In Vivo Evaluation of Guar Gum Hydrogel Incorporated Poly-Mushroom Extract for Chronic Wounds in Rats

[2]Saranya A, [2]Sreelakshmi S, [1]Ashok Kumar K, [1]Abirami G, [1]Suganthi M, [1*]Jayanthi M

Department of Biotechnology,
Vels Institute of Science Technology and Advanced Studies,
Pallavaram, Chennai – 600 117, Tamilnadu, India
Corresponding Author: Assistant Professor,
Contact No: +918056103828, rsjaya24@gmail.com
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Abstract- Chronic wounds are widespread. It is estimated that 1 to 2% of the population will experience a chronic wound during their lifetime in both developed and undeveloped countries. Studies in India have reported higher rates of wound infections from 23% to 38% due to chronic wounds. The conventional medicines used for the treatment of chronic wounds are Bacitracin, Neosporin, and Polysporin. Although widely used, serious side effects observed are severe pain, nausea, lack of appetite, significant emotional and physical distress, and lack of sleep. In this context we propose to explore the angiogenic property of poly fungal extracts that are already known for their biological properties such as antitumor, anticancer, antioxidant, and antifungal activities. Guar gum is a biopolymer derived from the seeds of the guar plant. It has been used in a variety of applications, including food, pharmaceuticals, and cosmetics, due to its unique properties such as thickening, stabilizing, and emulsifying. In recent years, guar gum has also been studied for its potential use in tissue regeneration. It is important to note that the use of guar gum hydrogel incorporated with poly mushroom extract for chronic wound treatment in humans has not yet been extensively studied. The study concludes that the poly mushroom extract incorporated with guar gum hydrogel appears to possess extremely good wound healing attributes and can be employed as a potential wound dressing aid and tissue regeneration.

Index- Terms: Guar gum, Chronic wound, Tissue regeneration, Biopolymer, Polyfungal.

I.INTRODUCTION

Wound healing is a complex process involving the reconstruction of damaged skin through the interaction of various epithelial and mesenchymal cells, along with cytokines, chemokines, and growth factors [1]. Keratinocyte growth factor (KGF) or fibroblast growth factor - 7 (FGF-7) is a vital paracrine growth factor, produced by various cell types, has multifaceted functions underscore in maintaining skin integrity and promoting healing in response to injury or stress [2]. A wound is a physical degradation of the body's natural integrity caused by an agent. It includes erosion, ulcers, and fissures. Erosion refers to isolated epidermal losses, fissures involve vertical fractures, and ulcers are localized lesions. The healing process depends on the patient's health, treatment, and underlying cause (Han and Kawai, 2018).

Mushrooms have been recognized for their healing properties for millennia, with ancient cultures in China, Eastern Europe, Mesoamerica, and Africa utilizing them for medicinal purposes. They exhibit antitumor, antibacterial, and antiviral effects, attributed to their bioactive compounds [3]. Medicinal mushrooms are often rich in vitamins, minerals, and other essential components that are hard to find elsewhere in nature. Mushrooms have been studied for their medicinal properties, with some species producing metabolites that inhibit or destroy cancer cells [4]. Polyherbal formulations offer a promising and safe alternative to conventional antibiotics in antimicrobial therapy, especially against resistant pathogen strains, due to their multi-target approach and synergistic effects. The exploration of innovative compounds for wound healing is a key focus in biomedical sciences. Many researchers are investigating phytomedicine, acknowledging the ability of various plants to enhance natural repair mechanisms. Over 70% of wound healing pharmaceuticals are plant-derived, while only 20% are based on mineral compounds [5].

The role of mushrooms in wound healing is not well understood, making it essential to investigate their effects. Study outcomes are influenced by factors such as the type and strain of fungus, the culture medium, the specific part of the fungus examined (spores, mycelia, or fruit bodies), and the extraction techniques used for active components. Several studies have been studied for wound healing activity on *Auricularia auricula*, *Boletus edulis, Cordyceps sinensis, Fomitopsis officinalis, Coriolus versicolor, Lentinula edodes, Tremella fusiform* and *Ganoderma lucidum,* many of the studies have been reported on their each specific efficiency. Further research is needed to identify and characterize more bioactive compounds that play a role in enhancing wound healing effectiveness[6].

II.MATERIALS AND METHODS

Sample Collection and Extract Preparation

Auricularia auricula, *Boletus edulis, Cordyceps sinensis, Fomitopsis officinalis, Coriolus versicolor, Lentinula edodes, Tremella fusiform* were collected from the Pushpagiri Research Centre, Thiruvalla. Further the samples were dried under shade and ground to fine powder. 10 g of each sample were extracted using Deionised water by Soxhlet extraction method. The resulting extract was condensed to a dry residue at room temperature under decreased pressure.

Biomaterial Preparation

Guar gum hydrogel as a blank were taken. Each 1 mg of the mushroom extracts were mixed together and incorporated with guar gum hydrogel to form a poly mushroom extract of study. Silver nitrate gel was used as a positive control and Saline solution were used as a negative control.

Experimental Model

Female Sprague Dawley rats weighing between 200 to 300 g were taken for the study.

In vivo Wound Healing Studies

Animal Grouping and Experimental Design

The wound healing capacity of the prepared samples was tested using six weeks old Sprague Dawley rats weighing approximately 250-300g.

The test groups were randomly divided into four groups each containing two rats.

The group details are given below:

GROUP 1- Treatment (Poly Mushroom extract)

GROUP 2- Blank (guar gum hydrogel)

GROUP 3- Positive control (silver nitrate gel)

GROUP 4- Negative control (Saline solution)

During the study, animals were examined repeatedly with gentle handling to minimize stress and to ensure assimilation to the laboratory environment. Each rat was housed in a single cage to prevent it from being injured by another rat. The rats were selected from an inbred group maintained under standard conditions of temperature (25°C) and humidity conditions. The animals were provided with standard laboratory animal feed (Champaka feeds and foods, Bangalore India), rat /mice pellet and UV-sterilized water. All animal experiments in this study were carried out with the prior approval of the Institutional Animal Ethics Committee (IACP/PIMS and RC/2016/17)

Animal Restraining

Proper restraining and handling techniques are essential for reducing stress for laboratory animals and the handler. Animals become much easier to handle if they are trained and accustomed to handling. It is necessary of handling the animals regularly even when no procedures are performed. Most rodents will attempt to bite when handled. Since rodent bites are painful and can become infective, care and proper technique in handling rodents are essential. Restraining devices or chemical restraining should be considered for prolonged potentially painful procedures.

ANESTHESIA

Loss of sensation of awareness that is induced for medical purposes. The mice were anesthetized by intramuscular injection of ketamine (10 units) and xylazine (5 units).

- Ketamine is a dissociative anesthetic that produces a trance-like state in the animal, often referred to as a "k-hole" state.
- Ketamine is often used in combination with other anaesthetics, such as xylazine, to provide a deeper level of Anesthesia.
- Xylazine is a sedative and analgesic that is commonly used in veterinary medicine to help calm animals before surgery or other procedures.
- It can be administered intravenously, intramuscularly, or subcutaneously, and its effects can last for several hour

XYLAZINE

This injection is recommended for inducing sedation and analgesia in various animals for routine observations, minor surgical interventions, and immobilization of animals.

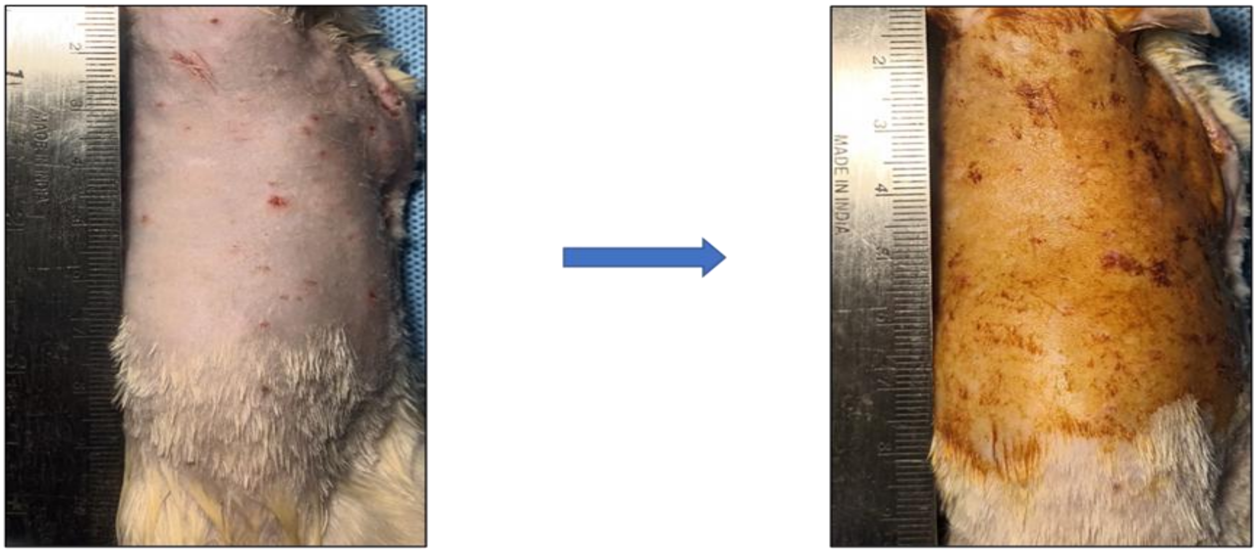
KETAMINE HYDROCHLORIDE

Ketamine hydrochloride injection is indicated as the sole anesthetic agent for diagnostic and surgical procedures that do not require skeletal muscle relaxation. Anesthesia was given to the rat through IP injection (intraperitoneal injection).

HAIR TRIMMING AND SURFACE STERILIZATION

An area was prepared for the excision of wounds. The surgical site where the hair was removed was sterilized using betadine, also known as povidone-iodine which acts as an antiseptic. Dorsal skin of the sedated rat is first shortened by sharp scissors and a suitable surgical blade which is used to remove the remaining fur. The Entire Dorsal Surface of the rat skin is sterilized with the Application of an Antiseptic Agent such as Betadine

Before Fur Removal **After Fur Removal**



BEFORE APPLYING BETADINE

APPLICATION OF BETADINE

Figure 1: Hair Trimming

Excision of Wound

Four separate wounds are created on the surface of the rat for implantation of study materials. Made 4 full thickness (10mm) wounds on the dorsal surface of the rats using a biopsy punch to expose the subcutaneous tissue. The dissected tissues were examined for histopathological analysis. Guar gum hydrogel as blank, Poly Mushroom extract as treatment, silver nitrate as positive control, and saline solution as negative.

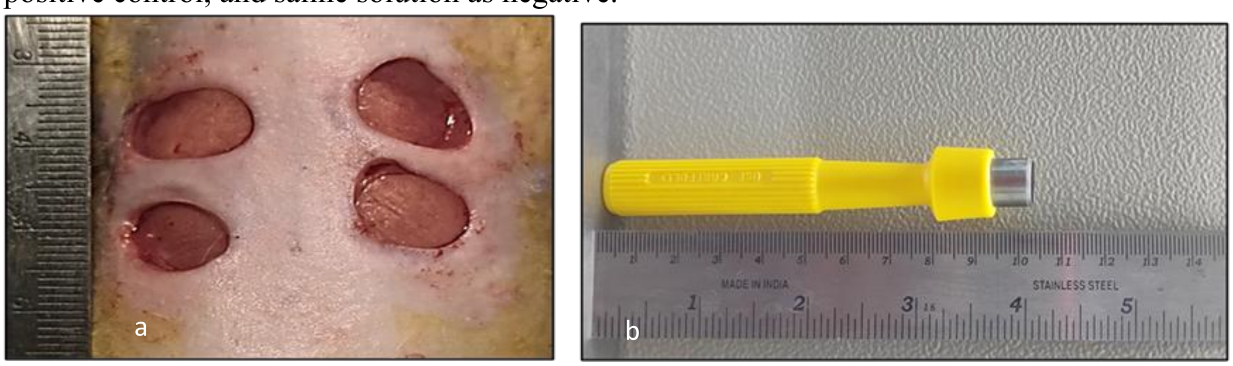


Figure 2: a. Fresh wound created on the dorsal skin of the rat model using 10 mm biopsy punch; b. Biopsy Punch

Application of Hydrogel

The wounds are cleaned with sterile cotton. The samples are loaded into the wounds using a sterile spatula in the order blank, test, and positive control, respectively to avoid cross-contamination. The wounds are individually covered with small gauze pieces. The entire dorsal surface of the rat model is covered with clear tape or Tegaderm.

Fig 3: Application of Hydrogel

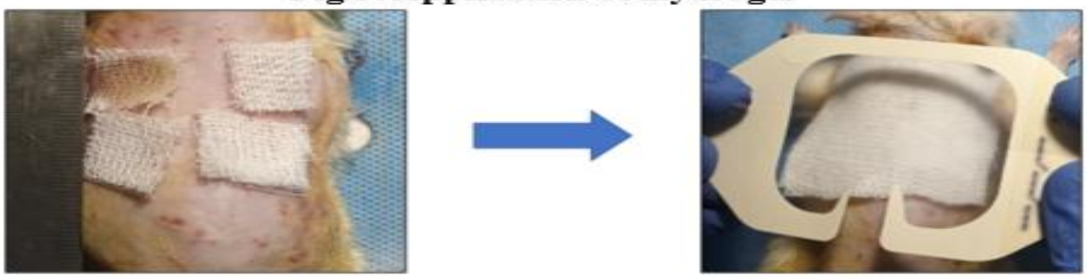


Fig 4: a. Wound individually covered with Gauze after the application of samples; b. Fully dressed dorsal skin by using Tegaderm

Wound Contraction Assessment

Wounds treated and untreated in the standard positions were photographed at relatively same heights. Visual inspection of the wound bed was carried out by taking photographs until the end of the experiment. All wounds were assessed on days 0, 5, 10, 15, and 20 until the wound was completely healed. At the end of the experiment, on the 21st-day tissues were dissected and examined for histopathological analysis.

Histopathology of Tissues

To evaluate the morphological changes, the excised tissues were fixed in 10% formalin and embedded in paraffin wax for pathological examinations section of 5 m thickness were prepared and stained with haematoxylin-eosin at the pathology laboratory, Pushpagiri Institute of Medical Science and Research Centre, Thiruvalla, Kerala, India.

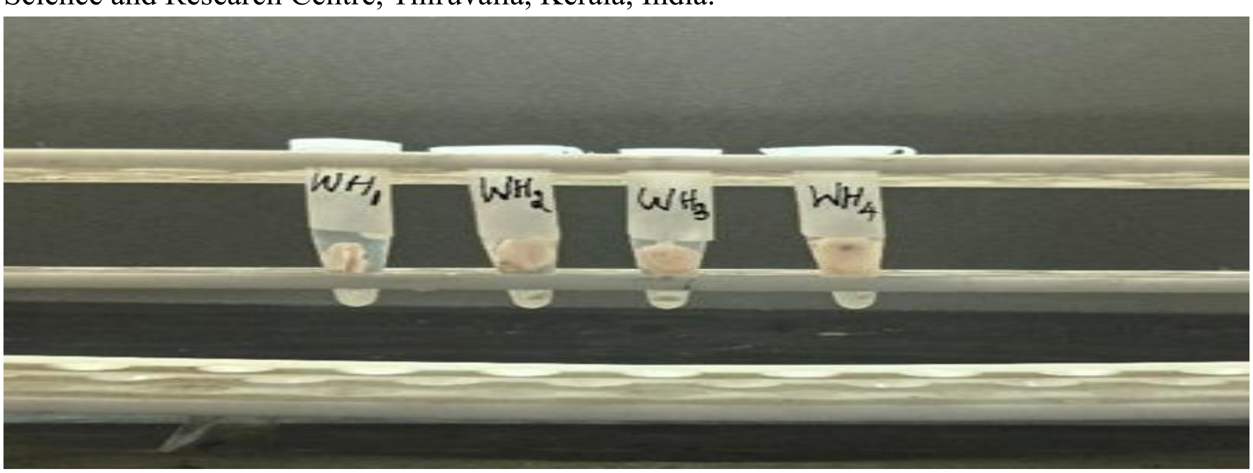


Fig 5: Skin Collected for Histopathology

III.RESULT AND DISCUSSION

In Vivo Visual Inspection

The wound is examined at five days intervals up to 20 days after the wound creation and observed data are recorded and the wounds are photographed. After that hydrogel samples are reapplied to the wound and dressed. After the 20th day mark the skin biopsy. Chronic wounds are a common and often serious medical problem, particularly among the elderly and those with underlying health conditions such as diabetes or peripheral vascular disease. These wounds can be slow to heal, which can lead to a prolonged healing process and increase the risk of complications such as infections and amputations. Despite advancements in wound care, there is still a need for effective treatments that can promote healing and prevent complications (Gao *et al*., 2002).

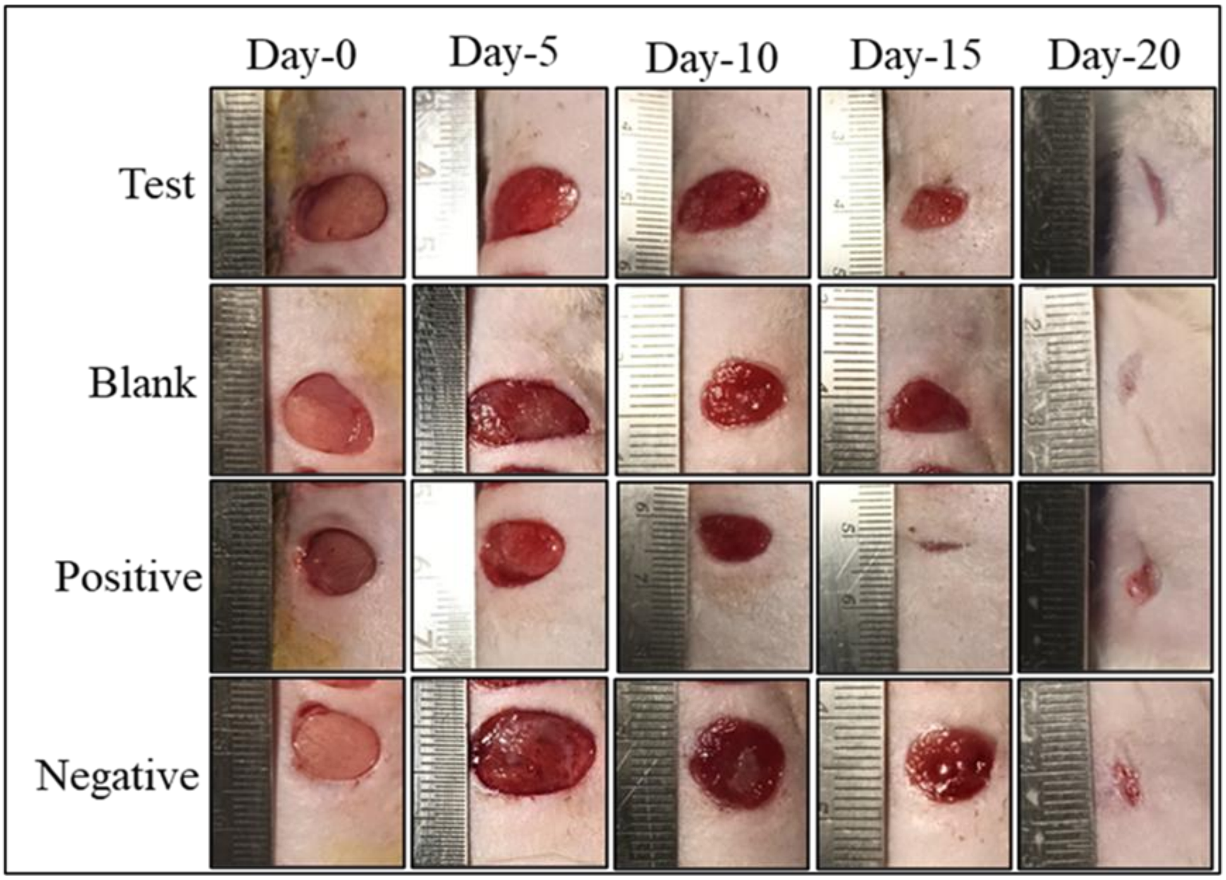


Fig 6: In vivo Wound Contraction Assay of Each Wound (Day 0^{th} – Day 20^{th})

Visual Inspection

A Rapid decrease in the diameter of the wound can be observed in which biomaterial was used. When compared to other wounds. On the 20^{th} day, the biomaterial wound was perfectly healed. But negative shows slow healing. On day 5 and 10 there were blood stains and pus on negative control and blank. Treatment and positive control show better results without any blood stain, pus and inflammation

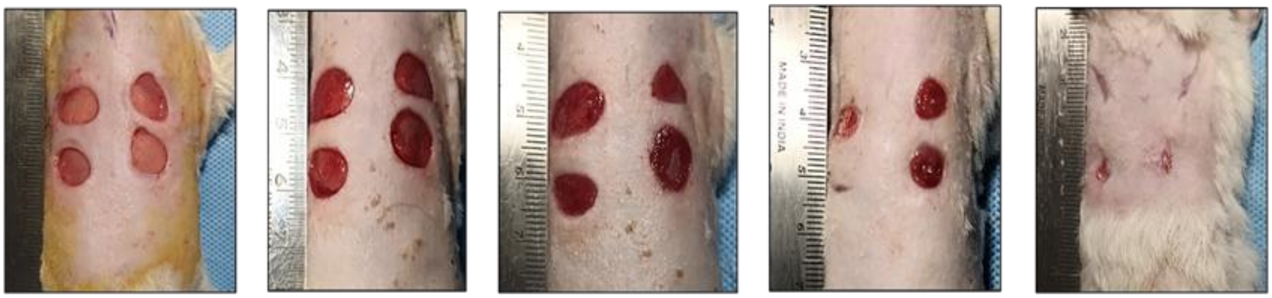


Fig 7: Wound Contraction Assay (Day 0th – Day 20th)

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