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Evaluating the wound healing Potency of Citrullus colocynthis within the Experimental Realm of Animal Model

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Abstract: This research rigorously investigates the wound-healing properties of Citrullus Colocynthis in a controlled experimental setting using animal models. Using a meticulous experimental methodology, we thoroughly examine the potential effectiveness of this plant-based substance as a catalyst for wound healing. As conduct a comprehensive examination, closely examining the complex processes and physiological reactions related to wound healing caused by Citrullus Colocynthis. Through the analysis of molecular complexities and the observation of physiological changes in animal models, our goal is to get a thorough knowledge of how this natural botanical substance influences the healing process.

The significance of our discoveries goes beyond the boundaries of the laboratory, providing essential knowledge about the extensive domain of natural treatments for wound healing. With this study, our aim is to make a significant contribution to both scientific understanding and medicinal applications. The prospective uses of Citrullus Colocynthis in wound treatment might lead to novel techniques in healing, bridging the gap between traditional botanical knowledge and modern medical breakthroughs. This research seeks to enhance the discussion on wound healing procedures by exploring the connections between science and nature. The ultimate objective is to advance our overall comprehension and improve healthcare practices.

Keywords: Wound healing, Citrullus Colocynthis, molecular complexities, herbal extract.

INTRODUCTION

Physiological discontinuities are caused by lesions to living tissues, such as skin, and are known as wounds. A variety of injuries, including chemical, microbiological, thermal, or immunological damage, as well as trauma, disease, attack, or exploitation of the skin, may occur. (1) There is a chain reaction of cellular and molecular effects after any kind of injury. (1) Skin injuries alter the normal anatomical structures and functional status while compromising the skin's integrity. Deeper wounds, including epithelial breaks or subcutaneous irruptions, may lead to the deterioration of several organs, including muscles, nerves, parenchymal organs, bones, and more. Even after the incision has fully healed, a scar will be evident. The Ayurvedic term for a wound is Vrana, whereas the Unani term is Zakham. Sixth, a burst in the dermal lining membrane is what the Ayurvedic texts mean when they talk about a wound.

It is critical to repair injured organs, tissues, wounds, and fractures in order to increase longevity. Restoring and regenerating dead or damaged tissue layers and cellular structures is an essential part of the complex, dynamic, and physiological process of wound healing. Cells and the matrix engage in a series of consecutive events to initiate wound healing. A variety of components, including cellular processes, the surrounding environment, growth factors, and physiological responses, make up the biochemical and physiological systems that facilitate healing (1,6). (1,2,3,4,5,6) While all tissues initiate healing instantaneously, the liver, eye, and skeletal tissues all follow different processes. It takes a lot of tissue-specific phenomena, such the rate of tissue regeneration, to determine how long it takes for different tissues to heal from wounds. For a wound to heal, a number of processes must take place, one of which is cell migration to the site of the injury. (3) The aggregation of different cell types and their metabolites is an essential component of wound healing. The process of lesion formation is preceded by the cell strain-dependent repair and regeneration phase. (5) The wound area's pH is crucial to the healing process; the most critical pH levels are alkaline, neutral, and basic. (4) As soon as feasible, the objective of wound care is to lessen pain, suffering, and scarring. (6) By killing off cells and bacteria in the area of the wound, free radicals help the body recover. (7) Controlling the healing process requires knowledge of how immunology, cell biology, and biochemistry interact with one another and with collagen metabolism and inflammation. (3) In most cases, there is a regular and predictable order to the healing process; when this order is disturbed, the healing period is slowed down, which increases the risk of chronic wounds and pathological scarring. (4) The scientific community has long recognised the medicinal value of using natural products as a complement to conventional medication. Because of their major role in health care systems around the globe, herbal plants must undergo standardisation, safety testing, and scientific validation prior to

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their usage in wound healing. The bio-active components included in medicinal herbs boost the body's own natural healing mechanisms and hasten the recovery process.

How the Body Heals Wounds

The physiological healing process is intricate, ongoing, and symbiotic, including coordinated interactions of the immune and biochemical systems. The process begins with an injury (22-26) and progresses through various time-dependent phases; in humans, it mainly affects the skin, liver, and nerves. However, it may facilitate fetal development or repair (3-13). Although the order and interplay of these components change during the process, the steps involved in wound healing remain consistent. Deposition of connective tissue matrix, contraction, and epithelialisation are other signs of the healing process (1-3, 27, 28). (three to fourteen).

Methodology

Materials

Formulation

1. Cetosteryl alcohol 2. Soft white paraffin 3. Hard soft paraffin

a) Ether for wound creation

c) Analysis of biochemical factors:

1. Sodium bicarbonate

2. Cuprous sulfate

S-D fine chem labs manufacture the following chemicals: epinephrine, hydrogen peroxide, para dimethyl amino benzaldehyde (PDAB), n-propanol, sulfuric acid, TES buffer, ninhydrin colour reagent (NCR), 1-propanol, phosphate buffer, tris base, 10% formalin, sodium bicarbonate, hydrochloric acid, methanol, S-D fine chem labs, DTNB reagent, National Chemicals, Adrenaline, Pharmacure Labs, Thiobarbituric acid, National Chemicals sodium lauryl sulphate, S-D fine chem Pvt. Potassium dihydro phosphate, dipotassium hydrogen phosphate, disodium hydrogen phosphate, triclosan, S-D fine chem labs, and trichloroacetic acid

Equipment's

a) Getting the water bath ready for the samples

a) Analysis of biochemicals.

Remi Electro Technique Ltd. developed a Teflon homogeniser Remi Electro Technology Ltd.'s R-8C Centrifuge. Autoclave UV-Visible Spectrophotometer.

Medications' acquisition and verification

In August 2017, we harvested fresh fruit from a natural garden that belonged to the cucurbitaecae family. The Secretary of the Hyderabad Unani Research Centre in Hyderabad, TS, certified the authenticity of the plant and its contents.

a) Sample processing We rinsed, peeled, and shade-dried the fresh bitter apples. After a week of air drying, the apples were crumbled.

b) Making pulp powder: After crushing the materials, they were ground into coarse particles. In order to get a fine powder, these particles were passed through sieve no. 60. Ointments were made from 5 milligrams and 10 mg of the fine powder, respectively.

a) Making the ointment:

(i) Ointment with a concentration of 5% citrullus colocynthis: 5 mg of 95 ml of citrullus colocynthis ointment with pulp extract.

(ii) 10% ointment: To make a 10% ointment, 10 mg of citrullus colocynthis pulp extract was mixed with 90 ml of simple ointment. Using a water bath heated to 1000C, 5 g of hard paraffin were melted to make a basic ointment. At the same time, 85 g of white soft paraffin were heated and combined with 5 g of cetosteryl alcohol.

Procurement animals

All of the animals came from the Sainath agency in Hyderabad, India, specifically Musheerabad. Before the trial, acclimatised for one week. The research involved healthy Wistar rats that weighed between 150 and 200 g. Pelletised chow and water were part of their usual diet. With the help of proper ventilation, a 12-hour light-dark cycle was maintained. Individual aseptic cages were used to house the animals, and

They changed their sheets every other day. Ethical practises dictated that the animals be treated with care. Throughout the investigation, we took all necessary precautions to prevent contamination and keep the atmosphere clean. The IAEC gave its stamp of approval to the experiment's protocol. All procedures in the experiment were carried out in strict accordance with the regulations outlined by the International Association for the Ethics of Experimental Animals (IAEC). Reference: (IAEC/SUCP/2018/05).

Grouping of Animals

31 healthy Wistar rats were included in the research and classified into five groups based on their treatment regimen. The animals were divided into two groups of 6, with the first group receiving a 5% dose of citrullus colocynthis ointment.

Animals in the second test group received a 10% solution of Citrullus colocynthis ointment; those in the standard group received a standard dose of silver sulfadiazine; and the control group received no treatment, including dressings or medicine.

In the negative group, animals have a wound but do not receive any treatment; they remain in this state. Light ether was used to anaesthetise the animals to make excision wounds. For the anaesthetic effect, the animals were put in the desiccator for about three to four minutes. The next step was to lay the animals on the dissection tray and use a shaver or trimmer to remove their hair from their dorsal surfaces. A circle of about 3 cm in diameter was traced on the shaved dorsal abdominal area using the marker. After that, the thicker contour was cut away from the dorsal skin using medical iris scissors and forceps to make an incision. Because the wound is already open, the first step in treating it is to apply the ointment topically.

Treatment

Until each group of animals had fully recovered, they were each given a unique set of therapies. The first set of test subjects had 5% citrullus colocynthis ointment administered topically to their wounds daily. The wounds of the test 2 group and control groups' wounds were treated externally with 10% citrullus colocynthis and silver sulfadiazine ointment, respectively. Once a scar formed or the wound had healed completely, the ointments were no longer used topically.

Groups	Treatment Given
Group 1	Topical application of 5% test ointment.
Group 2	Topical application of 10% test ointment.
Group 3	Topical application of standard ointment.
Group 4	No treatment.
Group 5	No treatment.

Sample collection and processing

On the 11th day of healing, samples were taken to estimate collagen. To get these samples, we first euthanised the animals and then carefully cut off their repaired tissues. We next preserved these tissues in tris buffer or phosphate buffer saline for biochemical examination. Using 10% formalin, the histology samples were stored.

Pharmacological Investigations

Wound Healing Rate

By planimetrically tracing the boundaries of the raw wounds on the transparent sheet, we were able to measure the rate of wound contraction in each group of injured animals. In order to keep the wound from being contaminated, the tracing was done every other day.

We calculated the percentage of wound shrinkage by comparing the traced wound area to the graph sheet.

Formula

Percentage Wound Contraction:

$$Nth\ day - 100 = \frac{Wound\ area\ on\ Nth\ Day}{Wound\ are\ on\ 1St\ day} X\ 100$$

Estimation of epithelialisation

The number of days it took for the eschar to come off the wound was used to assess the epithelialisation. Dead tissue and other debris should not be visible on the surface of the healed wound, and the wound itself should be clean. The duration is determined by the time it takes for a wound to heal from the day it is created until it is completely healed. (29)

Examination of granulation tissue for bacteria

The regenerated region had its damaged skin scraped off. After weighing the tissue samples, about 16.1 mg was mixed with 10 ml of aseptic saline and mixed well. Bacteria were counted per ml of the sample after serial dilution (12).

Estimation of hydroxyproline

The following approach was used to ascertain the tissue hydroxyproline content. All tissue samples were hydrolysed for three hours at 1100C using 6N HCl. This process was repeated for each group. A 200 µl volume of tissue hydroxylate was placed into test tubes with 0.3 ml of 2.5N NaOH, 0.3 ml of 0.001M $CuSO_4$, and 0.3 ml of 6% H_2O_2. Before being rinsed with cold water for 5 minutes, the tubes were immediately placed in a water bath set at 800C for 15 minutes. Then, 0.6 ml of PDAB and 1.2 ml of 3N sulphuric acid were added. The test tubes were submerged in flowing water and returned to the water bath. Spectrophotometer readings were taken at 540 nm compared to the blank to determine the colour intensity. We used the standard curve and real L-hydroxyproline in tandem to look at how much hydroxyproline was in the samples.

Concentration of sample was determined by using the following formula

$$Concentration\ of\ sample = \frac{OD\ of\ the\ Sample}{OD\ of\ the\ standard} X\ Concentration\ of\ Standard$$

Anti-collagen assessment

To find out what kinds of collagen each species of animal produced, researchers took tissue samples from each. The test tubes were filled with 0.1 ml of tissue hydrolysate and 5 ml of TES buffer. They were then placed in an incubator set at 300C for 5 hours. We transferred the resulting mixture to new tubes after passing it through a syringe filter; we then sealed them. The colour was established using 0.2 ml of test filtrate and 2 ml of ninhydrin. Then, after thoroughly mixing, the ingredients were cooked in a saucepan for 30 minutes. By adding propanol to each tube, the absorbance could be measured.

Studying antioxidants

Analysis of Tissue Homogenates

Each set of animals had tissue samples taken from their healed wounds. The isolated tissues were washed with a physiological solution that was cool to the touch. A motor-driven, Teflon-coated homogeniser was used to homogenise the tissue samples in a Tris HCL buffer (pH 7.4). We are now processing a 10% w/v tissue homogenate that was produced. For about 10 minutes, the homogenised tissue samples were spun in a centrifuge at 10,000 rpm and 4ºC. The sedimented remnants, known as the supernatant, are collected by centrifugation and used to test for antioxidant activity.

Estimation of catalase activity

The following approach is used to analyse the Catalase content in a specific sample: a cuvette was filled with 2.25 ml of phosphate buffer and 100 µl of tissue homogenate and then left to incubate for about 30 minutes. After the incubation period, about 0.6 ml of H_2O_2 is introduced, and the change in absorbance is recorded. We measured the absorbance of the blank and all the other groups of samples three times: once at the end of the first minute and again at the end of the second minute. To determine the concentration of catalase per millilitre of a sample, we took the absorbance value of a single sample three times.

Assessment of glutathione reductase activity

Procedure

A framework was used to measure the tissue's reduced glutathione (GSH) concentration. In a 1:1 ratio, the homogenate supernatant was mixed with 10% w/v TCA ruinous. For 10 minutes at 4 °C, the tubes were spun in a centrifuge at 1,000 g. The 0.5 ml of collected supernatant was mixed with 2 ml of 0.3 M Na2HPO4. Spectrophotometry was used to measure absorbance at 412 nm after mixing 0.25 ml of freshly sorted DTNB [5, 5-dithiobis (2-nitrobenzoic ruinous) isolated in 1% w/v sodium citrate] at 0:001 M. The data were presented as micromoles of reduced glutathione per mg of protein, and a standard curve was produced using 10-100 µM of reduced glutathione.

Formula for Dilution Factor

Formula for calculation of glutathione in tissue homogenate: (µM/g = n M/mg).

$$X = \frac{Y - 0.00314}{0.0314} X \frac{Df}{Tissue\ homogenate}$$

Where, Y= Abs^{412} of tissue homogenate.

Df = Dilution Factor

Malonaldehyde level test for thiobarbituric acid reactive compounds (TBARS)

As a measure of the circumlocutory extent of lipid peroxidation, the measurement of malonaldehyde was regulated by reacting it with thiobarbituric acid (TBA) [23]. To begin, three millilitres of TBA reagent consisting of 0.38% TBA (weight/weight), 0.25 ml of hydrochloric acid (HCl), and 15 percent trichloroacetic acid (TCA) was mixed with 1 millilitre of split supernatant in separate test tubes. The strategy was tossed and turned for 15 minutes, then cooled in an ice spray. The mixture was centrifuged at 3500 g for 10 minutes after cooling. At 532 nm, a spectrophotometer was used to study the accumulated overlying layer. The findings were presented as nanomoles of protein/mg. The amount of MDA/mg of protein was analysed.

Calculation

$$Conc. of\ \boldsymbol{MDA} = \frac{Abs532\ X\ 100\ X\ VT}{(1.56X105)\ X\ WT\ \ X\ VU}$$

Where, Abs 532 = Absorbance of tissue homogenate

VT = Total volume of mixture

WT = Weight of dissected brain.

VU = Aliquot volume.

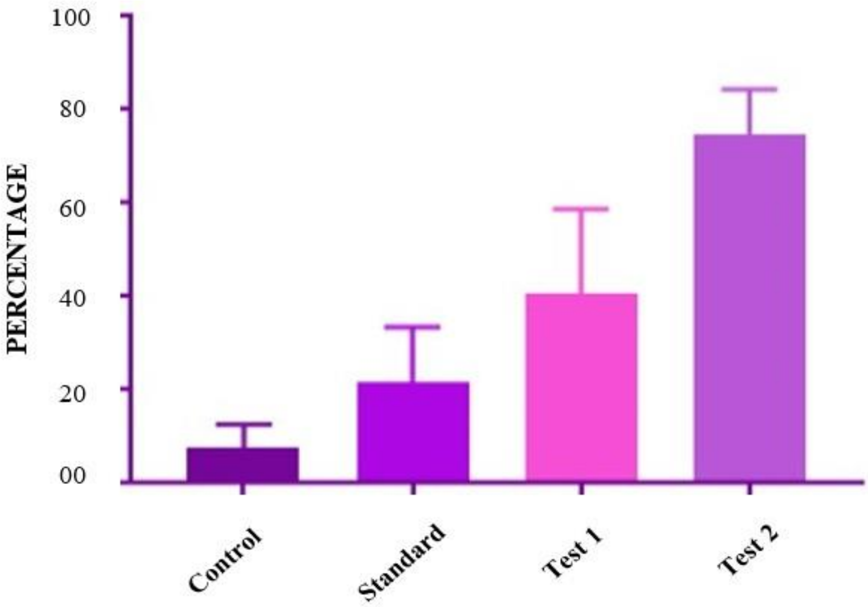
1.56 X 105 = Extinction coefficient.

Surgical pathology

Each animal group had its tissue samples taken and stored in 10% formalin. Hematoxylin and eosin (HE) were added after the samples were further processed and divided into pieces 5 micrometres in size. The proliferation of fibroblasts, synthesis of collagen, formation of epithelial cells, and keratin growth were the criteria used to classify the thin slices of materials under the light microscope.

Results

Wound contraction rate on day 3



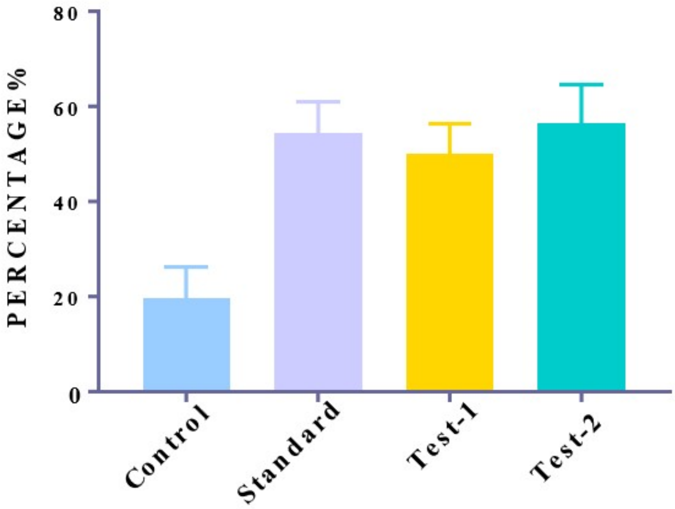
Sr. No	Group	Mean + SEM

1	Control	7.822 ± 1.957
2	Test 1	40.82 ± 7.343
3	Test 2	74.77 ± 3.929
4	Standard	21.53 ± 4.913

The wound contraction rate on day 3 in all the groups was compared as the Mean and SEM values was calculated. The percentage rate of wound contraction was found to be more in test 2 group of animals when compared to the negative groups. The P value was found to be significant.

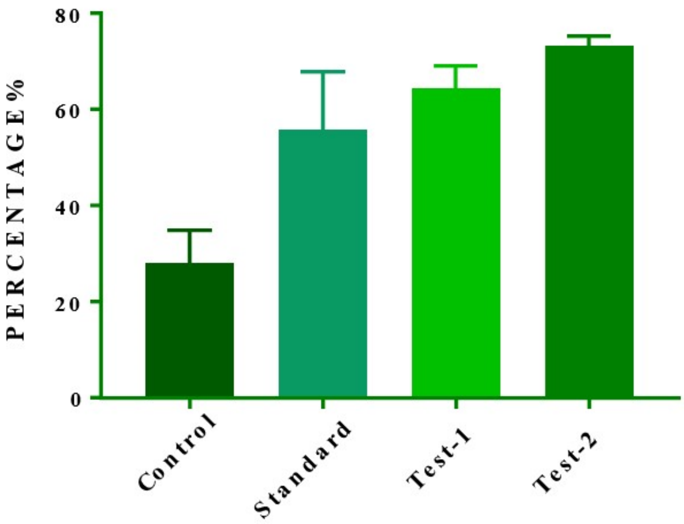
The values were expressed as Means ±SEM of n animals for each group. Statistical estimations of the data was compiled through one-way analysis of variance (ANOVA) succeeded by Dunnett's test. Variations were studied statistically vital when $P < 0.05$.

Wound contraction rate on day 5



Sr. No	Group	Mean ± SEM
1	Control	19.7 ± 2.66
2	Test 1	50.16 ± 2.504
3	Test 2	56.41 ± 3.37
4	Standard	54.41 ± 2.68

Wound contraction rate on day 10

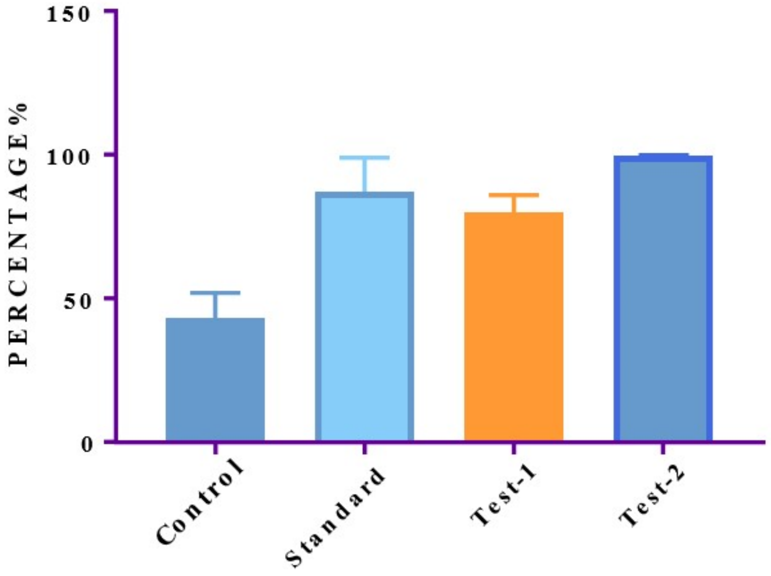


Sr. No	Group	Mean ± SEM
1	Control	27.97 ± 2.814

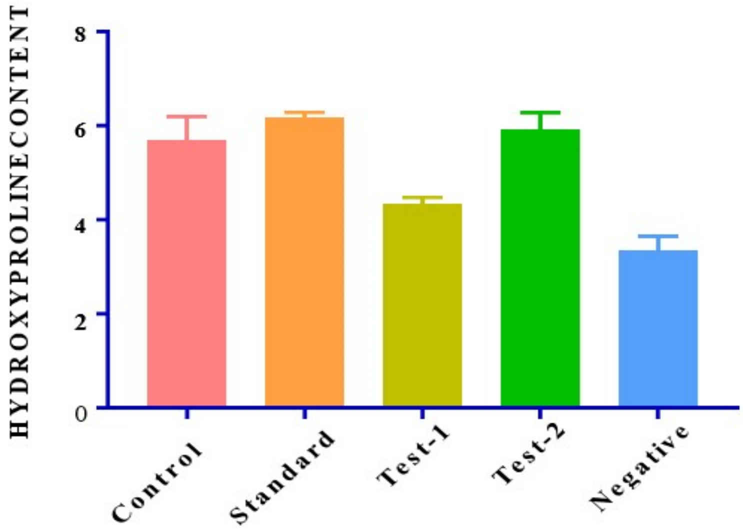
2	Test 1	64.31 ± 1.967
3	Test 2	73.32 ± 0.7772
4	Standard	55.76 ± 4.949

P value <0.0001

Wound contraction rate on day 15



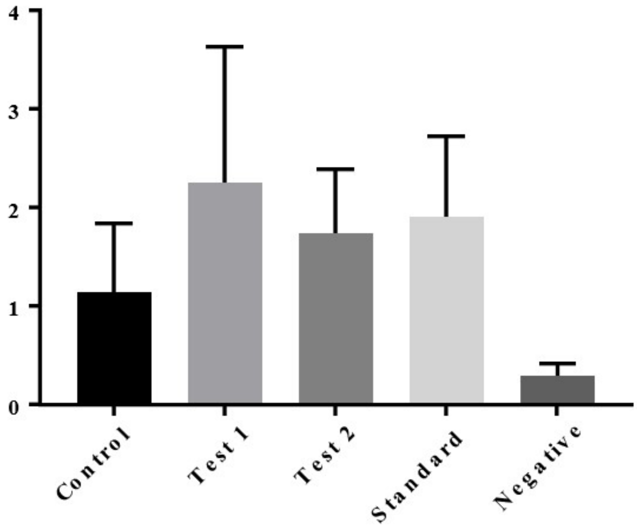
Sr. No	Group	Mean ± SEM
1	Control	27.97± 2.814
2	Test 1	64.31 ± 1.967
3	Test 2	73.32 ± 0.7772
4	Standard	55.76 ± 4.949



Hydroxyproline Estimation

Sr. No	Group	Mean ± SEM
1	Control	5.709 ± 0.2041

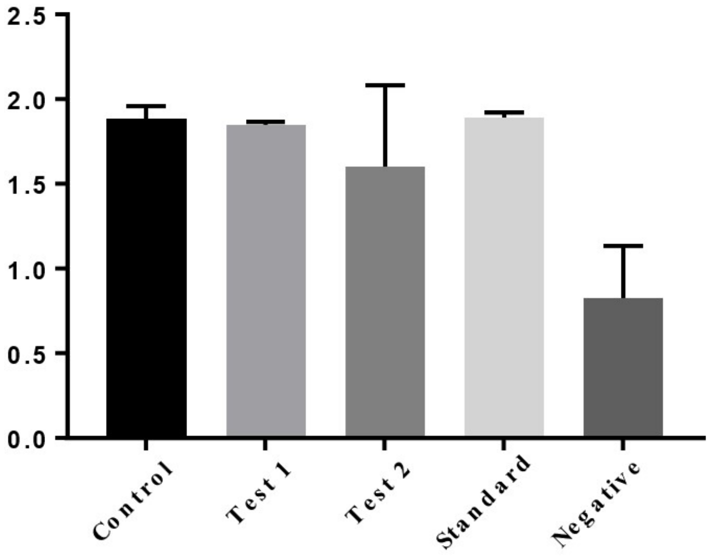
2	Test 1	4.345 ± 0.05764
3	Test 2	5.925 ± 0.1447
4	Standard	6.179 ± 0.04468
5	Negative	3.667 ± 0.1178



Catalase

Sr. No	Group	Mean ± SEM
1	Control	1.141 ± 0.283
2	Test 1	2.247 ± 0.567
3	Test 2	1.734 ± 0.267
4	Standard	1.906 ± 0.333
5	Negative	0.293 ± 0.0511

The oxidative enzyme catalase determines the antioxidant activity of citrullus colocynthis. Increased levels of catalase was graphically shown in the above graph. Highest concentration of catalase was noted in test 1 group of animals. Second highest concentration of catalase was seen in standard group of animals followed by test 2 and negative respectively.

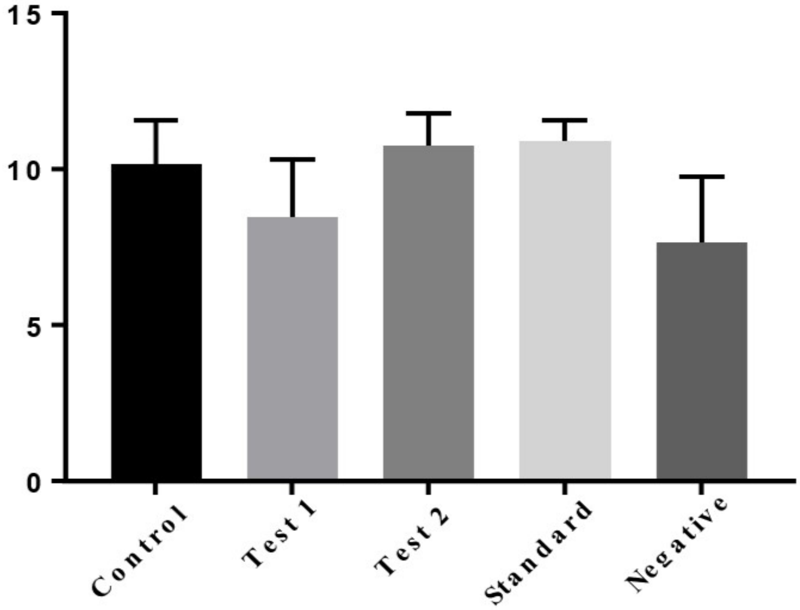


Sodium Level

Sr. No	Group	Mean ± SEM
1	Control	1.889 ± 0.029

2	Test 1	1.851 ± 0.007
3	Test 2	1.606 ± 0.1934
4	Standard	1.893 ± 0.0121
5	Negative	0.829 ± 0.123

SOD is the antioxidant enzyme that helps to reduce the levels of ROS in the body. Maximum concentration of SOD in standard group determines it highest antioxidant activity. P value was found to be significant.

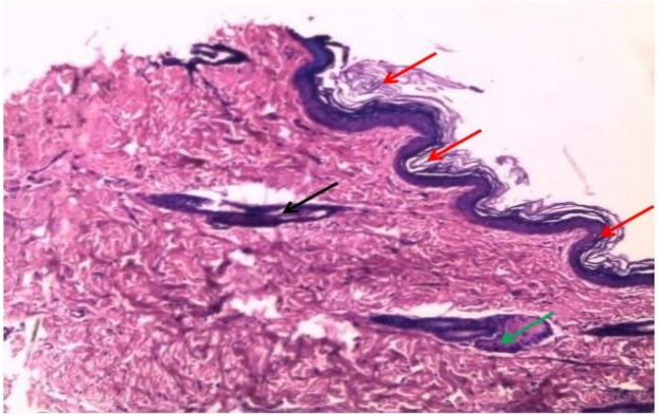


GSH LEVELS

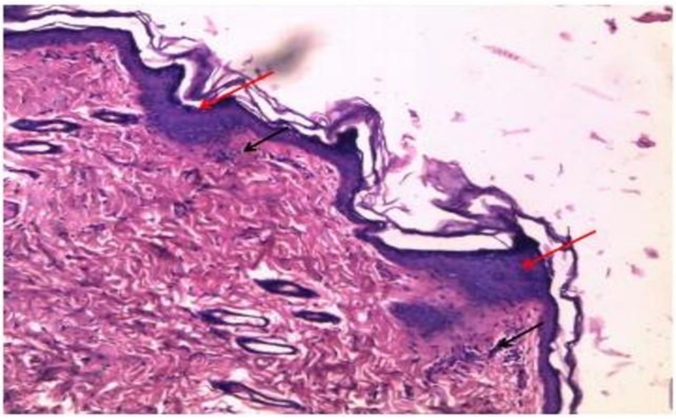
Sr. No	Group	Mean ± SEM
1	Control	10.16 ± 0.570
2	Test 1	8.479 ± 0.746
3	Test 2	10.77 ± 0.413
4	Standard	10.73 ± 0.290
5	Negative	7.641 ± 0.866

Histopathology

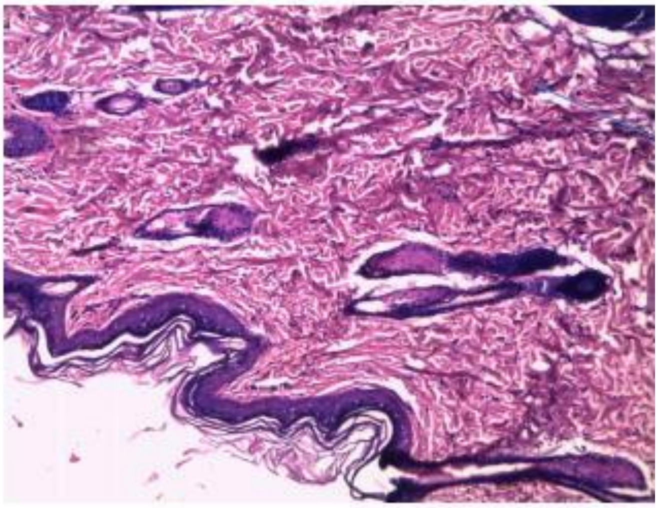
The histopathological results of test 1 shows the following



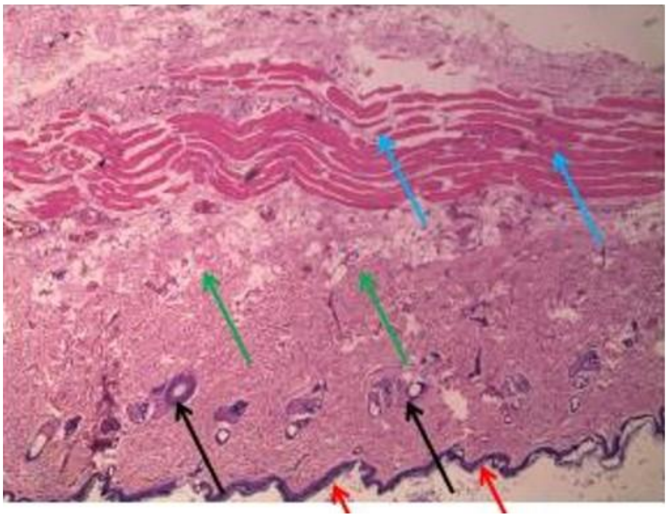
Moderate hyperkeratosis [thickening of keratin] in the stratum corneum layer of epidermis red arrow Normal dermal layer of skin with hair follicle [black arrow] and sebaceous gland [green arrow]



Mild epidermal Hyperplasia [increase size of stratum granuloma] red arrow along with foci of infiltration of inflammatory cells noticed in dermal region- black arrow.



Normal Cutaneous layer of skin tissue / No Inflammation



Normal epidermal [red arrow], dermal with hair follicle & sebaceous gland [black arrow], and muscular layer [blue arrow] no inflammation noticed in the entire layer of skin.

CONCLUSION:

Studies on wound healing revealed that the pulp of citrulllus colocynthis has biological activity. It had a wide range of traditional uses. The fruit pulp was prepared into an ointment and tested on animals to see how it would react to an excision wound model. The re-epithelialization process and the reduction of oxidative stress via the upregulation of oxidative enzymes were both uncovered in these studies. Wound healing research may now add to the plant's list of established uses as an antioxidant and anti-inflammatory.

Its ability to cure damaged wounds is attributed to the phytochemical components, including as flavonoids and phenols, which participate in the healing process. It is possible to draw the conclusion that this plant and its product have significant therapeutic value and might be used pharmaceutically for the treatment of wounds. Researchers found that histological and biochemical analysis of citrullus colocynthis pulp increased wound contracture levels.

In order to develop this plant into an effective medicine for wound healing, more pharmacological, pharmaceutical, and investigative investigations are urgently required. By doing the targeted research on the medicine, one may create a picture of its potential market. It would be great if this plant's evidence against wound healing could be confirmed in future clinical trials so it may be utilised as a primary therapy or as an alternate.

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