

UNLOCKING THE REGENERATION BENEFITS OF GUAR GUM: IN VITRO AND IN VIVO EVALUATION OF ITS ROLE IN WOUND HEALING

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Abstract

This comprehensive study aimed to evaluate the potential impacts of guar gum, on wound healing using a multi-faceted approach that included in silico, in vitro, and in vivo analyses. The in vitro experiments using the Vero cell line were conducted to evaluate the impact of guar gum treatment on cellular responses related to wound healing. The results revealed a significant increase in cell proliferation, as assessed by MTT assay, indicating the treatment's ability to promote cell growth. The crystal violet assay also demonstrated high cell viability in the guar gum-treated group, further supporting its positive effects on cellular activity. To evaluate the effects of guar gum therapy on wound healing in a physiological context, a rat burn model was used. The investigation showed that the treated group had a faster wound healing index than the control group, indicating a quicker healing time and better tissue regeneration. Furthermore, a significant decrease in Annexin V levels, a marker for apoptosis, was noticed in the treated group, suggesting a reduction in cell death and enhanced cell survival. These extensive findings from in vitro, and in vivo analyses collectively highlight the potential benefits of guar gum treatment in wound healing. The treatment exhibited favorable molecular interactions, promoted cell proliferation and viability in vitro, and facilitated faster wound healing with reduced apoptosis in vivo. While these results hold promise for the therapeutic application of guar gum in wound healing, further research is warranted to elucidate the underlying mechanisms and optimize treatment protocols. Additionally, larger clinical trials are necessary to evaluate the long-term safety and efficacy of guar gum treatment in human subjects. Finally, this study provides valuable insights into the potential of guar gum as a therapeutic intervention for enhancing wound healing outcomes, bridging the gap between, cellular responses, and physiological healing processes.

Keywords: *Cyamopsis Tetragonoloba*, Guar Gum, VERO, H₂O₂, Burn Wound.

1.0. INTRODUCTION

An injury known as a burn wound happens as a result of being exposed to heat, electricity, chemicals, radiation, or friction. The four categories used to categorize a burn wound's severity are first-degree, second-degree, third-degree, and fourth-degree burns (He *et al.*, 2021). The magnitude of the injury and the depth of the tissue damage is used to categorize the damage.

Medication can help with pain management, infection prevention, and wound healing, making it a crucial component of burn injury treatment. However, burn damage drugs can potentially cause negative effects, just like all other treatments. Certain burn damage treatments, such as topical creams, oral antibiotics, or pain relievers, may cause an allergic reaction in certain patients. From minor skin rashes to life-threatening symptoms like anaphylaxis, hives, and difficulty breathing, allergic reactions can range in severity. Gastrointestinal adverse effects from oral drugs might include nausea, vomiting, and diarrhea. Certain painkillers may have adverse effects on the central nervous system (CNS), including sleepiness, disorientation, and dizziness. Due to their potential increased risk of mishaps like falls, elderly folks may find this to be especially bothersome. Nonsteroidal anti-inflammatory drugs (NSAIDs), one type of medication used to treat burn injuries, can eventually harm the kidneys and liver. People who have liver or renal illnesses are more at risk. Blood thinners and other drugs can also interact with medications for burn injuries, increasing the risk of bleeding or other negative effects. In the traditional treatment, dead tissue is removed, the wound is debrided, antibacterial medicines are applied topically, and dressings are used to treat burn wounds. This method, however, has been found to be insufficient and frequently leads to wound infections, scarring, and sluggish recovery. Additionally, there is a critical need for alternate and more potent therapies due to the high expense of wound care and the rise in antibiotic-resistant germs.

The use of organic and biocompatible materials to treat burn wounds has gained popularity over the past few decades. These substances, including polysaccharides, proteins, and growth factors, have the potential to replace conventional therapies as they have been found to have significant wound healing characteristics. Guar gum, a polysaccharide produced from the seeds of the guar plant (*Cyamopsis tetragonoloba*), is one example of an organic chemical. Due to its outstanding thickening and emulsifying abilities, guar gum is frequently utilized in a variety of industries, including the food, pharmaceutical, and cosmetic sectors. Due to guar gum's biocompatibility, hydrophilicity, and capacity to encourage tissue regeneration, there has been a rise in interest in researching its possible use as a wound healing agent in the recent past.

The study aims were to assess the guar gum's efficacy and safety in aiding burn wound healing. This study looked into the potential mechanism of action in promoting wound healing.

2.0. MATERIALS AND METHOD

2.1. In-vitro study

2.1.1. Preparation of guar gum extract

The Pakistan Gum Industries (Pvt.) Ltd provided the guar gum powder which was used to prepare an extract. To make the extract, 5g of guar gum was mixed with 200 ml of 70% ethanol and left for 5 days before being filtered and dried in the shade. A stock solution of 100mg/ml was made by diluting 400mg of the guar gum extract with 4 ml of distilled water. From this stock solution, seven doses were prepared, 100µg/ml, 50µg/ml, 25µg/ml, 12.5µg/ml, 6.25 µg/ml, 3.12µg/ml, and 1.56µg/ml.

2.1.2. Cell culturing

The regenerative properties of guar gum extract were evaluated using the VERO cell line. Cryovials containing VERO cells were obtained from a liquid nitrogen cylinder and thawed prior to cell culture. An antibiotic-antimycotic solution 100X (Penicillin, Streptomycin Sulphate, and Amphotericin B in a 0.85% Saline) (Caisson Labs, USA), 100 ml, 1000 ml of distilled water, and 10% fetal bovine serum (FBS) (Gibco) were combined to create DMEM-HG media, which was then used to cultivate cells. The cell culturing media was added to a cell culturing flask and VERO cells were allowed to cultivate in the flask until they reached a confluency of 70-80%. To detach the cells from the walls of the culturing flask, 2-3 mL of phosphate buffer saline (PBS) was used to wash the cells, and trypsin-EDTA was added and incubated. The detachment of cells from the walls was observed under a phase contrast microscope, and 6-7 mL of DMEM medium, which contains sufficient FBS to inhibit the trypsin activity, was added and mixed well. The mixture was then put into a 15ml falcon tube and spun at 2000 rpm for 5 minutes. The pellet was re-plated after the trypsin-EDTA-containing medium was aspirated and transferred to a brand-new culture medium (Maqbool *et al.*, 2019).

2.1.3. Cell line injury

The cells were initially put in 96-well plates to adhere overnight before being subjected to the oxidant for the H₂O₂ cell injury. The cells were then given DMEN without serum and subjected for four hours to 6 mM freshly generated H₂O₂ after the culture media was removed. H₂O₂ was additionally added to the media in culture wells devoid of cells as a negative control. In contrast, H₂O₂-free media was used to cultivate the control cells.

2.1.4. Cell line treatment with plant extract

To treat VERO cells with guar gum extract, the H₂O₂-containing medium was first removed and replaced with different doses of guar gum. Four groups of VERO cells were then created, with the first group serving as the untreated control without any extract added. The remaining three groups of VERO cells were treated with different doses of the guar gum extract and incubated for 24 hours to observe the treatment effects. Following the 24-hour incubation period, the treated cell lines were analyzed using ELISA, cell proliferation assay, and crystal violet assay.

2.1.5. Cell proliferation assay

Following a 24-hour treatment with guar gum extract in a 96-well plate, the proliferative potential of the VERO cell line was assessed using the MTT assay. Phosphate-buffered saline (PBS) from Invitrogen Inc. was used to wash the cultivated cell monolayer in the beginning. Following this, 500 l of complete medium and 60 l of MTT solution (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) from Invitrogen Inc. were added to the wells, and the mixture was incubated for 2 hours. Following the MTT's transformation into a purple formazan, 10% SDS (sodium dodecyl sulfate) from Invitrogen

Inc. was used as a dissolving agent of it. The resultant solution's absorbance was then measured at 570 nm (Rebouças *et al.*).

2.1.6. Crystal Violet Assay

To assess the cell viability of the VERO cell line, the crystal violet staining method was used. First, phosphate buffer saline was used to wash the wells after each group's media had been removed. The wells were then covered with a solution of 0.1% crystal violet dye and 2% ethanol, which was then let sit for 15 minutes at room temperature. To prevent cell separation, the dye was gently removed, and the wells were delicately cleansed. The stain was then solubilized by adding 100 µl of 1% SDS to each well and incubating for 5–10 minutes. The absorbance at 540 nm was then measured using a microtiter plate reader.

2.1.7. ELIZA (Enzyme-linked immunosorbent assay)

VEGF and annexin V solid-phase sandwich ELISA were carried out in a 96-well plate. A coating buffer was first used to dilute rabbit polyclonal anti-VEGF and annexin V antibodies (Santa Cruz Biotechnology, USA) to a concentration of 2–10 µg/ml. After that, each well of the plate received 100 µl of the diluted antibodies, which were then incubated for 120 minutes. Following the incubation, each well was rinsed three times with 1X TBS-T (washing solution) for five minutes. The capture antibodies were then discarded. Each well of the plate was then filled with 200 µl of blocking solution (BSA), which was then removed after 30 minutes of incubation. Each well received culturing medium that had been collected from several experimental groups. After 30 minutes, the medium was discarded and each well underwent three-time five-minute thorough washings. After rewashing the plate, the primary detection polyclonal rabbit antibody was introduced and allowed to incubate at room temperature for an additional 1 to 2 hours. The plate was then incubated for 60 minutes after each well received 100 µl of an HRP-conjugated donkey anti-rabbit secondary antibody from Santa Cruz Biotechnology in the United States. After incubation, the secondary antibody was taken out, and each well underwent a five-minute, three-step washing procedure. Tetramethyl benzidine (TMB) (Invitrogen Inc., USA) was added (100 µl) to each well in preparation for HRP detection. The plate was then incubated for 20 minutes. After incubation, 100 µl of 0.18 M sulfuric acid (H₂SO₄) stop solution was added to each well to halt the TMB reaction. Then, using a microtiter plate reader, absorbance was measured at 450 nm.

2.2. In-vivo study

2.2.1. Animals

A total of eighteen white albino rats weighing between 200-250gm were procured from the animal house at the University of Lahore and grouped into sets of six, with three animals per set. The rats were housed in steel cages and provided with regular meals and ad libitum access to water.

Table 1: Categorization of rats into six groups

Groups	Group Name	Treatments	No. of Animals in each group
Group 1	Normal	Untreated rats	3
Group 2	Injured	Injured rats left untreated	3
Group 3	Placebo 50 mg/ml	Injured rats treated with a 50 mg/ml placebo	3
Group 4	Treatment 50 mg/ml	Injured rats treated with 50 mg/ml aqueous extract	3
Group 5	Placebo 100 mg/ml	Injured rats treated with 100 mg/ml placebo	3
Group 6	Treatment 100 mg/ml	Injured rats treated with 100 mg/ml aqueous extract	3

2.2.2. Burn injury

Six groups of albino rats were used in the experiment, with each group contained three rats (n=3 per group). One of these functioned as the control group and was spared any harm, while the other five groups all suffered acid burn wounds. 15 rats were burned with HCl acid after being given doses of xylazine (5 mg/kg) and ketamine (50 mg/kg), respectively, to induce anesthesia. The hair in the targeted region was removed, and a 1cm mark was made on the skin. Acid-soaked filter papers were then applied to the marked area for 20 seconds.

2.2.3. Treatment

Out of the five groups of rats, one group received no therapy, while the four other groups were administered either an aqueous extract of guar gum or normal saline in varying concentrations (50mg/ml and 100mg/ml) using a gavage tube. Two groups were given the aqueous extract of guar gum, and the remaining two groups were given normal saline.

2.2.4. Wound Index Measurement

On days 0, 5, 10, and 15, the wound index measuring assay was carried out to measure the wound's healing. The rats' wounds were first anesthetized, and a transparent plastic sheet was placed on the ventral side of the wounds. A pointer was then used to draw a line through the wounded area on the sheet (Cai *et al.*, 2014). Tow-way ANOVA was used in the statistical study. By comparing the initial and current wound sizes, the percentage reduction in the wound size was calculated.

2.2.5. Sandwich ELISA

After sacrificing the rat, ELISA was performed on its blood sample to isolate serum for skin regeneration. For the solid-phase sandwich ELISA of VEGF and annexin V, a 96-well plate was utilized. Rabbit polyclonal anti-VEGF and annexin V antibodies were diluted and added to the plate's wells, which were then incubated for 120 minutes. The capture antibodies were washed and blocked with BSA. Rat serum was next added, and then donkey anti-rabbit secondary antibody that had been HRP-conjugated. TMB substrate was added after washing, and it was then incubated for 20 minutes. The microtiter plate reader then assessed the absorbance at 450 nm for HRP detection after adding the H₂SO₄ stop solution (Alhabbab and Alhabbab, 2018).

2.2.6. Histopathology of Skin Sections

To conduct histology, skin samples of rats measuring 0.5-2cm were utilized. The injured portion of the skin was removed and placed in 10% neutral buffer formalin for fixation, left overnight. The samples were then exposed to increasing concentrations of isopropyl alcohol for 2 hours at 80%, 90%, and 100% for the following 3 hours. The tissues were then periodically shaken in acetone for 1-2 hours, and after being removed from the acetone, they were examined for a milky appearance. The dehydrated tissue samples were then placed into an L-block and molten paraffin wax was added and allowed to cool. Thin sections of about 2-8µm were obtained from the tissue samples using a microtome. The tissues were divided into sections, mounted on slides with Mayer's albumin solution, and heated to 60°C for two hours. After that, xylene was used to deparaffinize the slides for 20 to 30 minutes. After being submerged in water for three minutes, the tissues were repeatedly rehydrated using 100%, 90%, and 80% isopropyl alcohol for two to three minutes each. Staining was performed by immersing the slides in hematoxylin for 1-2 minutes, followed by tap water to remove excess hematoxylin, and then eosin stain for 30 seconds. After removing any extra eosin, the tissue was consecutively dehydrated for 20 minutes using 80%, 90%, and 100% isopropyl alcohol and xylene. A coverslip was placed on the slide with 1 drop of DPX, and it was left to dry overnight to produce the permanent slides.

2.3. Statistical analysis

The results of the studies, which were run in triplicate, are presented as mean SEM. One-way and two-way ANOVA as well as the Bonferroni's test were used in the statistical analysis for the various groups. The plots for the quantitative data derived from the molecular analysis were made using GraphPad Prism 8.0. For statistical analysis, a P value of less than 0.001 was regarded as significant. Software named Endnote X20 was employed for reference.

3.0. RESULTS

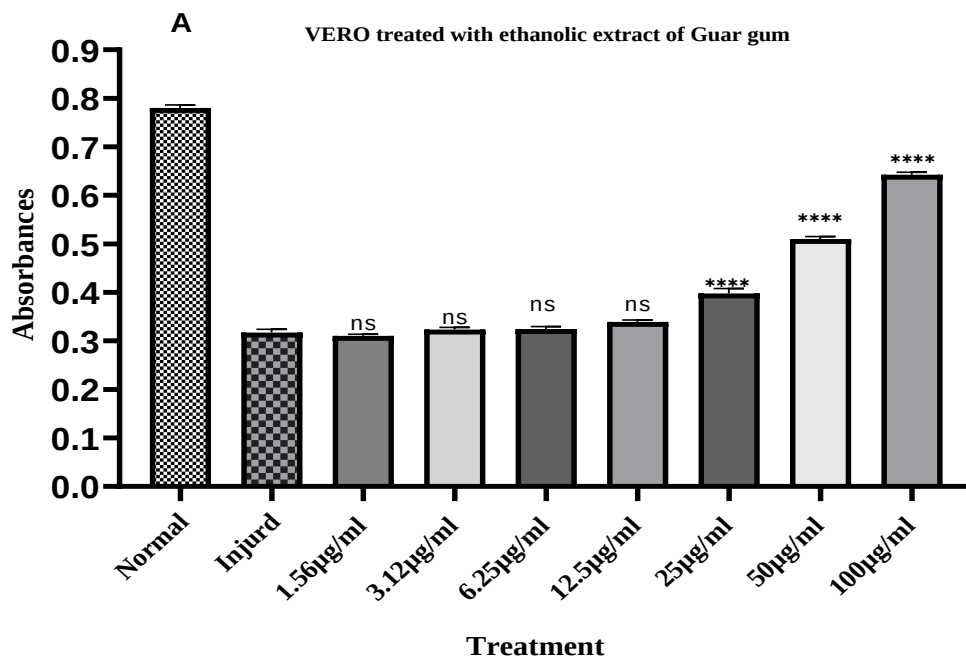
3.1. *In-vitro* results

3.1.1. Cell proliferation assay

According to the MTT reagent analysis, guar gum extracts at doses of 25 µg/ml, 50 µg/ml, and 100 µg/ml significantly increased the proliferation of wounded VERO cells in culture when compared to the control group. According to figure 1

Table 2: The mean ± SEM values of cell proliferation, obtained from the MTT assay, were reported

Normal	Injured	1.56µg/ml	3.12µg/ml	6.25µg/ml	12.5µg/ml	25µg/ml	50µg/ml	100µg/ml
0.78±0.006	0.32±0.004	0.31±0.003	0.32±0.004	0.32±0.005	0.34±0.004	0.40±0.010	0.51±0.005	0.64±0.005



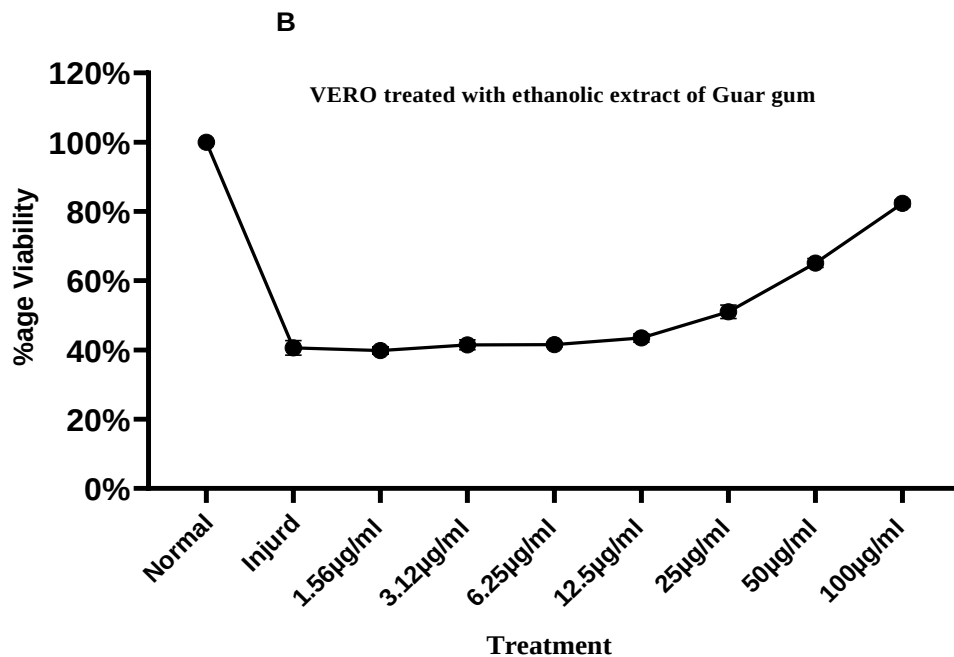


Figure 1: The proliferation rate of damaged VERO cells treated with various doses of guar gum extract was measured using the MTT test. (A) The MTT assay results for VERO cells treated with guar gum extract in ethanol at different doses (100 g/ml, 50 g/ml, 25 g/ml, 12.5 g/ml, 6.25 g/ml, 3.12 g/ml, and 1.56 g/ml). (B) The percentage values of different guar gum extract concentrations in ethanol. The information is displayed as the mean $\pm$ SEM of three experiments. (****P < 0.0001; ns, not significant)

3.1.2. Crystal Violet assay of *guar gum* extract

A noticeable increase in cell proliferation was seen in VERO cells cultured with 12.5 μ g/ml, 25 μ g/ml, 50 μ g/ml, and 100 μ g/ml of guar gum as compared to the injured group after adding an ethanolic extract of guar gum to H₂O₂-injured VERO cells and culturing them with various concentrations of guar gum extract separately.

Table 3: The data for cell viability after crystal violet staining, expressed as a mean $\pm$ SEM

Normal	Injured	1.56µg/ml	3.12µg/ml	6.25µg/ml	12.5µg/ml	25µg/ml	50µg/ml	100µg/ml
0.967 $\pm$ 0.0123	0.405 $\pm$ 0.005	0.424 $\pm$ 0.006	0.421 $\pm$ 0.004	0.439 $\pm$ 0.001	0.457 $\pm$ 0.017	0.567 $\pm$ 0.002	0.773 $\pm$ 0.007	0.872 $\pm$ 0.008

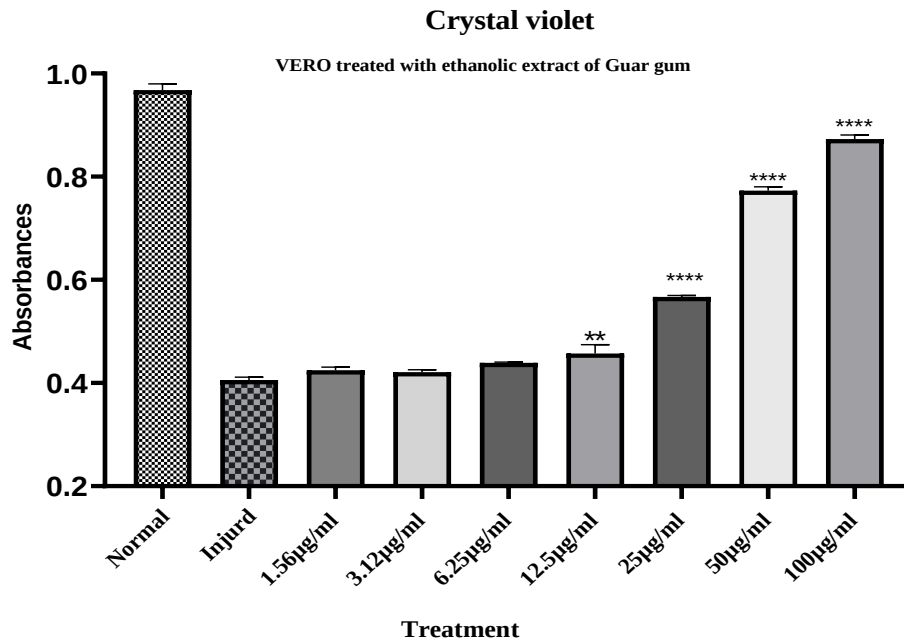


Figure 2: The values of the crystal violet assay in the treated group of VERO cells with selected doses of guar gum were compared to the levels in the injured group of cells. **** Symbols indicate that there is high statistical significance in the results ($P < 0.0001$). The mean $\pm$ SEM is used to express the values. (**** $P < 0.0001$; ** $P < 0.005$; ns, not significant)

3.1.3. Increased Angiogenic Potential of guar gum Extracts

Guar gum extracts at concentrations of 25µg/ml, 50µg/ml, and 100µg/ml were used to treat the VERO cell line, and this boosted the angiogenesis. VEGF (vascular endothelial growth factor receptor) was measured using an ELISA technique to determine the degree of angiogenesis. The findings showed that the treated groups' levels of the protein VEGF significantly increased when compared to the wounded groups' levels, suggesting an increase in angiogenesis. (Fig. 3)

Table 4: Angiogenesis level (mean $\pm$ SEM) values

Normal	Injured	1.56µg/ml	3.12µg/ml	6.25µg/ml	12.5µg/ml	25.0µg/ml	50.0µg/ml	100µg/ml
1.091 ± 0.015	0.388 ± 0.005	0.489 ± 0.001	0.389 ± 0.002	0.392 ± 0.006	0.402 ± 0.0008	0.550 ± 0.015	0.621 ± 0.007	0.843 ± 0.005

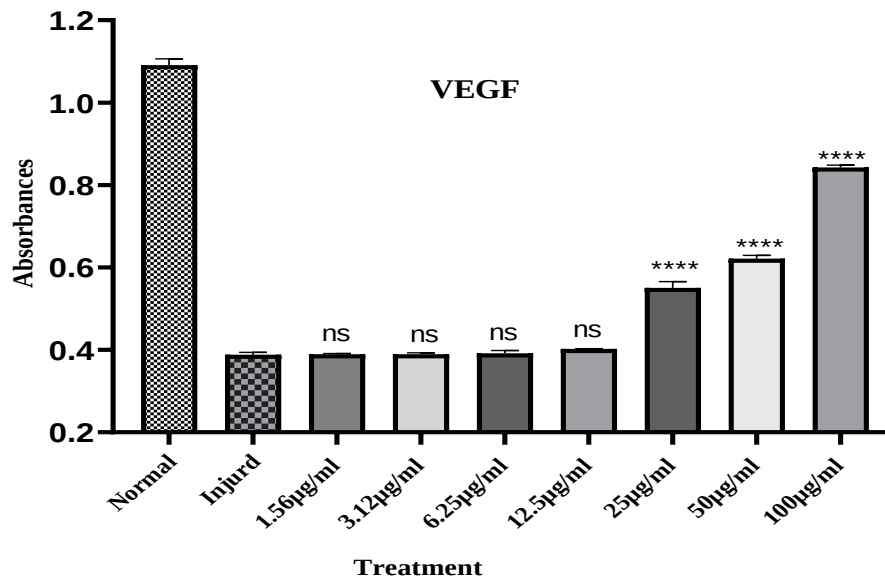


Figure 3: The levels of VEGF were compared between the treated group of VERO cells with selected doses of guar gum and the injured group. The mean $\pm$ SEM was used to express the values. (****P < 0.0001; ns, not significant).

3.1.4. Reduced apoptosis effect of guar gum extracts

An annexin-V antibody, a key component in the process, was used in an ELISA assay to measure the degree of apoptosis. In contrast to the wounded group, Figure 4 shows that the treated groups had lower levels of annexin-V protein. The level of apoptosis was the same in the injured group and the groups treated with guar gum extract at concentrations of 12.5µg/ml, 6.25µg/ml, 3.12µg/ml, and 1.56µg/ml. However, the level of apoptosis was significantly lower in the groups treated with guar gum extract at concentrations of 25µg/ml, 50µg/ml, and 100µg/ml than in the injured group

Table 5: Annexin V level measured via ELISA (mean $\pm$ SEM) values

Normal	Injured	1.56µg/ml	3.12µg/ml	6.25µg/ml	12.5µg/ml	25µg/ml	50µg/ml	100µg/ml
1.424 $\pm$ 0.018	2.427 $\pm$ 0.046	2.400 $\pm$ 0.021	2.292 $\pm$ 0.060	2.317 $\pm$ 0.022	2.319 $\pm$ 0.006	2.244 $\pm$ 0.028	2.197 $\pm$ 0.009	1.663 $\pm$ 0.049

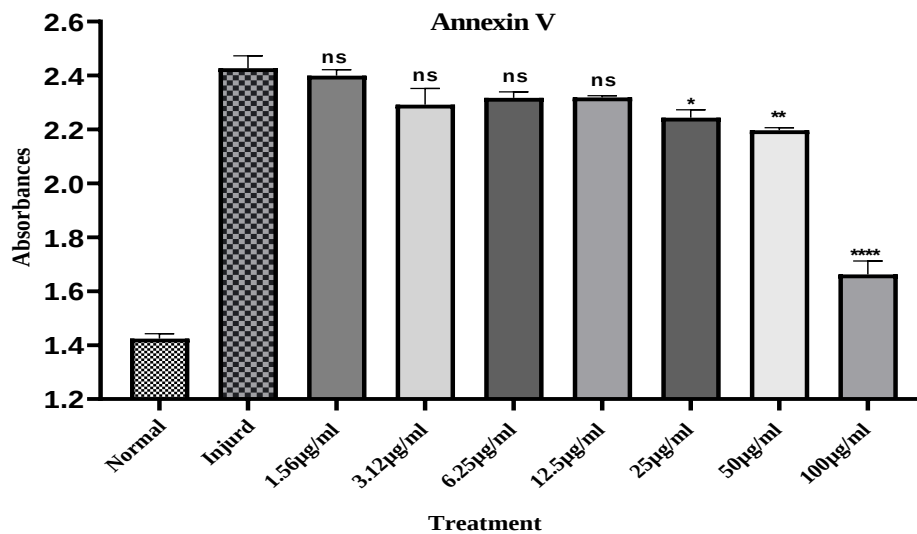


Figure 4: The graph compares annexin V levels in the injured and treated groups of the VERO cell line using selected doses of guar gum. It shows decreased annexin V protein in the treated groups with 25µg/ml, 50µg/ml, and 100µg/ml guar gum. Mean $\pm$ SEM is used to express the values.

(****P < 0.0001; ** P < 0.0015; * P < 0.011; ns, not significant)

3.2. *In-vivo* trial results

3.2.1. Wound healing index

The pace of wound closure varied noticeably amongst the six groups beginning on day 0. As the days passed, the treatment groups' rate of wound closure was noticeably quicker than that of the control groups.

Table 6: The values for the wound healing index of rats (mean $\pm$ SEM)

Days	Normal group	Injured group	Placebo 50 mg/ml	Treated 50 mg/ml	Placebo 100 mg/ml	Treated 100 mg/ml
0	0	10.00 $\pm$ 0.00	10.00 $\pm$ 0.00	10.00 $\pm$ 0.00	10.00 $\pm$ 0.00	10.00 $\pm$ 0.00
5	0	10.30 $\pm$ 0.26	10.80 $\pm$ 0.06	9.20 $\pm$ 0.17	10.43 $\pm$ 0.12	8.23 $\pm$ 0.20
10	0	11.03 $\pm$ 0.75	12.17 $\pm$ 0.18	7.50 $\pm$ 0.31	11.10 $\pm$ 0.12	7.13 $\pm$ 0.20
15	0	8.37 $\pm$ 0.20	10.60 $\pm$ 0.26	4.17 $\pm$ 0.23	9.60 $\pm$ 0.36	2.67 $\pm$ 0.19

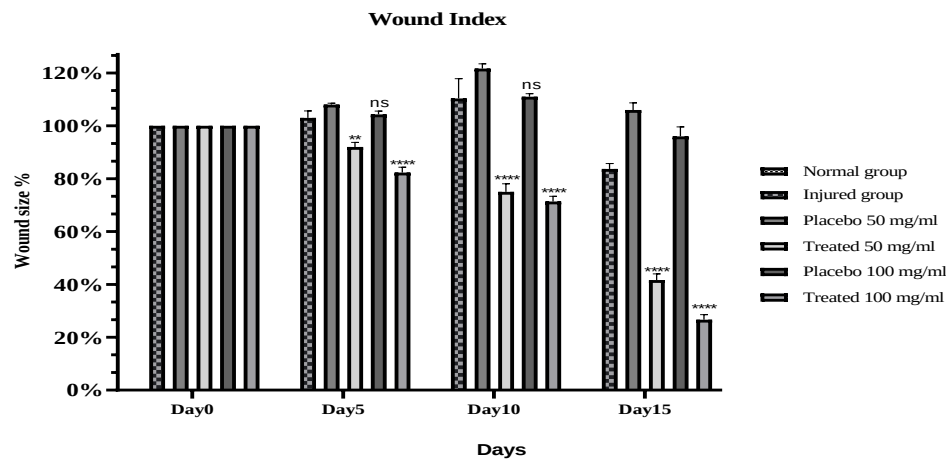


Figure 5: The levels of wound index in the treated groups of rats with selected doses of guar gum were compared to those of the injured group at different time intervals (n = 3). Results are presented as mean $\pm$ SEM

3.2.2. ELISA VEGF

The ELISA data show that, as compared to the control groups, the group of rats given different doses of guar gum had significantly higher levels of the VEGF marker.

Table 7: The mean $\pm$ SEM values of VEGF levels measured by ELISA

Groups and Doses	Normal	Injured	Placebo 50 mg/ml	Treated 50 mg/ml	Placebo 100 mg/ml	Treated 100 mg/ml
Values ($\pm$ SEM)	2.54 $\pm$ 0.027	1.96 $\pm$ 0.032	2.03 $\pm$ 0.033	2.22 $\pm$ 0.041	1.90 $\pm$ 0.054	2.59 $\pm$ 0.017

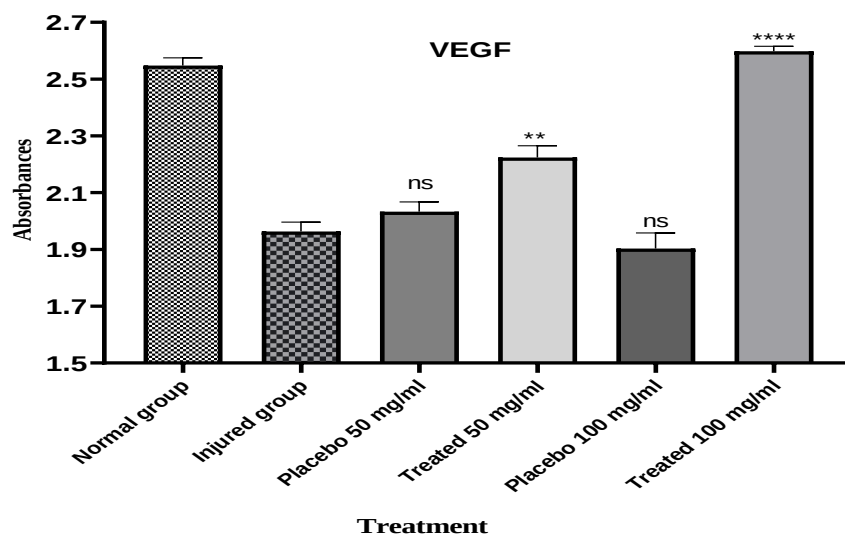


Figure 6: The VEGF levels in the treated group of rats with selected doses of guar gum were compared to the VEGF levels in the injured group of rats and the values are expressed as mean $\pm$ SEM. (****P < 0.0001; ** P < 0.0015; ns, not significant)

3.2.3. ELISA annexin V

The graphical data demonstrates that the group of rats treated with guar gum exhibits a lower level of apoptosis compared to the injured groups, indicating a potential protective effect of the treatment.

Table 8: The mean $\pm$ SEM values of Annexin level measured via ELISA

Groups and Doses	Normal	Injured	Placebo 50 mg/ml	Treated 50 mg/ml	Placebo 100 mg/ml	Treated 100 mg/ml
Values ($\pm$ SEM)	0.97 $\pm$ 0.008	1.67 $\pm$ 0.056	1.53 $\pm$ 0.020	1.37 $\pm$ 0.009	1.56 $\pm$ 0.036	1.08 $\pm$ 0.040

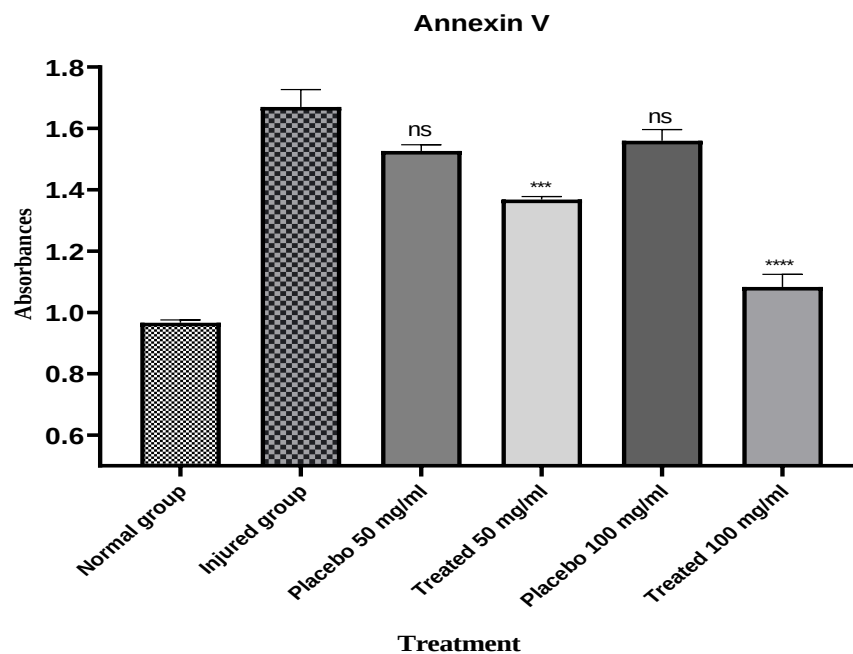


Figure 7: The levels of Annexin V in the treated group of rats with selected doses of guar gum were compared to the levels in the injured group of rats. The values are expressed as mean $\pm$ SEM.

(****P < 0.0001; *** P < 0.0005; ns, not significant).

3.2.4. Histopathology of Skin Sections

Hematoxylin and Eosin staining of the skin sections provided insight into the microenvironment of all the groups. In Figure 8A, the skin architecture of a normal rat is shown, with healthy and undisturbed glands. Figure 8B illustrates a depicted wound in the diagram, displaying an inflammatory reaction, rapid edema formation, compromised skin thickness, and infiltration of inflammatory cells. Similar findings were observed for the skin architecture in Figures 8C and 8D, where it was disturbed. In Figure 8E, a decrease in infiltration of inflammatory cells is observed, indicating a healing process and subsequent recovery. Finally, Figure 8F demonstrates a morphology that is very close to normal, with smooth skin architecture. The release of different factors by dendritic cells promotes early cell proliferation, facilitating rapid burn healing.

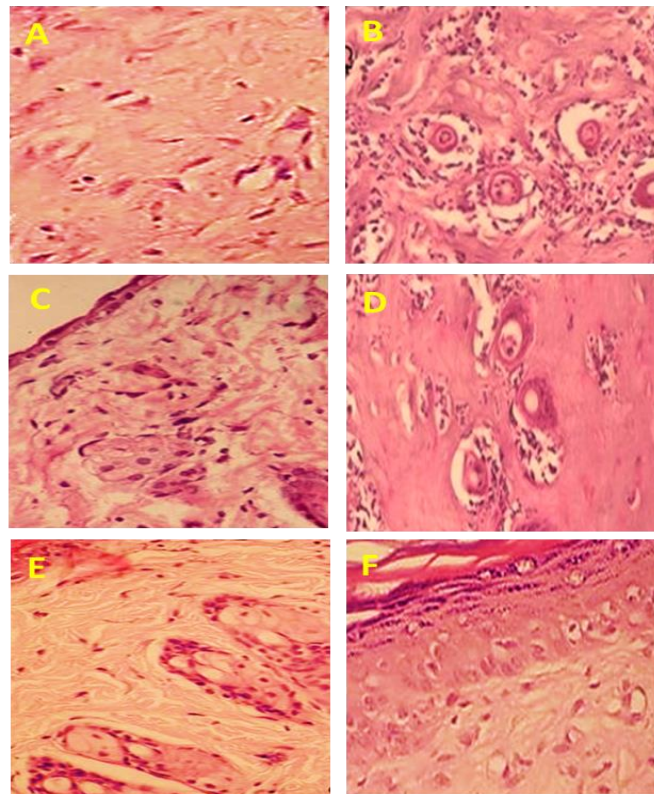


Figure 8: The hematoxylin and eosin staining of the post-treated group, providing insights into the architecture of the skin tissue. (A) A section of normal rat skin, as determined by histology. (B) A histology sample of rat skin damaged by HCL. (C) Histopathological slice of the 50 mg/ml placebo-treated rat skin from the control group. (D) A histology sample of 100 mg/ml placebo-treated rat skin from the control group. (E) A histological section of the 50 mg/ml-treated group's treated rat skin. (F) A histological slice is taken from the treated group's 100 mg/ml-treated rat skin.

4.0. DISCUSSION

There are several stages involved in the complicated process of wound healing, including inflammation, proliferation, and tissue remodeling. Effectively managing these stages is crucial for optimal wound healing outcomes.

One strategy to enhance wound healing is by controlling inflammation. Inflammatory responses play a vital role in clearing debris and pathogens from the wound site. However, excessive or prolonged inflammation can impede the healing process. Studies have demonstrated that guar gum, a substance derived from *Cyamopsis tetragonoloba*, possesses anti-inflammatory properties (Bains *et al.*, 2023). This suggests that guar gum may help regulate inflammation and create a more favorable environment for wound healing.

Additionally, promoting the proliferation of cells involved in wound healing is essential for efficient tissue regeneration. Guar gum has been studied for its potential to enhance cell proliferation in various contexts (Johnson *et al.*, 1984). Its ability to promote cell growth suggests its potential for accelerating wound healing.

The modulation of extracellular matrix (ECM) maturation is critical for wound healing. The ECM provides structural support and guidance for migrating cells during tissue repair. Manipulating ECM

remodeling can help promote wound closure and proper tissue regeneration. Although the specific effects of guar gum on ECM maturation in wound healing are not yet well-understood, its reported anti-cancer activities (Rebouças *et al.*, 2023). indicate its potential in influencing ECM dynamics.

The observed faster wound healing index in the treated group suggests that the guar gum has a positive impact on wound healing. This finding is consistent with previous studies that have reported the beneficial effects of the treatment on various aspects of the wound healing process, such as inflammation control, cell proliferation, angiogenesis, and anti-apoptotic activity (Bhubhanil *et al.*, 2021). The faster wound healing index observed in the treated group indicates that the treatment facilitates an accelerated healing response, leading to quicker closure of the wound and improved tissue regeneration. This may be attributed to the treatment's ability to promote cellular activities essential for wound healing, such as cell proliferation, angiogenesis, and anti-apoptotic effects.

The results of the *in vitro* study using the Vero cell line demonstrated a significant increase in cell proliferation and high cell viability in the guar gum treated group compared to the injured group, as evidenced by the MTT assay and the crystal violet assay, respectively. The enhanced cell proliferation observed in the guar gum treated group suggests that guar gum has a stimulatory effect on cell growth and division. This finding aligns with previous studies that have reported the proliferative properties of guar gum in various cell types (Bal-Öztürk *et al.*, 2023). The ability of guar gum to promote cell proliferation is crucial in the context of wound healing, as efficient tissue regeneration requires the rapid multiplication of cells at the wound site.

The high cell viability observed in the guar gum treated group, as indicated by the crystal violet assay, further supports its potential as a beneficial treatment in wound healing. Maintaining cell viability is essential for successful tissue repair, as it ensures the survival and functionality of cells involved in the healing process (Luchetti *et al.*, 2023).

Angiogenesis, the growth of new blood vessels, is essential for the healing of wounds as it ensures an adequate blood supply to the healing site, facilitating nutrient and oxygen delivery (Hassani *et al.*, 2023). The observed increase in angiogenesis in the treated group suggests that the administration of the treatment, such as guar gum, promotes the growth of new blood vessels.

The findings from both *in vivo* and *in vitro* analyses provide robust evidence supporting the pro-angiogenic effects of the treatment. In the *in vivo* analysis, the treated group exhibited a higher level of angiogenesis compared to the injured group, indicating the potential of the treatment to enhance the formation of new blood vessels in a physiological setting. Similarly, the *in vitro* analysis confirmed the pro-angiogenic properties of the treatment, suggesting that it directly promotes endothelial cell proliferation and vessel formation. These results have significant implications for wound healing, as adequate angiogenesis is crucial for proper tissue repair and regeneration. The enhanced angiogenesis observed in the treated group suggests that the treatment has the potential to promote the development of a well-vascularized wound bed, facilitating the delivery of nutrients and oxygen to support cellular activities necessary for efficient healing.

Annexin V is commonly used as a marker for apoptosis, which is programmed cell death. A decrease in Annexin V levels suggests a reduction in apoptotic activity. In the context of wound healing, a decrease in apoptosis can be beneficial as it indicates improved cell survival and reduced cell death at the wound site (Injarabian *et al.*, 2023).

The observed decrease in Annexin V levels in the treated group, as compared to the injured group, indicates that the treatment has a potential anti-apoptotic effect. This suggests that the administration of the treatment with guar gum may help preserve cells and prevent excessive cell death, thereby supporting the healing process. The findings from both *in vitro* and *in vivo* analyses

provide consistent evidence for the anti-apoptotic properties of the treatment. In the *in vitro* analysis, the treated group exhibited significantly lower Annexin V levels, indicating a reduced apoptotic response in the treated cells. Similarly, in the *in vivo* analysis, the treated group showed decreased Annexin V levels, suggesting a decrease in apoptosis in the treated tissue compared to the injured tissue. These findings have significant ramifications for wound healing because decreasing apoptosis can aid in cell survival, tissue regeneration, and wound healing as a whole.

These *in vitro* and *in vivo* results have significant implications for the potential clinical application of the treatment in wound healing management.

5.0. CONCLUSION

The findings from the various analyses conducted in this study highlight the potential benefits of the guar gum, in wound healing. The *in vitro* studies demonstrated that guar gum treatment led to a significant increase in cell proliferation and high cell viability, indicating its ability to promote cell growth and support cell survival. Additionally, the treatment showed pro-angiogenic effects, enhancing the formation of new blood vessels, which is crucial for adequate tissue regeneration. The *in vivo* analyses further supported the positive effects of the treatment on wound healing. The treated group exhibited a faster wound healing index compared to the control group, indicating an accelerated healing response and improved tissue regeneration. Moreover, the decrease in Annexin V levels in the treated group suggested a reduction in apoptosis, supporting cell survival and contributing to enhanced wound healing outcomes. The results suggest that the guar gum has multifaceted effects on wound healing. It promotes cell proliferation, angiogenesis, and anti-apoptotic activity, all of which are critical processes for efficient wound healing. These findings support the potential of the treatment as a therapeutic intervention to enhance wound healing outcomes.

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