framycetin sulfate cream 1 % w/w Reference standard	325.45 \pm 4.77**
3	Test formulation	305.18 \pm 2.36**

Values are mean \pm S.E.M of 6 animals in each group. ** $p < 0.001$ vs respective control by student 't' test.

In incision wound model study, significant increase was observed in the skin tensile strength of aqueous extract of *Abelmoschus esculentus* treated group on 10th post wounding day when compared to control.

The results of aqueous extracts of *Abelmoschus esculentus* on both excision and incision wound model showed significant acceleration in the process of wound healing by decreasing the surface area of the wound and increasing the tensile strength.

Our present study emphasized the present need of medicinal plants against synthetic drugs on wound healing potentials.

CONCLUSION

From the above mentioned studies it can be concluded that, The wound healing activity of aqueous extract of *Abelmoschus esculentus* was studied by using excision and incision wound model and the extract showed significant wound healing activity when compared to control and similar to standard FSC (Framycetinsulphate ointment).

Moreover the extract did not produce any adverse effect and because of this it can be strongly recommend in different wound healing models like burn wound, dead space wound, injury by X-ray radiation and ultraviolet light etc.

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