SAFETY, EFFICACY & COMPARATIVE STUDY OF VARIOUS WOUNDS WITH DIFFERENT WOUND HEALING AGENTS-FORMULATION IN SPRAGUE DAWLEY RATS

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ABSTRACT

Aim: Safety, efficacy & comparative study of various wounds with different wound healing agents/formulation in Sprague Dawley Rats

Material and method:

Material: Chitosan (2%), RTX [Rutoheal-D] (0.04%), HOCL [Hypochlorous acid] (0.006%), STS [Sodium thio Sulphate] (0.25%), Betadine (5%).

Method: Excision Wound Model was used for comparative profile for experimental animals (Sprague Dawley male rats). We evaluated the anatomical, macroscopically, histopathological alterations in wounds of experimental rats, epithelialization of wounds and hematological parameters in experimental rats. Treated with negative control (test formulations) such as: FA1, FA2, FA3, FA4 and positive control is Betadine (FA5). The test formulation primary evaluated by some parameters, following as: pH, spreadability, drying time of gel and skin irritation test.

Conclusion: The group treated with chitosan (FA1) and HOCL (FA3) solution showed better wound healing activity as compared to control group as well as Betadine. The all formulations showing wound healing effect but chitosan and HOCL showing significant improvement in epithelial tissue. Wound re-epithelization was found 86% wound healed with chitosan 2% gel and 83% healed with HOCL in 7 days. Chitosan and HOCL having higher degree to healing dermal wound and use for the treatment in wound healing.

Keywords: Chitosan gel, RTX gel, STS gel, HOCL solution, Betadine, Wound healing, Histopathology, Epithelization.

I. INTRODUCTION

The external surface of the body is covered by the skin. Skin acts as water repellent, help in thermoregulation, and also synthesize various beneficial compounds like vitamin D and most significantly it serves as a protective covering between the outer environment and internal body tissues. According to medical dictionary; due to any accident, act of violence or surgery, breaching of skin or underlying tissue takes place which is called as wound. Following an injury, the skin has a tremendous capacity to heal. A wound is any destruction or damage in the lining of the skin. Wounds can be:

Accidental for example, burns, abrasions, paper cuts, skin tears.

Surgical for example an incision to remove a diseased appendix.

Occur because of underlying disease for example diabetic and vascular ulcers.

Some skin conditions may also develop into a wound for example eczema or psoriasis.
Generally, wounds are of two types: acute and chronic. Acute wounds converted into chronic wounds when acute wounds could not progress through the stages of healing normally. They may recover at a very slower rate, heal only partially or reoccur after partial or complete healing. These chronic wounds are almost always associated with underlying chronic diseases that affect either the blood supply or how the cells function at the wound site. Wounds that take a long time to heal need special care. It is important to not only treat the wound but also diagnose and address the underlying condition causing the wound to minimise risk of further chronic wounds.

In history of wound healing before the 1960 were considered by the lethargic product having minimum wound healing role. The revolutionary research initiated the role of Wound healing concept and active contribution in environmental wound repair. [1] In since 1980’s, different types of wound dressing created, but some effects cannot have treated by these dressings such as antibacterial effect, wound healing promotion and another effects. But in 2019 by Wan-Yi Zhao MM treating all these effect by the chitosan-calcium alginate dressing (CCAD). They permute wound healing effect. [2] Skin is a primary protective barrier against the environmental germs and toxic substance. Skin injury or illness causes and disability, sometime death. [3] A wound is a disruption in the epithelial reliability of the skin and may be attended by disruption of the structure and function of underlying normal tissue. [4] A wound shows many result from a contusion, hematoma, laceration or abrasion, it is caused by burns, ruptures, animal bite, etc. Sometime Unnecessary blood flow during wound is a major cause of mortality. Cellular immunity has important work in wound healing such as protect against infection, tissue repair and increases phagocytosis, it’s done by pro-inflammatory cytokines such as Interleukin-1, Interleukin-8 and Tumor necrosis factor (TNF). Cytokines are regulated by fibroblasts and endothelial cell. [5] excessive amount of neutrophils leads to larger amount of degradative matrix metallic proteinases (MMPs), exclusively MMP-8 and neutrophil-derived elastase. The MMPs delayed wound healing process. [6] Slow wound healing or non-healing wounds and some injuries is the typical condition for animals and humans.

Basically wound healing have some steps to heal the wound. These steps are inflammation phase, epithelialization phase, tissue granulation, collagen synthesis phase and wound contraction phase. [7] Healing of wound has main 4 stages for wound ending Hemostasis, Inflammation, The Proliferative phase (re-epithelialization, granulation and neo-angiogenesis) and The Remodeling phase (Maturation Phase). Firstly, cytokines releases surrounding to the wound tissue site, then fibrin collecting by fibroblast cell known as collagen and providing the power for regenerating tissue. [8] Endothelial cells, fibroblast, monocytes and lymphocytes cells are involved in wound healing. [9] These cells form nitric oxide and its shows antibacterial activity, according to wound impaired models. [10] In the early stage in wound healing, amino acid found to cell proliferation. [11]

II. MATERIALS AND METHODS

Reagents and Chemicals
Chitosan (INMAS laboratory, DRDO), Sodium-Thio-Sulphate (Thomas Baker, Mumbai), Hypochlorous Acid Solution (INMAS, DRDO Delhi), Betadine Ointment 5% USP (win Medicare Pvt, Ltd). Glycerol (fisher scientific), Carbapol (Thomas Baker, Mumbai), HPMC K4M (Merck Pvt, Ltd), Acetic Acid (Merck Pvt, Ltd).

Percentage (%) of test and standard formulation using in experiment

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Abbreviation</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitosan gel</td>
<td>FA1</td>
<td>2% w/v</td>
</tr>
<tr>
<td>RTX gel</td>
<td>FA2</td>
<td>0.04% w/v</td>
</tr>
<tr>
<td>HOCL solution</td>
<td>FA3</td>
<td>0.006% v/w</td>
</tr>
<tr>
<td>STS gel</td>
<td>FA4</td>
<td>0.25% w/v</td>
</tr>
<tr>
<td>Betadine</td>
<td>FA5</td>
<td>5% w/v</td>
</tr>
</tbody>
</table>

PREPARATION OF FORMULATION
Chitosan Gel (2%) w/v {FA1}
Gel of chitosan was prepared by concentration (2%) w/v. Chitosan was dissolved in 0.5% of acetic acid (v/v). Then add Glycerin for the preservation and also effect as humectant for the skin. Glycerin also helps in wound healing. Stir properly until gel was completely formed. It was then sonicated till all air bubbles were removed. [12]

RTX Gel (0.04%) w/v {FA2}
HPMC K4m (2%) was dissolved in boiling water, then keep it suddenly in freezer until clear phase is found, then add Carbapol (0.5) w/v and stirring continuously. Add Rutoheal-D tablet powder (0.04%) w/v. Then stir until gel form prepare. Sonicate gel formulation for removal of air bubbles. Preparation of RTX gel, take Rutoheal-D powder (0.04%) h)

HOCL (Hypochlorous acid) (0.006%) V/W {FA3}
HOCL is taken from INMAS (DRDO) lab, its pre-formulated for a clinical trial. HOCL Preparation is done by mixing the chlorine with water under the dissolution process. HOCL contain some active ingredient such as: bleach and chemical having base (OCl-), they are responsible for anti-micro-organism. Solution is stored in amber color bottle because its light sensitive solution.

STS gel (0.25%)W/V {FA4}
HPMC K4m (2%) was dissolved in boiling water at 60c, and then freeze suddenly to dissolve, until HPMC solution becomes clear. Then it was stirred continuously& Glycerine (0.2%)v/v was added. Add STS (Sodium-Thio-Sulphate) powder (0.25%) w/v weighed quantity in 1 ml methanol & added in to the HPMC and glycerine mixture. After this mixture was stirred by magnetic stirrer until the hydrogel was completely not prepared. STS Gel formulation was prepared, using bath sonicator. Bath sonicator help to eliminate extra air bubbles.

Betadine ointment (5%) W/W
Antiseptic for the topical treatment or prevention of infection in minor cuts. It releases iodine which results in the death of microorganisms. It was purchased from local medical store. Betadine is used in this experiment as a positive control drug.

EVALUATION OF TEST FORMULATION
All formulations FA1, FA2 and FA4 characterized by the following parameters

Organoleptic property
Organoleptic characterization was done manually and some parameters were identified such as: color, texture, solubility and odor. a small quantity of the formulated gels was tested by pressing between the index finger and thumb, to evaluate the consistency of test gel formulations. Gel formulation feel sticky and have gel like texture. [13]

pH Evaluation
Evaluation of the pH was done by taking 1 gm gel from each gel formulation and mixing with 25mL of deionized water. then clean the pH electrode with deionized water. Determination of the pH using pH meter (LAQUA PH1100, HORIBBA). The pH evaluation process was repeated three times and taking a mean value to defining the actual value of each formulation. The pH was adjusted with standard buffer solutions (pH 4, 7, and 10) before using for each formulation. [13] & [14]

Spreadibility test
Spreadibility of all gel test formulations was evaluated by the spreading diameter of gel. Firstly, take two horizontal glass slide and pour 1 gm of gel. The gel pours only one side of the slide and place a second glass. Then applied a standard weight on the both glass side, the standard weight is 25g for one minute. This procedure repeated for three time for the standard mean value. [15]

Drying time of gel
The drying time is a time consuming process. All test gel (FA1, FA2, FA4) take 2 ml of each formulation and placed in an unabsorbed surface and glass slide. Pour 2 ml of gel in the surface and measure the surface area of
poured gel. Stay until dry (at least) 12 hours then again measure the surface area of gel. 16 & 17. Because HOCL is a light sensitive solution, so its evaporate in air contact.

Skin irritation test

Test of Skin irritation was performed on (Adult Sprague Dawley male) rat for comparative evaluation potential of test gel with marketed formulation. Weighing rats acclimatized before the study. Dividing rats in to the seven group (n=6). Follow as:

Group 1: No application of any drug.
Group 2: Wound control
Group 3: Chitosan Gel (FA1)
Group 4: RTX Gel (FA2)
Group 5: HOCL Solution (FA3)
Group 6: STS Gel (FA4)
Group 7: Betadine ointment USP (FA5)

Before the experiment dorsal side of rat shaved and leave rats for 24 hrs. according to divided groups all test formulation applied on each rat by a sterile spatula. After the applied test formulation leave the rats in the cages for 24 hrs. The formulation applied area should be 4cm. the rat skin showing any observable changes such as: redness and edema, that’s mean the test formulation showing irritating effect against the skin. [18] & [19]

In Vivo Experimental Studies Animal

Excision wound model

The all experiments performed According to the guidelines for Care and Use of Animals in Scientific Research (Indian National Science Academy 1998, Revised 2000). In this experiment 42 Adult Sprague Dawley male rats and all rats under (150gm -250gm) weight. All rats divided into groups of 7 (n=6) and the cages of animal properly clean with corn beading. The all cages placed on suitable temperature at 25 ± 2°C. Animals were allowed to access standard laboratory diet (Lipton Feed, Mumbai, India) and water present in the cages whole the duration of the study. The protocol of experimental was permitted by the Experimental Animal House, Institute of Nuclear Medicine and Allied Sciences, DRDO, New Delhi, India.

Before the experiment group 2,3,4,5,6 & 7 of rat anesthetized by the route of intramuscular administered xylazine and ketamine. The dose of xylazine and ketamine 4-5 and 40mg/kg, individually by giving mixture. Firstly, mark the dorsal site of rat on each group, then hair clean by the using surgical razor form the skin surface area. Before the shaving the hair, the razor was disinfected with 70% methanol 24 hour.

On the dorsal clean area clean by disinfectant and a deep surgical cut by the using punch biopsy device after the administration of general anesthesia. Wound area around 1 cm and A circular wound measuring around 0.8 - 1 mm in diameter, then dermal and epidermal layer of skin should be separated. Each rats of all groups preserved under observation to the 0- 14th day and measure some changes such as: wound size, behavior changes after given wound and appearance were recorded. Before the drug applied the wound area clean by disinfectant. Every Animals of all group was treated with test and standard formulation defining in table: 2. [20]

After given wound all animal of each group were given treatment by applying the respective observation day 0 to day 14th wound size, weight variation, eating, drinking, sleeping behavior, facial changes photos and wound healing photos click until the wound ending.

days.

Table: 2. Wound healing in-vivo experimental designing

<table>
<thead>
<tr>
<th>So.no.</th>
<th>Groups (n=6)</th>
<th>Drug formulations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Group 1</td>
<td>Non-treated</td>
</tr>
</tbody>
</table>
ANALYTICAL PARAMETERS FOR IN-VIVO EXPERIMENT

Weight variation: Firstly, all rats divided into the 7 (n=6) respective groups. Each group having 6 animals were weighed on daily basis. The weight was recorded each day before dosing to analyze the increase or decrease in weight of animals during 14 days’ study. The animals were dozed with the formulation twice daily for 14 days.

Measurement of Wound Contraction and Epithelialization Period: The all divided rats treated with both test and standard formulation. The formulation applied twice in a day and wound covered with a no adhering bandage. The bandage replaces in 24 hrs. adhering bandage use for the covering the wound site, its protect the wound area for any other infection. The bandages replaced in 24 hrs (after one day). Before the application of formulation all rat wound measure by the scale and the scale marked under millimeters (mm). wound size measure the alternate days 0, 3, 7, & 14th. wound area contraction was calculated using Equation (1):

\[
\% \text{ wound contraction} = \left( \frac{A_1 - A_2}{A_1} \right) \times 100
\]

A1= Initial wound area, A2= Specific wound area.

Biochemical and hematological studies: Firstly, all rats divided randomly into 1-7 groups, each group having (n=6). all groups dividing according to the (table 10) The treatments were given two time daily for 14 days. Rats were monitored daily. Day 1 collecting blood for pre hematological study and day 14 collecting blood for post hematological study. Collecting blood from the rat eye, the part is call orbital sinus. Blood collecting through a glass capillary. The collected blood stored in EDTA (K2) and (Gel + BCA) tube.

Histological Examination: For the histopathological examination, firstly prepare 10% of formalin solution for the storage of wound area tissue. Then fixing expunged wound sites 10% formalin, and entrenched in paraffin. A small section takes from the wound cut area and stained by hematoxylin. Slide stained section placed under microscope and take photograph at 40x.

Statistical Analysis: All data were represented as mean ± SEM (standard error of mean [n = 6]). Statistical analysis was done by one-way analysis of variance (ANOVA) followed by t-tests using the Graph Pad Prism version 8.4.3. P < 0.05 was considered as statistically significant.

III. RESULT & DISCUSSION

Organoleptic properties

The organoleptic properties help to identify odor, texture, color and solubility of compound. It’s a primary screening of test formulation.

<table>
<thead>
<tr>
<th>S.no.</th>
<th>Drug</th>
<th>Odor</th>
<th>Color</th>
<th>Solubility</th>
<th>Texture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chitosan</td>
<td>Pungent</td>
<td>Pale yellow</td>
<td>Acetic acid</td>
<td>Springiness</td>
</tr>
</tbody>
</table>
ANALYTICAL EVALUATION OF TEST FORMULATION

Post-Formulation characterization of an all test formulation was done by some parameters such as: Organoleptic property, pH, spread ability, drying time and skin irritation test of all the test gel preparations {FA1, FA2, F3, FA4}.

pH Evaluation

All the formulations pH of was favorable to the skin and any irritating effect not sowing on the applied area, that’s mean the all formulation value is not more than acidic and not less than basic range. The all formulation pH results showing in (Table.4).

<table>
<thead>
<tr>
<th>S.no</th>
<th>Test formulation</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chitosan gel</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>RTX gel</td>
<td>6.4</td>
</tr>
<tr>
<td>3</td>
<td>HOCL solution</td>
<td>6.5</td>
</tr>
<tr>
<td>4</td>
<td>STS gel</td>
<td>6.2</td>
</tr>
</tbody>
</table>

Spreadibility test

Spread ability of gel is very much important for show the behavior of gel comes. The values of spread ability shown in (table.5) Small amount of gel spread in small area indicates the polymers use in the gel. If concentration increasing of gel that’s means deceasing the spreadibility and lower the diameter of gel in between glass sides.

<table>
<thead>
<tr>
<th>S. no</th>
<th>Gel</th>
<th>Spreadibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chitosan {FA1}</td>
<td>3.24</td>
</tr>
</tbody>
</table>
Drying time

Drying time of test gel is easily dry at room temp within 10-12 hrs. showing in fig: 3 because test formulation (FA1, FA2, F4) prepared with polymers, after drying gel formulation showing a transparent film of polymer. Measure a gel film area, after and before the drying of gel. Procedure repeated three time and take a mean value.

Table:6. Drying time of test formulation

<table>
<thead>
<tr>
<th>S. no</th>
<th>Test formulation</th>
<th>Drying time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0-hrs</td>
</tr>
<tr>
<td>1</td>
<td>Chitosan {FA1}</td>
<td>1.91</td>
</tr>
<tr>
<td>2</td>
<td>RTX{FA2}</td>
<td>0.53</td>
</tr>
<tr>
<td>3</td>
<td>STS {FA4}</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Primary skin irritation

Skin irritation and epidermal thickness in hairless rats treated with all test formulations. The primary skin irritation testing is more favorable for gel testing, it is used for identification of gel having higher acidic pH and
irritating material in gel formulation, which showing slight sing or irritation after applying gel for 48 h. the present test formulation (FA1, FA2, FA3, FA4) not visualize redness, edema up 72 hrs. because all test formulations having neutral pH (6 to 7) and they don’t have any irritating material in test formulation. The result come out after primary skin irritation test not showing any sing (edema, redness) after applying test formulations on the rat skin.

IN-VIVO EVALUATION STUDY

In the wound developed animal model, a single skin wound (about 1 cm in diameter) was create on the dorsal surface of the rats. The rate of wound contraction was evaluated by Eq:1. Determination the open wound area healed with in time (showing in Figure 3 and 4) by applying the control (FA5) and treatment formulation (FA1, FA2, FA3 and FA4). The wounds treated with FA1 and FA3 were showing contract at much higher as compared with other groups of rats with different treatments showing in the figure. On day 3, wounds treated with formulations, but FA1 and FA3 formulation had more wound contraction and epithelial tissue. Than wounds with any other treatment. These two formulation FA1 (51%) and FA3 (45%) to the other formulations FA2, FA4 and FA5 (control group). On day 7, treatment with FA1 and FA3 a remarkable difference was observed in wound contraction FA1 (86%) and FA3 (83%) wound closure on the dorsal surface (Figure 3). Same as compare, wound control group(G2) rats receiving no treatment on wounds, then the wound contraction and wound epithelization result shows slowly and around 64% wound closure. when treated with (FA1, FA2, FA3, FA4 and FA5) On the 14th day of the experiment, wound of the GA3 (99.9%), GA5 (99.9%), group was totally healed with minimal scarring but GA2 (89%) and GA4 (86%) was not completely healed.

<table>
<thead>
<tr>
<th>Table:7. Showing efficacy of test formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Day 3</td>
</tr>
<tr>
<td>Day 7</td>
</tr>
<tr>
<td>Day 14</td>
</tr>
</tbody>
</table>

ANALYTICAL PERAMETERS FOR IN-VIVO EXPERIMENT

Weight variation

The weight variations in different groups were noted and it was observed that there was a significant decrease in weight of these animals when compared with the control group (untreated). This data in Fig:4 clearly depicted that as the strength of dose increases there was a noticeable loss in weight of Sprague Dawley rat animal indicating. On the other hand, there was an increase in weights up to a certain level of the animals after treatment with all test formulation. It was observed that there was a substantial increase in weight in the group 1, 3 and 5 after the day 7th and 14th respectively. Moreover, the animals of groups 2, 4, 6 and 7 showed weight increases.

<table>
<thead>
<tr>
<th>Table:8. Showing weight variation on different days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>GROUP 1</td>
</tr>
<tr>
<td>GROUP 2</td>
</tr>
<tr>
<td>GROUP 3</td>
</tr>
<tr>
<td>GROUP 4</td>
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<tr>
<td>GROUP 5</td>
</tr>
<tr>
<td>GROUP 6</td>
</tr>
<tr>
<td>GROUP 7</td>
</tr>
</tbody>
</table>
Figure 4: Evaluating weight variation in GA1 (NON-treated), GA2 (Wound treated), GA3 (Chitosan treated), GA4 (RTX treated), GA5 (HOCL treated), GA6 (STS treated) and GA7 (Betadine treated).

Wound measurement and re-epithelization:
Percentage (%) of all group measure by the GA1 (no wound and no treatment), GA2 (only wound or non-treatment), GA3 (FA1 treated), GA4 (FA2 treated), GA5 (FA3 treated), GA6 (FA4 treated), GA7 (FA5 positive control treated). In the 14 days’ observation, wounds without any treatment showed very less contraction of size of wound on day 3. The process of Wound healing started further conformed epithelization and scar. The control wounds (GA2) as compared the epithelialization of wounds treated with either FA1, FA2, FA3, FA4 and FA5. The rate of re-epithelialization increased on day 14 showing in (figure: 5). Wound contraction evaluating by eq. no:1.

Table 9. Wound contraction in test and control group

<table>
<thead>
<tr>
<th></th>
<th>Group 2 (non-treated)</th>
<th>Group 3 (Chitosan gel)</th>
<th>Group 4 (RTX gel)</th>
<th>Group 5 (HOCL)</th>
<th>Group 6 (STS gel)</th>
<th>Group 7 (Betadine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 3</td>
<td>31%</td>
<td>51%</td>
<td>30%</td>
<td>44.90%</td>
<td>30%</td>
<td>38%</td>
</tr>
<tr>
<td>Day 7</td>
<td>44%</td>
<td>86%</td>
<td>56%</td>
<td>83%</td>
<td>60%</td>
<td>61%</td>
</tr>
<tr>
<td>Day 14</td>
<td>71%</td>
<td>99.90%</td>
<td>87%</td>
<td>99.90%</td>
<td>88%</td>
<td>86%</td>
</tr>
</tbody>
</table>

Figure 5: Percentage of wound contraction for in vivo wound healing experiments. The rate of wound contraction in control and experimental rats at day 3 to 14 days. Values are expressed in (mean ±)
The figure represents size of wounds measured on day 1, 3, 7 and 14th of the study respectively.

The group treated with Chitosan and HOCL solution showed better wound healing activity as compared to control group as well as Betadine. It was observed that there was a significant decrease (~95%) in the size of the wound of the group treated with chitosan gel and HOCL solution, which means that it heals the wound faster as compared to other groups. Firstly, the chitosan gel decreased the wound size at a faster rate as compared to the control and groups treated with other formulations within 1 to 14 days.

Biochemical and Hematological parameter test.

Table: 10. Showing pre-hematological and biochemical parameter of rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Hemoglobin</th>
<th>Total RBC</th>
<th>Total WBC</th>
<th>Platelet Count</th>
<th>BUN</th>
<th>Serum Creatinine</th>
<th>S GOT</th>
<th>S GPT</th>
<th>A.P</th>
<th>Blood Urea</th>
<th>Bilirubin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.41</td>
<td>7.35</td>
<td>33.9</td>
<td>8.6</td>
<td>18.08</td>
<td>0.91</td>
<td>266.83</td>
<td>82</td>
<td>366.66</td>
<td>28.33</td>
<td>0.90</td>
</tr>
<tr>
<td>2</td>
<td>14.75</td>
<td>7.93</td>
<td>20.6</td>
<td>7.76</td>
<td>14.55</td>
<td>0.92</td>
<td>264.66</td>
<td>69.5</td>
<td>366.33</td>
<td>31.16</td>
<td>0.28</td>
</tr>
<tr>
<td>3</td>
<td>14.38</td>
<td>6.72</td>
<td>35.1</td>
<td>17.11</td>
<td>13.60</td>
<td>0.94</td>
<td>306</td>
<td>75.3</td>
<td>379.66</td>
<td>34.86</td>
<td>0.25</td>
</tr>
<tr>
<td>4</td>
<td>13.93</td>
<td>7.57</td>
<td>19</td>
<td>7.76</td>
<td>24.29</td>
<td>0.93</td>
<td>235</td>
<td>82.5</td>
<td>601.5</td>
<td>45.16</td>
<td>0.24</td>
</tr>
<tr>
<td>5</td>
<td>16.41</td>
<td>6.47</td>
<td>39.8</td>
<td>17.9</td>
<td>19.82</td>
<td>16.66</td>
<td>267.33</td>
<td>119.16</td>
<td>707.16</td>
<td>31.33</td>
<td>0.27</td>
</tr>
<tr>
<td>6</td>
<td>14.56</td>
<td>6.37</td>
<td>40.8</td>
<td>6.92</td>
<td>16.06</td>
<td>1.01</td>
<td>250.15</td>
<td>78.1</td>
<td>502.16</td>
<td>32.33</td>
<td>0.23</td>
</tr>
<tr>
<td>7</td>
<td>14.7</td>
<td>7.28</td>
<td>38</td>
<td>7.79</td>
<td>15.90</td>
<td>1.02</td>
<td>237.83</td>
<td>69.6</td>
<td>361</td>
<td>30.26</td>
<td></td>
</tr>
</tbody>
</table>
Figure: 7 showing hemoglobin, total RBC, WBC, platelet counts and BUN in pre-hematological study.

Figure: 8 Showing SGOT, SGPT, A.P and Blood urea.
Figure 9: Showing bilirubin and serum creatinine level in pre-hematological data

Table 11. Post-hematological and biochemical parameter of rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Hemoglobin</th>
<th>Total RBC</th>
<th>Total WBC</th>
<th>Platelet Counts</th>
<th>BUN</th>
<th>Serum Creatinine</th>
<th>SGOT</th>
<th>SGPT</th>
<th>A.P</th>
<th>Blood Urea</th>
<th>Bilirubin</th>
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<tr>
<td>1</td>
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<td>7.72</td>
<td>13.6</td>
<td>13.6</td>
<td>7.80</td>
<td>12.3</td>
<td>0.98</td>
<td>238</td>
<td>83.8</td>
<td>404.16</td>
<td>23.3</td>
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<td>2</td>
<td>14.71</td>
<td>7.04</td>
<td>12.1</td>
<td>12.1</td>
<td>7.95</td>
<td>10.7</td>
<td>0.89</td>
<td>240.16</td>
<td>87.3</td>
<td>434.33</td>
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<td>14</td>
<td>8.29</td>
<td>14.3</td>
<td>14.3</td>
<td>9.00</td>
<td>12.3</td>
<td>0.94</td>
<td>275.66</td>
<td>90</td>
<td>412.33</td>
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<tr>
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<td>6.99</td>
<td>13.2</td>
<td>13.2</td>
<td>7.98</td>
<td>17.3</td>
<td>0.91</td>
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<td>86</td>
<td>442</td>
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<tr>
<td>5</td>
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<td>7.68</td>
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<td>11.4</td>
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<td>13.7</td>
<td>0.9</td>
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<td>101.15</td>
<td>136.16</td>
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<td>0.91</td>
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<td>38.1</td>
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<td>7</td>
<td>15.01</td>
<td>7.10</td>
<td>15.0</td>
<td>15.0</td>
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<td>11.8</td>
<td>0.90</td>
<td>188.66</td>
<td>64.5</td>
<td>392.66</td>
<td>27.5</td>
</tr>
</tbody>
</table>

Blood Parameters
Pre and post hematological study done by using an automatic hematological analyzer (KX-21N, Sysmex, Japan). The blood including different parameters such as: red blood cell count, white blood cell count, hemoglobin, lymphocytes, absolute lymphocyte, platelet, and platelet volume. Firstly, the blood was centrifuged at 1480rpm at room temperature for 15 min and the blood divided in to the two parts, then the blood serum expunction. After the expunction serum was stored at -20°C. The blood has some parameters such as: Hemoglobin, WBC, RBC, Platelets count, blood urea nitrogen, alkaline phosphate, blood urea, bilirubin and creatinine. biochemical kits in post hematological result showing SGOT, SGPT, AB & blood urea level increases in post hematological analysis. The result showing pre bilirubin level is more than the post bilirubin level. SGOT, SGPT, AP, Blood urea increases in post hematological results. The result showing pre- serum creatinine level is lower than the post serum creatinine level. The difference between in pre and post-hematological parameter showing in table no.10&11.

**HISTOPATHOLOGY**

After completion of the study, on day 14 the tissue from healed wound was excised for studying the microstructural changes after healing in each group.
Fig: 13 Histopathological photographs of epithelial tissues stained with H& E., Group 1(Control), Group 2(wound control), Group 3(chitosan), Group 4(RTX), Group 5(HOCL), Group 6(STS), Group 7(Betadine)
The histopathology results of positive control and test formulation treated Group on day 3rd of experiment and 14th day of experiment. The histopathology results showing different phases of wound healing on different days because wound imitated by various factors in during skin tissue healing process. According to histopathology examination on day 3rd skin showing completely epidermal disruption, it's showing inflammatory cells and exudates. Histopathology results found wound filled with two components such as necrotic materials and a granulation tissue. According to the histopathology results, the group of animals treated with chitosan FA1 & FA3 showing higher epithelization better than the FA2, FA4 & FA5.

The histological results indicated and supported that the healing in the chitosan (GA3) and (GA5) HOCL group was better and more rapid when compared with the other groups. Collagenase activity was found higher in the healing wound tissues of Chitosan and HOCL in comparison to other groups suggesting, the formulation is effective for promotion of wound healing by inducing more production of collagenase on the site.

IV. DISCUSSION

The scope of the present study, however, was to study the comparative efficacy evaluation of few wound healing formulations. Injuries are a complicated healing process, they including different phases and various growth factor. A wound area taking a time period for proper healing and this time various factors occurs for proper wound healing. Wound healing process including molecular and cellular process including and causing different chemical changes. [26] The wound causing discomfort, infection, and other obstruction. Then using Selected agents’ i.e. Chitosan, it is a natural occurring polymer and it’s a biodegradable polymer. They respond at biological level and showing physiochemical interaction. [27]

RTX gel prepared by minimum quantity of Rutoheal-D tablet and the Rutoheal-D containing four components, such as Trypsin, Bromelain, Rutoside and Diclofenac. It’s a digestive protein and its naturally secreted from small intestine. Trypsin containing peptide bond and easily hydrolyzed in to amino acid. Trypsin have two main compound after breakdown such as: (a) Pepsin (b) Chymotrypsin. [28]

HOCL responsible for health care. Its work as antiseptic and helping in wound healing promotion. HOCL useful in microbial eradication. It is use in different areas such as disinfectant, antimicrobial and antiseptic. HOCL is a germicidal agents and using foe wound healing. Hypochlorous acid is made from sodium hypochlorite. Hydrogen peroxide produce converse reaction. The HOCL used in this study was concentrated at 300 ppm. [29-35]

STS is a bactericidal and disinfectant & antimicrobial activity. The hydrogel of sodium thio-sulphate with HPMC polymer. STS hydrogel is useful for wound healing and its work as disinfectant. The all gel prepared as hydrogel and they all are applied on topically. The gel applied easily on the wound area and its effective.

Betadine is a standard formulation and betadine were compared for their wound healing efficacy in Sprague Dawaley rats. According to this experiment method, preparing the all test formulation against the betadine ointment. After comparing the efficacy of all test formulation as compared to marketed formulation.

The process of wound healing by using test formulation, primarily showing protein synthesis, penetration of microbe cell membrane, binding of ribosomes to the bacteria, m-RNA codon and peptide chain with amino acid bind in to cell membrane and start bacterio lysis. All test formulation shows flow rate, which is reproduced by the high viscosity values. The pH value of all formulation was favorable for skin and all formulations will be nonirritant. Spreadibility parameter calculate the values are considered an important point, the formulations are satisfying for the topical and easily spread on the wound area. All gel formulation evaluating a drying time value, this evaluation helps to evaluate the polymer is compatible for hydrogel and they how many time to taking drying. Drying time evaluation helps to evaluating the gel drying after applying on the wound. The basically the process of acceleration the healing process Completed with N-Acetyl-D-glucosamine.

They all formulation having a chemical reaction and its caused by enzyme lysozyme. Its helps in transport to wound sites and helping in closer the wound area. Rat Increasing weight pattern in all the treatment groups demonstrated healing in all the animals along with anti-inflammatory actions of developed formulations, which again verifies the safety/non-toxicity of drug/formulations prepared.
All the preparation has a suitable range under physicochemical properties such as: pH of all formulation is neutral and non-irritant to the skin when applied on the all group of rat. All the formulation exhibited desired characteristics w.r.t. speradibility suitable for topical application.

The Rate of drying shown that wound treatments of gels. Its helps in determine the drying time of acetic acid and glycerol on the wound area. The study was conducted up to 14 days and results in terms of wound size and other related parameters were considered for comparison on day 1, 3, 7 and 14th. On the basis of comparative analysis and data obtained it was found that Chitosan and HOCL was the most potential formulation where the wounds were healed completely at day 14th followed by Chitosan > HOCl > RTX > Betadine >STS > Control, where complete wound healing was not obtained till day 14.

In histopathological examination of tissues from wound site, tissue integrity, microstructure, number of fibroblast cells, inflammatory cells, were observed to determine the collagen synthesis in the healing wounds of animals of different groups. Histopathology showing Chitosan and HOCL group was effective and faster wound healing action with the comparison of other groups. histopathology results observed fibroblast cells produce collagenase enzyme at the wound site.

V. CONCLUSION:
The data obtained from the comparative wound healing study suggests that the formulation Chitosan and HOCL is a potential wound healing and has shown superior response in comparison to the other drugs/formulations tested for the purpose. Moreover, the non-irritability of the prepared Chitosan and HOCL formulation made it a pickiest formulation to be used as effective wound healer. Ingredients of Chitosan and HOCL have probably contributed significantly for to showing excellent wound healing potential of the prepared formulation. Histopathology evaluates acceleration wound as compare the positive control group betadine.

REFERENCES

www.turkjphysiotherrehabil.org 18547


27. Serhan Sakarya, MD1 ; Necati Gunay, MS2 ; Meltem Karakulak, MS3 ; Barcin Ozturk, MD1 ; Bulent Ertugrul, MD1,( 2014) “Hypochlorous Acid: An Ideal Wound Care Agent With Powerful Microbicidal, Antibiofilm, and Wound Healing Potency” Article in Wounds: a compendium of clinical research and practice, Vol.26 no.12, pp.342-350


33. Dr. Amit Sharma, Dr. Santosh Kumar Verma, Mrs. Neha Tyagi, Surbhi Sharma, Om Prakash Yadav, Covid The Third Wave, INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH IN TECHNOLOGY,8(1): 198-200,2021.

34. Dr. Amit Sharma, Dr. Santosh Kumar Verma, Mrs. Surbhi Sharma, Om Prakash Yadav, Yellow and White Fungus (Alarming infections), AEGAEUM JOURNAL, 9(5): 389-392,2021.