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Investigation of Wound Healing and Anti-Inflammatory Effects of Phytohemagglutinin Protein and Chitosan

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Abstract:- Open-wound complications - ranging from mild infections to amputations and organ failures- claim the lives of approximately 5 million people annually[5]. Surgical intervention remains the primary treatment for wound-caused severe bleeding, but access to healthcare is limited in many parts of the world, necessitating the development of alternative mobile solutions. This research projectaimed to address this issue by designing a bandage tailored for the treatment of severe bleeding and wound recovery. Utilizing phytohemagglutinin (PHA) protein and chitosan, the bandage exhibited exceptional wound healing rates as well as infections prevention in both the in vitro and in vivo trials, showcasing it as a promising solution for wound management and haemorrhage control in diverse healthcare settings.

I. INTRODUCTION

Wounds are a type of external injuries that cause a partial discontinuity of skin and body tissues .They are classified as acute wounds and chronic wounds, both varying in there severity and average recovery time. While acute wounds typically heal promptly, chronic wounds persist, posing complex challengesand complications.

Chronic wounds, characterized by prolonged healing and often exacerbated by underlying medical conditions, present a substantial public health concern. Beyond their prevalence, they impose substantial physical and economic burdens on individuals and society at large. In the United States alone, chronic wound care incurs an annual cost exceeding 50 billion dollars, affecting approximately 6.7 million people. On the other front, uncontrollable bleeding remains a global health challenge, claiming the lives of around 5 million individuals annually.

Traditional bandages, which have played a pivotal role in wound care throughout history, often fall short in effectively addressing chronic wounds and controlling severe bleeding. Similarly, modern wound dressings, while incorporating a wide array of drugs, frequently exhibit side effects, making them unsuitable for many cases. This research seeks to bridge this gap by designing a novel bandage that overcomes the limitations of previous wound dressings, offering a reliable solution for chronic woundsand their complications.

Our choice of solution is based off the shortcomings of previous approaches in successfully managing severe injuries while being biocompatible for most cases. To achieve this, we have harnessed the potential of two naturally occurring organic compounds: Phytohemagglutinin protein (PHA), found abundantly in some legumes specifically white and red kidney beans (Phaseolus vulgaris), and Chitosan polysaccharide, sourced from the exoskeletons of marine animals such as shrimps and crabs. In the following sections, we will delve into the methodology, testing, and findings of our investigation into the wound-healing effects of these substances.

II. MATERIALS AND METHODS

A. *Materials*

- Red kidney beans (Phaseolus Vulgaris): PHA protein source.
- Shrimp shells: Chitosan source.
- Medical dressing.
- Monosodium Phosphate (NaH_2PO_4); used in PHA extraction.
- Disodium Phosphate (Na_2HPO_4); used PHA extraction.
- \Potassium Carbonate(K_2CO_3); used in Chitosan extraction.
- Nitric Acid (HNO_3); used in Chitosan extraction.
- RPMI complete medium; used in cell culture (Fig.2).
- WI-38 cells.
- Anaesthetic liquid for inhalation (Fig.1). Different blood types samples.

B. *Instruments and Apparatuses*

- Lyophilizer machine, shown in Figure 3.
- Homogenizer, shown in Figure 4.
- Centrifugation apparatus.
- Cell Incubator.
- Lab grinding mill.
- Sensitive balance.
- Pipets.
- Light microscope.
- Neubauer haemocytometer, shown in Figure 5.

Fig 1 ANAHAL Anaesthetic Liquid

Fig 2 RPMI Complete Medium

Fig 3 Lyophilizer Machine

Fig 4 Homogenizer Machine [PRO Scientific, 2023]

Fig 5 Neubauer Haemocytometer

C. Methods

In the next subheadings, the methods and procedures for this study are explained.

- *Phytohemagglutinin (PHA) Extraction & Purification:*

The protein extraction process was carried out to obtain a high-quality Phytohemagglutinin protein. Initially, 170 grams of red kidney beans (Phaseolus vulgaris) were meticulously ground in the laboratory's grinding mill, as shown in Figure 6. Next , the ground beans were immersed in a previously prepared phosphate buffer, made from monosodium and disodium phosphates, with a pH of 7.2. This mixture was allowed to rest at a temperature of 4°C for 2 days, as illustrated in Figure 7.

Subsequently, to ensure thorough homogenization, the combination of the buffer and beans was subjected to a rigorous homogenization process for a period of 5 minutes until the beans were finely ground.

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Fig 6 Ground Phaseolus Vulgaris

Finally, the solution underwent centrifugation to separate its components, resulting in the formation of a PHA protein -rich supernatant solution, as illustrated in Figure.8. This supernatant solution was collected and was furtherly subjected to lyophilization, for the purposeof eliminating any liquid impurities and undesired proteins.

Fig 7 Buffer and Beans Mixture after 2 Days

Fig 8 PHA-rich Supernatant

➢ *Hemagglutination Assay*

This step was carried out to ensure the presence and efficiency of the PHA protein in the protein extract. The Hemagglutination assay (HA) tests the agglutination properties of the protein by adding it to serially diluted concentrations of different human blood types in a 96-well plate and leaving it to set for 10-15 minutes. After that period, if the Phytohemagglutinin protein is present, the red blood cells in the blood samples should clump together forming a suspended red lattice without any sickling or precipitation. The HA test was preformed as shown in Figure.9 and the RBCs in all blood samples formed a floating lattice instead of a precipitation, ensuring the abundance and effectiveness of the PHA protein in the purified protein powder, and the absence of any foreign, undesirable proteins.

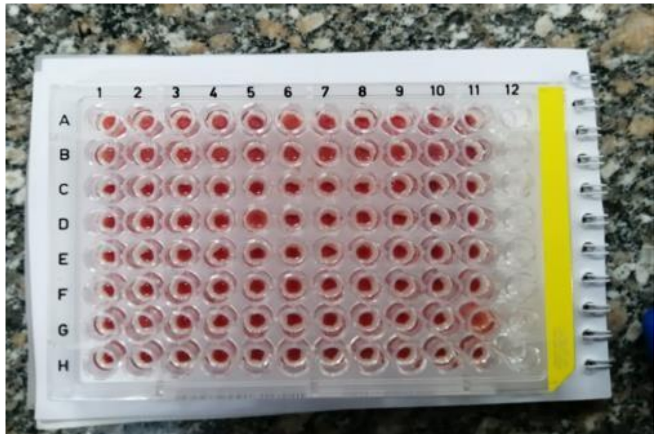


Fig 9 Hemagglutination Assay after Setting for 15 Minutes

➢ *Chitosan Extraction Procedure:*

The chitosan extraction started after the shrimp shells were deliberately washed with distilled water to remove any impurities. it had three steps which were deproteinization, demineralization and deacetylation. These steps aim to liberate the chitosan in the shrimp shellsfrom the compounds bound to it, which are mainly proteins, minerals ($CaCO_3$), and acetyl- groups ($-COCH_3$).

- *Deproteinization:*

In the deproteinization process, a 5% potassium carbonate (KCO_3) solution was addedto the dried shells at a solid-to-liquid ratio of 1:10 (g/mL) as shown in

Figure.10. The reaction proceeded with continuous agitation for a duration of 3 hours. Following the reaction, the solid material was carefully filtered, and rinsed with distilled water until a neutral pH was achieved. At this stage, the resultant solid material comprised a mixture of chitin - the sourcefor the chitosan - and calcium carbonate ($CaCO_3$).

- *Demineralization:*

Fig 10 Shrimp Shells and KCO3 Mixture

Demineralization was carried out by adding 1 L of 5% HNO3 to the deproteinized shrimp shells. The reaction proceeded at room temperature under agitation at 250 rpm for 2 hours producing chitin. Afterward, the chitin-containing shells were filtrated and washed with distilled water until neutral pH is achieved.

- *Deacetylation:*

Finally, the deacetylation of chitin was accomplished by immersing the chitin-containing shells in a 50% potassium carbonate (KCO3) solution at a controlled temperature of 120°C for an approximate duration of 6 hours, resulting in the formation of chitosan.

➢ *In Vitro Test*

This test was carried out compare the cell proliferation effects of chitosan, Phytohemagglutinin, their mixture, and the control sample while they are absent. It also allowed us to determine the perfect concentrations of each one mentioned above. A 24-well plate was used to carry out the test as shown in Figure.11. Each well contained approximately 30000 WI-38 cells, 1 ml of RPMI complete medium, and one of either PHA, chitosan, or their mixture with ratio of 1:1; except for the control sample that nothing else was added to it. Each row was serially diluted starting with the concentration of 1 mg/ml all the way to 31.25µg/ml. After each well was scratched and put in the incubator for 48 hours, the best results in all 24 wells were present in the 1:1 mixture well with concentration of 31.25µg/ml as shown in Figure.12. This concentration of 31.25µg/ml happened to be the concentration at which all of the wellspreformed the best at as shown in Table.1.

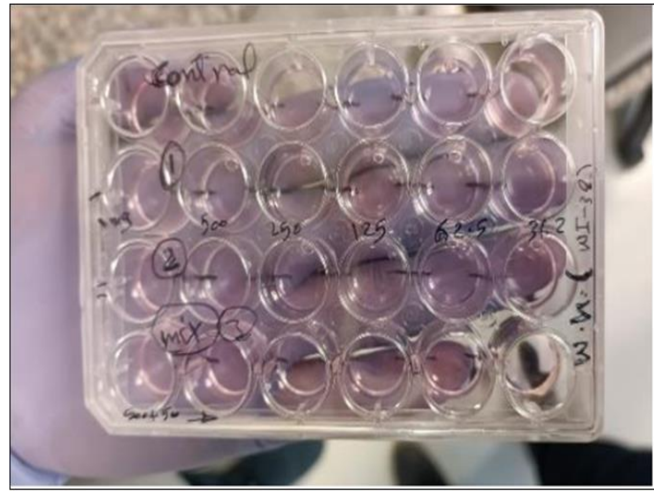


Fig 11 In Vitro Test

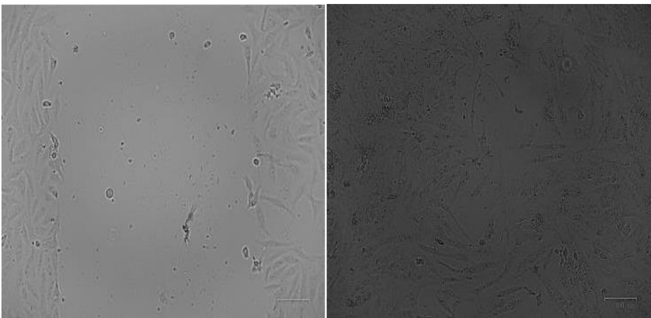
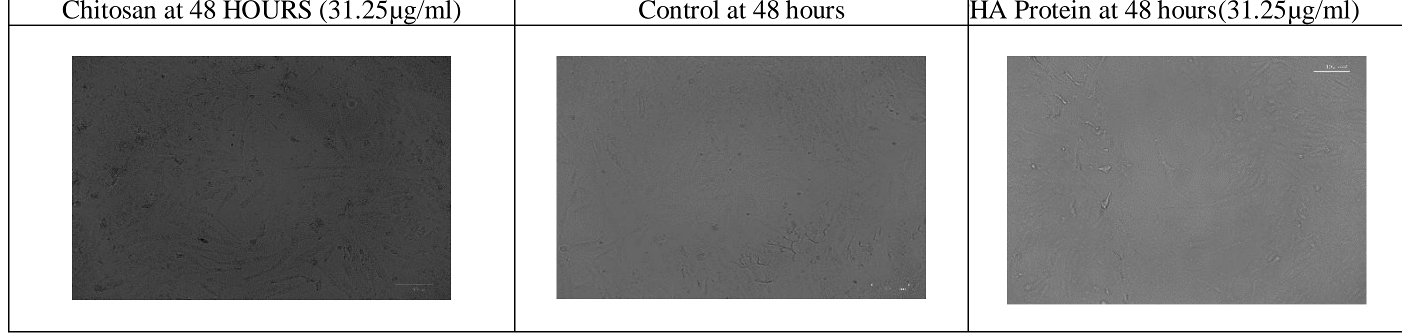


Fig 12:- 1:1 Mixture well with Concentration of 31.25µg/ml. On the left is the well on zero time and in the right is the well after 48 hours.

Table 1 Chitosan, PHA, and Control Samples after 48 Hours

Chitosan at 48 HOURS (31.25µg/ml)	Control at 48 hours	HA Protein at 48 hours(31.25µg/ml)



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- *In Vivo Test*

After determining the perfect ratio and concentration of PHA protein and chitosan mixture in the *in vitro* test, it was time for implementing the same test on living organisms in order to observe and screen for any abnormal reactions or effects it might have on them. The chosen animal species was the albino rat as it served as a perfect subject for our test. The rat experiment involved two rats each having two medium sized wounds. The four wounds were each covered with bandages labelled as control, PHA protein (31.25µg/ml), chitosan (31.25µg/ml), and chitosan-protein mixture (31.25µg/ml). The bandages were changed every 48 hours, and the wounds were periodically photographed. This experiment had similar results to the *in vivo* test since the control wound did not show significant improvement after 48 hours or 5 days. Conversely, the chitosan bandage showed a moderate effect, with a slight reduction in wound size and rapid clotting but incomplete healing on the fifth day. The protein bandage, on the other hand, showed a significant healing effect, with the wound shrinking almost being completely covered after 5 days, but blood clotting was delayed. Finally, the bandage containing both protein and chitosan demonstrated the most effective combination of the positive effects observed in the other bandages. The phytohemagglutinin protein in the bandage contributed to rapid healing and disinfection as it activates the lymphocytic cells in the blood stream, while chitosan's anti-inflammatory and blood clotting abilities helped stop the bleeding instantly, and the injury was fully healed in 5 days. The following table shows the progression of each wound healing over the five days.

Table 2 Comparison of the Four Wounds after 2 and 5 Days of Healing

WoundType	Control	Chitosan	PHA protein	1:1 Mixture
Wounds After2 days				
Wounds After5 days				

III. FUTURE PROSPECT

While the results have shown promising outcomes, there are several future directions that we plan to pursue to further improve the efficacy and safety of this treatment.

One area of interest is the recycling of red kidney beans' peels in the extraction of protein and purification of phytohemagglutinin protein. This has the potential to reduce waste and make the production of the wound dressing more sustainable.

In addition, we plan to improve the design of the bandage to optimize its healing properties. This could involve exploring different materials or structures that could enhance the effectiveness of the chitosan-protein mixture. Furthermore, we intend to conduct further studies to evaluate the safety and effectiveness of this treatment in human patients. This will involve investigating potential allergic reactions or adverse side effects and testing the wound dressing in larger, more diverse patient populations.

Finally, we believe that there is potential in combining the chitosan-protein wound dressing with other treatments or therapies to enhance its healing properties even further. We plan to explore this possibility in futureresearch to see if we can achieve even better outcomes for patients with various types of wounds.

IV. CONCLUSIONS

The findings of this experiment suggest that the use of a chitosan-protein mixture in wound dressings could have significant benefits for wound healing and, consequently, for healthcare in general. The rapid healing properties of phytohemagglutinin protein combined with the disinfectant and clotting abilities of chitosan could potentially lead to faster and more effective wound healing, reducing the healing time and overall healthcare costs. The promising results of the rat and rabbit experiments also suggest that this

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treatment has the potential to be a valuable addition to the existing wound care options, providing better outcomes for patients. Further research and clinical trials are needed to fully explore the potential benefits and ensure the safety and effectiveness of this treatment in human patients. The bandage will also replace the stitching of wounds that need professionals in order to perform them so that people that don't have access to efficient health care can use this bandage in order to recover from wounds easily, which can help reduce the severely bad health problems that wounds could possibly cause and reduce their effect on the vital functionsof the body.

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