

## Original Research Article

# PRECLINICAL ASSESSMENT OF WOUND HEALING AND ANTI-INFLAMMATORY ACTIVITIES OF *EICHHORNIA CRASSIPES* IN WISTAR ALBINO RATS

## ABSTRACT

**Aim:**An essential medicinal plant, *Eichhornia crassipes* is a member of the Pontederiaceae family and is used to treat a wide range of disorders. Using various animal models, this study sought to investigate the anti-inflammatory and wound-healing benefits of an ethanolic extract of *E. crassipes*.

**Study Design:**This study engaged *in vivo* studies to investigate the wound healing and antiinflammatory studies of the extract when compare with standard treatment.

**Place and Duration of Study:** Department of Pharmacology, Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, Telangana, India.

**Methodology:**The optimal dosage of MEEC (Methanolic Extract of *Eichhornia crassipes*) was established by conducting acute toxicity tests. The efficacy of wound healing was assessed in this study by means of excision and incision wound models. For the purpose of inducing paw oedema and testing the anti-inflammatory efficacy, we utilised carrageenan and formalin, respectively.

**Results:**Experiments on acute toxicity indicated that the extract was acceptable at doses up to 2000 mg/kg. Two gel concentrations of the extract, 5% and 10%, were applied topically. Wounds healed significantly faster with 10% MEEC gel than with the conventional 10% povidone iodine ointment. Similar to the gold standard drug diclofenac, MEEC at doses of 200 mg/kg b.w. and 400 mg/kg b.w. demonstrated strong anti-inflammatory effects in the two animal models.

**Conclusion:**The benefits of MEEC on wound healing and inflammation may be attributed to its chemical components, which include alkaloids, flavonoids, triterpenoids, and sterols.

**Keywords:***Wound healing, Anti-inflammatory, Methanolic Extract of Eichhornia crassipes, Excision wound model, incision wound model, Carrageenan induced paw edema, Formalin induced paw oedema model*

## 1. INTRODUCTION

Wounds can be either open, where skin is ripped, sliced, or punctured, or closed, where contusions are caused by blunt force trauma, and they heal rather quickly. In pathology, it describes an injury to the skin's dermis caused by a pointed object [1]. The complex process by which injured skin or other organ-tissues regenerate themselves is known as wound healing. In healthy skin, the outermost layer, also known as the epidermis, and the innermost layer, the dermis, are in a constant state of balance

with one another and provide a defence against the environment. Upon piercing the protective barrier, the body's natural healing mechanisms kick in [2].

When vascular tissues encounter potentially dangerous stimuli like infections, damaged cells, or irritants, they engage in a multi-step biological response that includes inflammation. Inflammation is a reaction that occurs when immune cells, blood vessels, and chemical mediators work together to protect the body. When an injury occurs, the body's immune system responds by destroying the offending agent, clearing the area of dead cells and tissues, and then starting the healing process [3].

Chronic inflammation occurs when inflammation lasts for more than a few weeks or months. Proliferative inflammation is defined as inflammation that lasts for weeks or months. In a state of chronic inflammation, cells often secrete chemicals that promote the growth of new tissue, including collagen and blood vessels. Many of these changes reflect the healing process, blurring the line between chronic inflammation and the entire repair process. Chronic inflammation is characterised by simultaneous inflammation, tissue damage, and attempts at repair [4].

The water hyacinth, or *Eichhornia crassipes*, is a perennial aquatic herb that grows freely in water and is endemic to tropical and subtropical South America. It is a member of the Pontederiaceae family given in figure 1. Tannins, flavonoids, alkaloids, and saponins are bioactive components found in plants that exhibit antimicrobial activity, according to phytochemical research. Alkaloids and flavonoids exhibit antiviral, antimicrobial, and anticancer properties[5].



**Fig 1. *Eichhorniacrassipes***

## **2. MATERIALS AND METHODS**

### **2.1 Plant collection and drying**

Collecting the whole *Eichhornia crassipes* plant was done near Balanagar, in the Sundalavanikunta River. We identified and authenticated the plant. After cleaning the debris, the plant was chopped into pieces, left to dry in the shade for approximately seven days, and then ground coarsely in a mixer grinder. The material that had been ground into powder was used for the extraction procedure.

### **2.2 Preparation of methanolic extract of *Eichhornia crassipes***

Continuous extraction is accomplished using the Soxhlet apparatus. The 500 g of plant powder is either put straight into the Soxhlet equipment or placed in a thimble of filter paper. Before heating up, methanol is allowed to syphon once for powder settling. The solvent is kept from bumping by adding new activated porcelain bits to the flask. When the liquid reaches the point of return, a syphon is set up in the syphon tube, transferring the contents of the extractor chamber to the flask. The vapours

travel through the side tube, and the condensed liquid progressively raises the level of liquid in the extractor. To achieve effective extraction, one can repeat this process of evaporating the solvent and syphoning it back multiple times without needing to replace the solvent. This approach, while continuous, is really only a sequence of brief macerations. We maintained room temperature while evaporating the resulting organic extracts [6].

### **2.3 Preliminary Phytochemical Analysis**

Phytochemical tests were conducted to determine the variety of phytoconstituents present in *Eichhornia crassipes*.

### **2.4 Animal procurement**

Wistar rats (weighing around 200 to 250 g) were obtained from the Albino research centres in Hyderabad. The present research was conducted in an animal facility that is CPCSEA-accredited at Gokaraju Rangaraju College of Pharmacy in Bachupally, Hyderabad, India. ("Reg. No. 1175/PO/Re/S/08/CCSEA").

### **2.5 Experimental animals**

For the pharmacological actions, Wistar albino rats weighing 180–250 g was utilised. In polypropylene cages, they were maintained at a temperature of  $25 \pm 2^\circ$  C, with a relative humidity of 45–55%, and subjected to 12-hour light and dark cycles. All the animals were acclimated to the laboratory environment for one week before use. They had free access to water and normal animal feed. Gokaraju Rangaraju College of Pharmacy obtained Institutional Animal Ethics Committee (IAEC) approval for all experimental protocols. We conducted the studies in accordance with the requirements of laboratory animal care guidelines and obtained prior approval from the IAEC. The current investigation was carried out in an animal facility at Gokaraju Rangaraju College of Pharmacy in Bachupally, Hyderabad, India, which is CCSEA-authorized.

### **2.6 Acute toxicity studies**

We conducted the investigation following the guidelines set out by the OECD. According to OECD Guideline 425, there is an up-and-down approach that is used to assess acute oral toxicity. Acute toxicity tests were conducted in accordance with the OECD 425 guidelines, which involve an up-and-down approach.

### **2.7 Evaluation of wound healing activity**

#### **2.7.1 Excision wound model**

A total of four groups of wistar Albino rats ranging in body weight from 180 to 250 g were chosen for this study. G-1 is the disease control group, G-2 is the test group 1, G-3 is the test group 2, and G-4 is the standard group. It is necessary to use an electrical clipper to remove the dorsal fur from rats. Use sterile scissors and forceps to make a circular incision of 500 mm<sup>2</sup> and 2 mm deep on previously shaven skin. One group will receive a gel base treatment, two groups will receive 5% and 10% MEEC

gel treatments, and group four will receive povidone iodine ointment treatments. We will administer the extract and standard medication once a day. To determine the wound closure rate, we traced the wound on the 0th, 3rd, 6th, 9th, 12th, 15th, and 18th post-wounding days using transparent paper and a permanent marker. Then, sketch the wound region again on a 1 mm<sup>2</sup> graph sheet using the tracing paper. Calculating changes in the wound area determines the rate of wound contraction. When the dead tissue flakes off with no trace of the raw wound left behind, that's another way to measure the epithelialization times. The procedure is represented in figure 2 [7].



(a) Anesthetized rat (b) Shaved skin (c) Making of wound (d) Excision wound

**Fig 2. Excision wound model**

### **2.7.2 Incision wound model**

Wistar Albino rats, ranging in weight from 180 to 250 grammes, were chosen for this research. A disease control group (G-1), test group-1 (G-2), test group-2 (G-3), and a standard group will make up the four groups. An electrical clipper is used to shave the rats' dorsal fur. In addition, make a 5-centimetre-long paravertebral incision into the skin and cutaneous tissue of the back. Proceeding with the incision, a curved needle and surgical thread are used to sew the skin that has been divided, with a distance of 1 cm between each stitch. Wounds will remain unclothed. Administer the gel formulations topically to the wound once a day. Apply the formulations to the wounds until the 10th day after wounding, and remove the sutures on the 8<sup>th</sup> day. In the evening of the tenth day following the final treatment, take measurements of wound-breaking strength. The procedure is represented in figure 3 [7].



(a) Anesthetized rat (b) Shaved skin (c) Making of incision (d) Incision Wound

**Fig3. Incision wound model**

### **2.7.3 Evaluation of parameters of wound healing**

#### ***2.7.3.1 Measurement of Wound Contraction***

Graph paper was used to estimate the area, and translucent paper was used to outline the excision wound margin once the wound was created. The percentage of the healed wound area was used to express the wound contraction, which was measured every two days until the wound healed

completely. After that, we used the assessed surface area to determine the percentage of wound contraction, with the initial size of the wound set at 100%, by applying the following formula:

$$\% \text{ wound contraction} = \frac{\text{Initial wound size}}{\text{Initial wound size}} \times 100$$

### **2.7.3.2 Epithelialization period**

The number of days it took for the eschar to fall off the wound surface without leaving a raw wound behind was used to evaluate it [7].

## **2.8 Evaluation of Anti-inflammatory activity**

### **2.8.1 Carrageenan-induced paw oedema**

Wistar Albino rats, ranging in weight from 180 to 250 gm, were chosen for this research. The animals were split up into four groups, i.e., the disease control group (G-1), test group 1 (G-2), test group 2 (G-3), and the standard group (G-4). They were separated and given a week to adjust to the lab environment. In order for an appropriate inflammatory response to emerge, it is crucial to maintain low stress levels. An indelible pen will be used to label the tail of every animal. Group-1 received carrageenan (1% w/v) and saline as the disease control; Group-2 received MEEC and carrageenan (1% w/v); Group-3 received MEEC and carrageenan (1% w/v); and Group-4 received diclofenac sodium and carrageenan (1% w/v). Subcutaneously administer 0.05 ml of 1% carrageenan in the sub-plantar area of each rat's left hind paw. Each rat received an oral dose of the corresponding test medication one hour before administering the carrageenan. The right paw served as control. Digital plethysmographs were used to measure paw oedema hourly for up to four hours in order to examine the influence of the plant's methanolic extract on inflammation. The real amount of oedema is determined by subtracting the readings taken at 0 and 3 hours.



(a) Paw volume (b) Injection into the sub plantar region (c) oedema formation

**Fig 4. Carrageenan induced paw oedema**

### **2.8.2 Formalin induced paw oedema**

Wistar Albino rats, ranging in weight from 180 to 250 grammes, were chosen for this research. The animals were split up into four groups, i.e., the disease control group (G-1), test group 1 (G-2), test group 2 (G-3), and the standard group (G-4). After being separated, the animals were given a week to adjust to the lab environment. In order for an appropriate inflammatory response to emerge, it is

crucial to maintain low stress levels. An indelible pen will be used to label the tail of every animal. Group-1 received saline and formalin (1% w/v) as a disease control; group-2 received formalin and MEEC; group-3 received formalin and MEEC; and group-4 was administered diclofenac sodium as well as formalin (1% w/v). Each rat's left hind paw will get a subcutaneous injection of 0.05 ml of 1% formalin in the sub-plantar area. An hour before administering formalin, we orally administered the appropriate test medication to each rat. The right paw served as control. To investigate the impact of the plant's methanolic extract on inflammation, paw oedema was measured hourly for up to four hours using a digital plethysmograph. There is a disparity between the readings at 0 and 3 hours, indicating the presence of real edema [8].



(a) Paw volume (b) Injection into the sub plantar region (c) Formation of Edema

**Fig 5. Formalin induced paw edema**

### **2.8.3 Calculation of percentage inhibition**

$$\text{Percent inhibition} = X 100 \frac{(V_t - V_o \text{ control} - (V_t - V) \text{ treated})}{(V_t - V_o) \text{ control}}$$

Where,

$V_t$  is the mean paw volume for each group after treatment.

$V_o$  is the mean paw volume for each group before any treatment.

## **3.RESULTS**

### **3.1 Preliminary photochemical analysis**

Alkaloids, flavonoids, sterols and triterpenoids were found in the preliminary phytochemical assessment of MEEC.

### **3.2 Acute toxicity test**

Acute toxicity studies indicated that the extract was safe at doses up to 2000 mg/kg. Two dosages for the current study were chosen: 200 mg/kg and 400mg/kg bd.wt.

### **3.3Wound healing activity**

The impact of MEEC on wound healing activities was investigated. Two wound models - the excision wound model and the incision wound model - were employed for this activity. The tables display the outcomes that were attained in these two models.

#### **3.3.1 Excision wound model**

**Table1. Percentage wound contraction**

Treatment	Percentage (% of Wound Contraction)					
	3 <sup>rd</sup> day	6 <sup>th</sup> day	9 <sup>th</sup> day	12 <sup>th</sup> day	15 <sup>th</sup> day	18 <sup>th</sup> day
Disease control	10.0±0.3	58.2 ± 0.4	67.4 ± 0.3	76.2 ± 0.2	85.5 ± 0.4	96.7 ± 0.1
MEEC 10% gel	24.6 ± 0.5*	95.2 ± 0.2*	100 ± 0.0	-	-	-
Povidone iodine 10%	34.4 ± 0.5*	100 ± 0.0	-	-	-	-

Wistar albino rats per group (n=6), values are presented as Mean ± SEM, (\*=p<0.01) compared with the control.

**0 DAY    9th DAY        12th DAY        18th DAY**



**CONTROL GROUP**



**TEST GROUP**



**STANDARD GROUP**

**Fig 6. Pictorial illustration of percentage of wound contraction**

**Table 2. Period of epithelization**

Treatment	No of days (Mean ± SEM)

Disease control	24 ± 0.25
MEEC gel 10%	13.8 ± 0.40
Povidone-iodine 10%	11.6 ± 0.21

Wistar albino rats per group (n=6), values are presented as Mean ± SEM, (\*= P<0.01) when compared with the control

### **3.3.2 Incision wound model**

**Table 3. Percentage of wound contraction**

Treatment	Tensile Strength (grams)
Disease control	291.66 ± 0.66
MEEC gel 5%	300.83 ± 0.60 #####
MEEC gel 10%	311.83 ± 0.47 #####
Povidone-iodine	325.0 ± 0.42###

The values are given as Mean ±SEM, with (n = 6). performed the statistical analysis using ANOVA, followed by conducting Dunnett's test. Results were expressed as (### = P<0.001) vs. the disease control group and (### = P <0.001) vs. the standard group.

A significant (P <0.001) rise in tensile strength was seen between the wound diabetes control group and the MEEC treatment groups at two different dosages of 5% gel and 10% gel. Comparing the Metrogyl-treated group to the control group, there was a substantial boost in tensile strength (P <0.001).

0' DAY 8th DAY 10th DAY



**CONTROL**



**Fig7. Pictorial illustration of Incision wound model**

### **3.4Anti-inflammatory activity**

The anti-inflammatory efficacy of MEEC was investigated. For this study, two models of paw oedema - one induced by carrageenan and the other by formalin - were employed. The tables provide the outcomes for these two models.

#### **3.4.1Carrageenan induced paw edema**

**Table 4.Impact of MEEC on paw edema induced by carrageenan**

Treatment	Change in paw volume at different hours (mL)				Percentage inhibition at 3hrs
	1hr	2hr	3hr	4hr	
Disease control	1.20± 0.01	1.2 ± 0.012	1.16 ± 0.01	1.03 ± 0.03	0.0
MEEC 200mg/kg bd.wt	0.92 ± 0.01*	0.87 ± 0.01*	0.79 ± 0.01*	0.66 ± 0.01*	31.8
MEEC 200mg/kg bd. wt	0.83 ± 0.01*	0.77 ± 0.01*	0.70 ± 0.01*	0.58 ± 0.02*	39.6
Diclofenac 10mg/kg bd. wt	0.78 ± 0.01*	0.71 ± 0.01*	0.62 ± 0.01*	0.54 ± 0.01*	46.5

Each value represented as mean ± SEM; (n=6). (\* = P<0.01) when compared with the control

The principal test for screening novel anti-inflammatory drugs is frequently carrageenan-induced paw oedema, which is thought to be biphasic. Histamine or serotonin release is responsible for the first

phase of oedema, while prostaglandin release is responsible for the second phase. According to the study's findings, rats' paw oedema caused by carrageenan was considerably lessened (dose-dependent) by the MEEC. As a result, the process of action might involve blocking the formation of prostaglandins, histamine, or serotonin. Sterols, triterpenoids, and flavonoids may all contribute to anti-inflammatory action.

### **3.4.2 Formalin induced paw edema**

**Table 5. Impact of MEEC on paw edema induced by formalin**

Treatment	Change in paw volume at different hours (mL)				Percentage inhibition at 3hrs
	1hr	2hr	3hr	4hr	
Disease control	1.70± 0.04	1.60± 0.04	1.40± 0.05	1.10± 0.05	0.0
MEEC 200mg/kg bd. wt	1.30± 0.04*	0.80 ± 0.06*	0.70± 0.01*	0.70± 0.01*	46.42
MEEC 200mg/kg bd. wt	1.10± 0.04*	0.80 ± 0.03*	0.60 ± 0.02*	0.50± 0.01*	52.10
Diclofenac 10mg/kg bd. Wt	1.0± 0.03*	0.70± 0.03*	0.60 ± 0.01*	0.50± 0.01*	55.0

Each value was represented as Mean±SEM (n=6). (\* = P<0.01) when compared with the control

Over the span of the observation period, the test drug-treated groups in this investigation showed suppression of formalin-induced paw oedema. The study's findings show that MEEC considerably (and dose-dependently) decreased the paw oedema that rats developed from formalin. Consequently, the suppression of prostaglandin, serotonin, or histamine synthesis could be the mode of action. Sterols, triterpenoids, and flavonoids may all contribute to anti-inflammatory action.

## **4. DISCUSSION**

The main causes of poor healing in rats include elevated metalloprotease levels, hypoxia, dysfunctional fibroblast and epidermal cells, and decreased angiogenesis [9]. Different plant chemicals that have health benefits can be found in the MEEC. These include saponins, alkaloids, flavonoids, phenols, carbohydrates, triterpenoids, and tannins. By improving epithelization, quercetin (a flavonoid) significantly increases wound contraction. Quercetin's capacity to raise tissue antioxidant levels, which could lead to enhanced healing of wounds, is likely responsible for this effect [10].

Flavonoids (luteolin) help wounds heal faster by increasing collagen production, encouraging collagen cross-linking, lowering soluble collagen degradation, speeding up the change from soluble collagen to insoluble collagen, and stopping the breakdown of soluble collagen [9]. Gallic acid (phenols) increases the amount of collagen in the wound, which stimulates the growth of fibroblast cells and speeds up the remodelling and epithelialization stages. It also has the potential to accelerate wound

healing by scavenging free radicals produced during the inflammatory phase. Tannin helps wounds heal by removing free radicals, making wounds contract, encouraging the growth of capillary vessels and fibroblasts, and increasing the number of keratinocytes. Through improved collagen synthesis and epithelialization, alkaloid exhibits a considerable improvement in % closure [10].

Saponin can stimulate interleukin-8, an inflammatory chemokine that influences the function and recruitment of different inflammatory cells, fibroblasts, and keratinocytes, resulting in faster wound contraction. It can additionally boost the gap junctional intracellular communication in fibroblasts and accelerate the maturation of granulation tissue. Due to improved granulation tissue formation, significant fibroblast proliferation, enhanced vascularization, and collagen fibre deposition, luteol (triterpenoid) increases wound contraction [11].

The antibiotic povidone iodine ointment is effective against both aerobic and anaerobic microorganisms. A wound-free environment can be maintained with its support. In the phase of proliferative growth, povidone iodine ointment works by stimulating fibroblast proliferation, which in turn promotes vascularization and collagen fiber density. The metalloproteases are inhibited by povidone iodine ointment [12].

During regular biological activity, cells in neutrophils and other leukocytes produce reactive oxygen species (ROS). Damaged cells in neutrophils, along with other leukocytes, produce ROS. Damage to DNA, lipids, proteins, the extracellular matrix, and healing cytokines slows cell proliferation and the closure of wounds. It is becoming more and more apparent that oxygen-derived species are responsible for cell injury or death, and this is especially true in the presence of low antioxidant concentrations. The byproducts of lipid peroxidation have the potential to harm DNA [13]. The ratio of ROS produced to the antioxidants available often causes an increase in LPO and a decrease in antioxidant protection. Cytotoxicity, allergies, mutagenicity, carcinogenicity, and prolonged diabetic wound healing are all possible outcomes of such an adverse event. It is possible that the increased collagen concentration and fibre stabilisation are responsible for the increased tensile strength observed in saponin. At the location of the wound, the newly synthesised collagen molecules are cross-linked by the fibres. Collagen remodelling and the establishment of permanent intramolecular and intermolecular crosslinks are the two main mechanisms by which wound strength is obtained. Flavonoids may stimulate collagen production, potentially explaining the rise in tensile strength. A possible explanation for the increased tensile strength observed in alkaloid materials is the stabilisation of fibres and an increase in collagen content [14]. Healing tissues produce collagen, which is an essential component of developing cells. A higher tensile strength indicates greater wound healing [15, 16].

MEEC considerably enhanced the tensile strength at two dosage levels: 5% gel and 10% gel. The presence of saponins and steroids in MEEC may have improved the wound healing processes. Tanning agents, alkaloids, flavonoids, carbs, phenols and triterpenoids Two dose levels greatly enhance the anti-inflammatory action of the MEEC: 200 mg/kg bd. Wt. and 400 mg/kg bd. Wt.

Because of its high concentration of saponins, steroids, alkaloids, flavonoids, phenols, carbs, triterpenoids, and tannins, MEEC may have lowered inflammation.

## 5.CONCLUSION

The anti-inflammatory and wound-healing properties of methanolic extract of *Eichhornia crassipes* were assessed. MEEC was shown to be non-toxic in acute toxicity experiments, and its LD<sub>50</sub> was reported to be greater than 2000 mg/kg bd. wt. After conducting dose selection tests, we determined that the ideal dose is 200 mg/kg bd. wt. The ideal dose was increased by twofold to provide a second dose of 400 mg/kg bd. wt. since we required two dose levels. A review of the chemical components and therapeutic potential of the *Eichhornia crassipes* methanolic extract has been conducted. Its primary chemical ingredients include alkaloids, flavonoids, and sterols, which may have anti-inflammatory and wound-healing properties. The wound healing activity showed that the MEEC gel speeds up wound contraction and cuts down on the time it takes for epithelization in both the excision and incision wound models. These findings justify the inclusion of this plant in wound healing therapy. The MEEC greatly decreased the carrageenan and formalin-induced paw oedema in rats, according to the results of its anti-inflammatory activities. The study discovered that the MEEC has anti-inflammatory and wound-healing properties. Accordingly, the current study backs up the conventional utilisation of the plant for its anti-inflammatory and wound-healing properties. To find out exactly how the methanolic extract of *Eichhornia crassipes* works, more research needs to be done using purified fractions of the bioactive ingredient.

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