# ANTICANCER AND SKIN REPAIR POTENTIAL OF GUAR GUM

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# ANTICANCER AND SKIN REPAIR POTENTIAL OF GUAR GUM


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**By**

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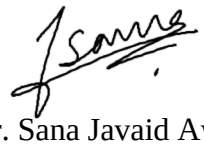

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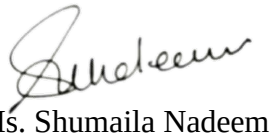

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# ABSTRACT

Guar gum is one of the medicinal plants that has been used for various diseases treatments. The purpose of this study is to assess the guar gum plant's capacity to wound healing process and prevent cancer in acid-burned rats. The wound healing capacity of Guar gum extract at different doses (50 and 100 mg/ml) was estimated by using different biochemical experiments. Wound index measurement was done to study wound healing ability of guar gum powder aqueous extract and results were significant indicating the wound healing ability of plant. The maximum potential of healing was observed at 100 mg/ml dose that was (2.2±0.24). MTT assay calculations were used to determine the anticancer effectiveness. The angiogenesis was assessed using the ELISA procedure and antibodies against VEGF (2.6±0.052) and Annexin (1.1±0.033). Biochemical analysis shows that wound become better after treatment with guar gum powder. The high dose level of guar gum plant powder treatment in the current study suggests that it has both anticancer and wound-healing properties.

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# List of Abbreviations

| No. | Abbreviations | Full Forms |
|---|---|---|
| 1 | VEGF | Vascular Endothelial Growth Factor |
| 2 | cDNA | Complementary DNA |
| 3 | dNTP | deoxyribonucleotide triphosphate |
| 4 | DNA | Deoxyribonucleic Acid |
| 5 | RNA | Ribonucleic Acid |
| 6 | ELISA | Enzyme-Linked Immunosorbent Assay |
| 7 | TBS | Tris Buffered Saline |
| 8 | BSA | Bovine Serum Albumin |
| 9 | HPR | Horseradish Peroxidase |
| 10 | TMB | Tetramethylbenzidine |
| 11 | HCl | Hydrochloric Acid |
| 12 | RNA | Ribonucleic Acid |
| 13 | DNA | Deoxyribonucleic Acid |
| 14 | TAE | Tris-acetate-EDTA |
| 15 | GITC | Guanidinium Thiocyanate |
| 16 | DEPC | Diethyl Pyrrocarbonate |
| 17 | cDNA | Complementary DNA |
| 18 | dNTPs | Deoxyribonucleotide Triphosphate |

# Chapter 1
# INTRODUCTION

A brand-new agricultural compound made from cluster bean endosperm is guar gum. In the food, oil well drilling, cosmetics, pharmaceutical industries, paper, textile and explosive, guar gum powder is a frequently used ingredient. Guar gum is suitable for industrial usage since it can make H2 bonds with H2O molecules. This is therefore mostly utilized as a stiffener and equilibrium. It helps with the treatment of a several of well-being conditions, including colon cancer, heart disease, diabetes, and bowel movements. Guar gum's production, processing, content, traits, food applications, and health benefits (1). The antibacterial and wound-healing properties of a hydrogel made of guar gum and silver nanoparticles stabilized by curcumin are exceptional. When compared to commercial antibacterial gels, in vivo studies on rats demonstrate that the hydrogel-nanoparticle composite accelerates less than 40% wound cessation and lessens 60% infectious counts (2).

Wounds are physical injuries that cause the skin to open or break, such as acute trauma or surgery. Patients experience non-trivial health problems as a result of subsequent infections, prompting important advancements in wound therapy (3, 4). The return of functional status to the skin, the decrease of healing time, the prevention of infection, and the restoration of damaged anatomical stability all depend on effective wound healing. As damaged tissues are repaired, including throughout the regeneration and replacement stages, a variety of cell types experience inflammation, propagation, and migration. After an injury, the inflammatory stage begins with vasoconstriction, which promotes homeostasis and releases inflammation mediators (5).

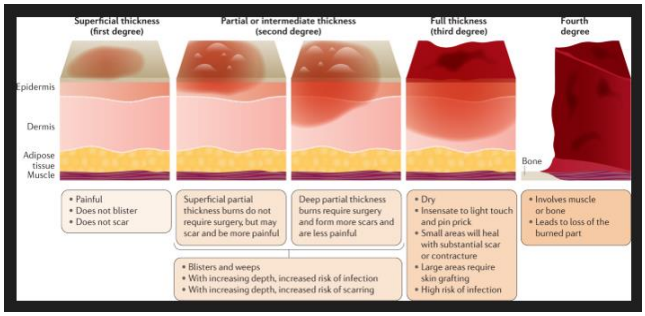



**Figure 1.1:** Stages of Acid Burn Wounds

Angiogenesis is the development of blood vessels by vascularization. Angiogenic therapy can fight cancer, heart disease and more than 70 deadly diseases (10). Although all of these vessels, including the aorta, are derived from capillary action, the "angiogenesis" often mentions only development of new vessels (11). Increased capillary function improves tissue oxygenation and thereby increases low energy production, leading to decreased capillary function and hypoxia, as well as oxygen deficiency in tissues.

To burn the metabolic substrates needed for energy, all bodily tissues need a constant flow of oxygen. Blood capillaries carry oxygen to these tissues; more capillaries will result in better tissue oxygenation, which will increase energy generation; fewer capillaries will result in hypoxia or even anoxia in the tissues. This indicates that angiogenic remedies, which were developed to manage the development and regression of vessels, can be used to restore the survival of injured tissues in the human body (heart, brain, bones, muscles, and so on), as well as to eliminate undesired cells (12).

The tentative finding that gradual growth of solid lump and their evolution is dependent on constant generation of new capillary blood vessels from the host has piqued interest in angiogenesis during the last era (13, 14). According to the Angiogenesis Foundation, angiogenic therapy has the potential to enhance at least 1 billion people's lives people around the world. The following findings have been made as a result of efforts to understand the process of angiogenesis: (a) Angiogenesis is a multi-phases process that, in some ways, resembles blood coagulation (15) and (b) Tumors are not the only physiological and pathologic processes that persuade neovascularization; they are also linked to delayed hypersensitivity, corpus luteum maturation, chronic inflammation, and wound healing (16-18).

Angiogenesis appears to be required for the completion of physiologic processes, such as the creation of the corpus luteum, and then decreases or is turned off once the process is accomplished. Angiogenesis is excessively protracted yet self-limiting in certain diseased conditions that are not malignant. Examples include retrolental fibroplasia, keloid formation, and pyrogenic granuloma. On the other hand, angiogenesis in cancer is not self-limiting. Angiogenesis that is triggered by a tumor can persist indeterminately till the lump is totally removed. However, the exact process underlying the development of new vessels

in reaction to a tumor resembles the capillary proliferation found in benign angiogenesis in a startling way (19).

Vascular endothelial growth factor (VEGF) is a heparin-binding angiogenic growth factor with a great endothelial cell particularity (22, 23). VPF (vascular permeability factor) is a protein that has been linked to tumor-associated proteins (24). In 1989 and others, Hauser revealed that a single VEGF-one gene encoded both Endothelial cell growth factor and permeability-inducing factor, and that the VEGF isoforms were triggered by the alternate splicing arrangement of disulfide-bound homodimers by this gene (25-27).

While VEGF receptors are mostly found on preglomerular, glomerular, and peritubular endothelial cells, VEGF expression is highest in glomerular podocytes and tubular epithelial cells of the kidney. The role of VEGF in typical renal physiology is largely unclear. Although it has been proposed that VEGF may play a part in the development and maintenance of glomerular capillary endothelial fenestrations, the absence of noticeable consequences of VEGF blockade in healthy experimental animal's points to a limited role during homeostasis. VEGF and its receptors are increased in both type 1 and type 2 diabetic humans and experimental animals. Diabetes-induced functional and structural alterations are improved by VEGF inhibition, suggesting that VEGF is detrimental to the aetiology of diabetic nephropathy (34). The primary goal of the current study is to determine the effects of giving guar gum plant powder to albino rats in order to test their ability to fight cancer and wound healing.

# AIMS AND OBJECTIVES

- Formation of aqueous extract of Guar gum.
- Evaluation of Wound index in rats
- To study of Anticancer property of Guar gum

# RATIONALE

Most medicinal plants have been used for a number of health benefits, like minimizing the probability of cancer due to their antioxidant activities and preventing cardiovascular diseases. In previous studies, guar gum has been used for the curation of intestinal ulcer, obesity and diabetes. The goal of the study was to investigate the anticancer effects along with its wound healing properties.

# CHAPTER 2
# Literature review

## 2.1 Plant Overview

The seeds of the drought-tolerant plant Cyamopsis tetragonoloba, a member of the Leguminosae family are used to make guar gum (39). Despite the fact that Hymowitz proposed concept of trans-domestication, this plant has no broad agreement on its origin (40). The theory explains how the guar gum, was adopted to survive in the extreme dry period from African species. The indigenous species is most frequently related with Pakistan and India, where it has been spread for decades as a source of nutrition for people and animals (41).


**Figure 2.1:** (A) Guar gum Plant (B) Guar gum leafs (C) Guar gum Seeds (D) Guar gum Powder

In the 1940s and 1950s the guar gum industry grew in United States (42). Prior to World War I guar was mostly imported to the United States as green compost but Prior to 1943, it had no industrial applications, which is likely the main reason for its limited research (43). Locust bean gum, which was once introduced from North Africa and Europe and was extensively utilized in textile and paper industries, had become scarce and was difficult to find (44).

This inquiry led to a reexamined of guar, that was determined to be the problem's best solution. During World War II, commercial development took place at the University of Arizona (45). Whistler analyzed the gum at Purdue near the end of the war. He studied the molecular structure and, after evaluating the qualities of guaran, a pure polysaccharide, he saw its vast economic potential and suggested that the plant be developed as a local crop for manufacturing as a replacement for palm oil (46). Studies show that gum is a helpful papermaker's adjunct for providing temporary wet strength in sheets, like paper towels, and it also helps hydrate different pulps while they are being beaten. The annual drought-resistant leguminous plant known as guar, or Cyamopsis tetragonolobus, which grows three to six feet tall and has been used as cattle and horse fodder in some regions of India for millennia, produced the best results (47).

Some varieties of paper are reported to benefit from the addition of guar flour to beaters to increase their tensile strength (48). According to reports, guar also possesses qualities that could be advantageous for printing pastes, warp sizing and a few finishing jobs. The endosperm of the seed must be separated from the exterior, mostly fibrous components, in order to produce the gum (49). Although guar gum consumption improved quickly, it was the creation of cationic and anionic derivatives of guar gum as well as their application in the stimulation of gas and oil wells that gave guar gum its current commercial significance (50). Guar gum thickens dye solutions used in textile and carpet printing, enabling the creation of patterns with greater clarity. As a water-blocking ingredient in explosives, guar gum was first introduced to dynamite more than 25 years ago (51). It has mostly taken the place of other gelling agents in recent years in explosives. The pulping process is developed when low quantity of guar gum are added. It functions as dry-strengthening agent and fibre deflocculent (52).

The endosperm of this plant is crushed to produce guar gum (55). The guar plant likes the sun and can survive high temperatures, but it is sensitive to frost. Maximum development requires soil temperatures between 25 and Ideal conditions would be 30 °C and a dry atmosphere with little to no rain but constant sunshine (56). Rain is required before and after sowing, guar plant growth to promote seed maturity. Guar beans of lesser quality are

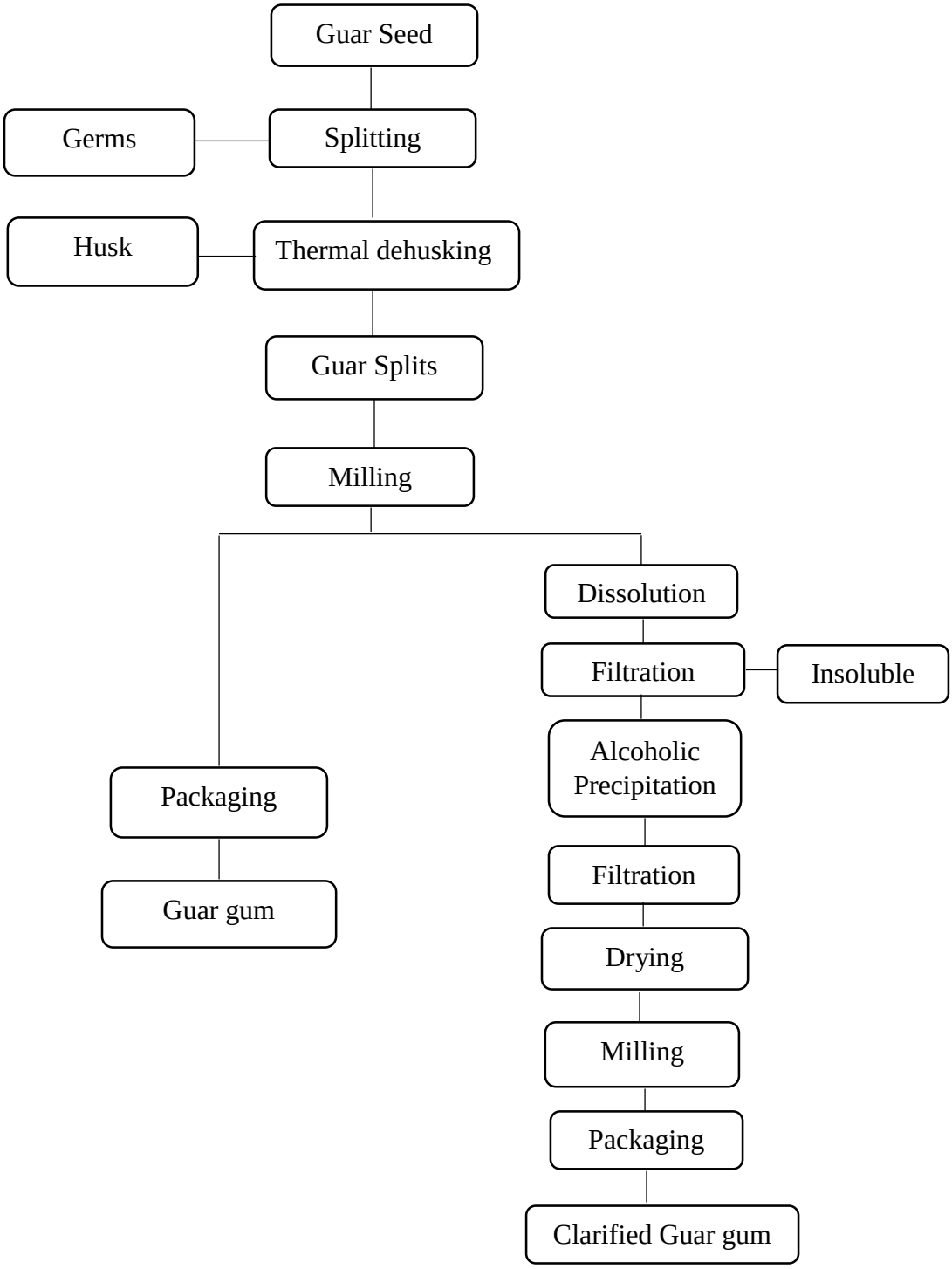
produced when there is too much moisture in the early stages of germination and after the seeds have matured (57).

The monsoon rain pattern in northern Pakistan and India often offers the best circumstances for guar growth. Pakistan and India produce more than 90% of the world's guar (58). The crop's unique necessity for the proper quantity of rain at the right time of growing and evolution renders it highly reliant on yearly rainfall patterns, resulting in periodic big swings in guar availability and pricing (59). In semi-arid areas of the southern hemisphere, including Brazil, Australia, and South Africa, and parts of the United States, including Texas and Arizona, guar is also farmed. It is anticipated that these countries will produce 15,000 metric tonnes of guar seeds annually (60).

In Australia, the agro-climatic conditions are also favorable for guar cultivation. Similar to this, there are rumors that Thailand, China are trying to grow guar. India and Pakistan may eventually lose their stranglehold on guar as a result (61). Rajasthan grow for 70% of the guar production, which is produced by India as a whole. Guar is cultivated in the northern Indian of Punjab, Haryana, Rajasthan and Gujarat. India produce most of guar gum (62). Guar was also grown on a regular basis in states of (UP), (MP), and Orissa throughout the 1970s. Due to the closure of processing plants in the U.P. and M.P., these states' cultivation is presently minimal (63). Guar cultivation is no longer practiced in Orissa. During the previous three years, annual production of guar varied between 11,00,000 and 12,87,000 MT (64).

Prior to the 90’s, approximately 80% of the guar in Pakistan was grown under sprinkling, which led to a higher yield per acre (65). At that time, guar was grown in Central, North and south Punjab. Guar gum is produce in lakhs (66).

From one factory to the next, guar gum is processed in a different way. The basic steps in the production of guar gum are as follow, when seeds are extracted, they are smaller than pea seeds, sphere-shaped in shape, and brown in colour. Firstly, the germs and husks are removed from guar seeds, and then the process of splitting is started. Guar seed is split by thermal dehusking and then milled until fine particles or powder form by the processes of dissolution, filtration, alcoholic precipitation, filtration, drying, and packaging (67)




Commercially, the gum is removed from seeds mostly via a mechanical method that entails boiling, grinding down, sifting, and improving. The endosperm and germ are split apart when the seeds are cracked open (68). Endosperm from each seed is produced as two halves

(69). Guar meal, a byproduct of the production of guar gum powder, is fed to cows. It is composed of the guar seed's hull (husk) and germ (70).

Depending on the desired outcome, the polished guar rifts are next processed and polished into powders using a variety of routes and processing processes (71). Before being properly dispersed to an ultra-fine mincer, the pre-hydrated guar rifts are smashed in a flaker mill. This prevents the splits from becoming overheated while grinding. The ground material is dried, then graded based on particle size by being run through screens (72). When choosing a grade, one should take into account the following factors: colour, mesh size, viscosity potential, and hydration rate. Extrusion is also used in the industrial processing of guar gum before hydration and flaking (73). Grinding and drying are completed after these procedures. The addition of extrusion results in guar gum powder with a higher hydration rate (74).

Due to the development of numerous guar gum derivatives, including anionic and cationic derivatives, guar gum demand has increased in recent decades (75). Sand can be transported into cracked rock by fracturing fluid that has been thickened by guar gum. This gap continues to be open because of the sand, enabling the flow of gas or oil into the well bore. Fracturing fluids use the guar derivatives carboxymethyl hydroxypropyl and hydroxypropyl guar (47).

Guar gum condenses dye solutions used in printing, enabling the creation of patterns with greater clarity. In the recent past, it has mostly replaced other crystalizing agents in water-based slurry combustible (55). The ability of guar gum to prevent water and to swell, gel, and swell allows it to be used as combustible material. Ammonium nitrate, nitroglycerine, and oil explosives can all have their explosive qualities maintained even in moist environments by mixing them with guar gum. The papermaking process is enhanced by adding guar gum in little amounts to the mixture (58). According to studies, guar gum can be processed to create complexing reagents. High-viscosity gel complexes are created when these chemicals combine with guar gum (76). The three layers that make up the guar kernel are the endosperm (34%–40%), the germ (43%–46% of the guar kernel), and the outer husk (16–18% of the guar kernel). In the seed's germ, protein predominates, whereas in the endosperm, galactomannan takes centre stage. Galactomannans, which are linear

chains of (1-4) polysaccharides with a high molecular weight, make up the majority of guar gum (77).

The mannose and galactose units make up the portion of the seed endosperm. At first, the side groups were thought to be used as replacement with the mandibular spine. However, experiments using guar enzyme degradation spectroscopic approaches and computer simulation indicate a very arbitrarily dispersion of side groups (78).

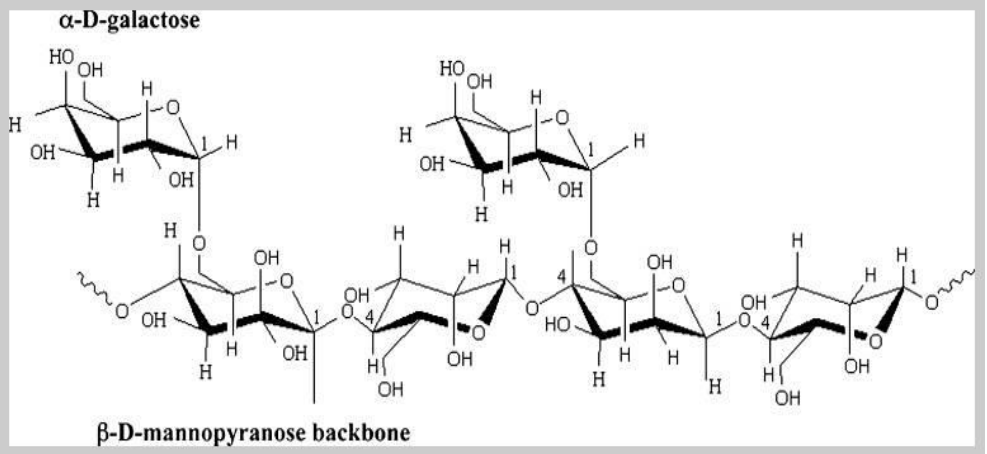



**Figure 2.2:** Composition of Guar gum

Among all the naturally occurring water-soluble polymers, guar has high weight. Depending on the galactomannan molecular weight, commercial guar gum formulations have a wide range of viscosifying effects. Early literature claims that while guar gum typically has a molecular weight between 0.25 and 5.0 million, it depends on the method applied (79).

Absolute approaches, like as light scattering techniques, have also been employed to quantify molecular weight, which are similarly helpful for giving the polysaccharide's physical structure. The typical molecular weight, however, appears to be in the 106 to 2 x 106 range, according to most latest information gathered by scattering and chromatography (80).

## 2.2 Physio-chemical properties

Their biological characteristics are determined by how guar β - glucan and other carbohydrates behave in an aqueous media. Guar gum forms stronger hydrogen bonds with

the polar liquids it is diffused in during the process of enlarging or resolving. Only in nonpolar liquids does it produce weak hydrogen bonds. The formation of stickiness and the rate of guar gum dissolving typically rises when temperature, pH, and particle size all decrease. Hydration rates are slowed down by dissolved salts and other substances like sugar that bind to water (81).

### 2.2.1 Rheology

It shows how materials behave by the impact of outside forces, including flow and deformation. Guar gum, like many heavy polymers, exhibits pseudo plastic or thinning behaviour in aqueous solutions, meaning that consistency reduces as sheerness increases. Guar gum aqueous solution exhibits shear-thinning behaviour as its polymer content and molecular weight rise (82). The yield stress features of guar gum aqueous solutions are likewise absent. Guar gum aqueous solutions at 1% concentration display typical complex molecular biopolymer activity, wherein in the lower frequency range, loss modulus (G) predominates over storage components (G). These components, however, outweighs the reduction of components. The storage components and loss components of guar gum aqueous solutions decreased over time (83).

### 2.2.2 Viscosity

Guar gum's most notable feature is the capacity to moisten quickly in water, resulting in very sticky solutions (76). When entirely hydrated, guar gum creates a viscous mixture dispersal that is a low-viscosity fluids system. Solutions with guar gum content of less than 1% have low-viscosity than solutions with guar gum content of 1% or higher. Schlakman and Bartilucci looked at thirteen distinct commercial samples and discovered a lot of variance in viscosity, particle size, and hydration rate. Good-quality guar gum can have a viscosity of up to 10,000 cp in an aqueous dispersion at 1% (84).

### 2.2.3 Hydration rate

Guar gum's rate of hydration varies. In practical applications, a 2-hour hydration period is required to achieve maximal viscosity. The rate of hydration is mostly determined by the size of the powder particles. As a result, very high quality gums are accessible for quick

initial viscosity. However, for optimal hydration and viscosity, a significant time period is still required (85).

### 2.2.4Hydrogen bonding activity

The OH- group in the gum is causes the substance's H-bonding abilities. It creates H bonds with minerals. Any system that has guar gum added to it will see a dramatic change in its electro kinetic properties. Guar gum's steric hindrance is increased when hydroxypropyl groups are put in their place, which lowers hydrogen bond stability (75).

## 2.3 The variables that determine thickness and hydration rate

Guar gum's thickness and hydration rate do not remain constant with changes in temperature, pH, solute, concentration, and other factors.

### 2.3.1 Temperature

The temperature has a significant impact on both the high consistency and the moisture content. Plant mixtures reach their extreme thickness much more quickly when created at greater temperatures than when made at lower ones. But it is also believed that persistent heat has a degrading effect (76). The final thickness of heated guar gum solutions is typically lower than that of heated solutions made with cold water and slowly hydrate. The ideal temperature range for guar gum dispersion's maximum viscosity is 25–40 °C. A 0.5 percent guar solution has a viscosity that is much higher at 25 °C than it is at 37 °C (86).

### 2.3.2 Concentration

The consistency of this plant solution is increased, even at low amounts. A concentration of less than 1% is advised for the majority of food applications. The guar gum concentration increases in direct proportion to the consistency of the solution. The water molecule and the galactose side chain of the guar molecule combine to cause this. Higher guar gum concentration promotes intermolecular chain interaction or entanglement, which increases viscosity (87).

### 2.3.3 PH

Solutions containing guar gum are constant at pH from 1.0 to 10.5; this results from the substance's neutral charge. The thickness of gum is unaffected from pH, although the

moisture content is affected by pH and changes accordingly. The quickest hydration happens between pH 8 and 9, while the less moisture happens less than pH 10 and (89).

### 2.3.4 Salt

Other than water, salt is the most commonly used element in foods, and its impact on guar gum has been widely researched. Saline guar gum solutions behave similarly to guar gum solutions in water. Although salt has little effect on the rate of hydration, sodium chloride marginally enhances the guar gum's final viscosity after being thoroughly hydrated (91). When a physiological buffer, such as Krebs bicarbonate, is added to a 0.25 percent guar gum solution, the viscosity is reduced when contrasted to the thickness of gum in H2O alone. Salts interfere with the guar gum solution's ability to hydrate. Srichamroen discovered that combining salts with a 0.5 percent guargum solution rises its viscosity. Salts can facilitate intermolecular interactions because they modify the gum's charge density and structure (92).

## 2.4 Usage in Food

Guar gum is a unique food stabilizer that is utilize in the many products of food to stabilize foods and as a fibre source in the industry of food. Due to its low cost and natural makeup, it is well-liked by both producers and consumers. It is utilize as a food stabilizer because it modifies the water’s behaviour, a common ingredient in many dishes (93).

### 2.4.1 Beverages

It is utilized in beverages for viscosity control and thickening due to its many natural possessions. Its primary quality is its opposition to deterioration in less pH settings, such as those present in beverages. Guar gum is perfect for use in the production of beverages since it is soluble in cold water. It increases the drinks' shelf life (94).

### 2.4.2 Dairy products

For stability, guar gum is mostly utilized in frozen foods. Guar gum is incredibly important for the stability of ice cream because of its capacity to bind water. It is perfect to be utilized in HTST (high temperature, short time) procedures, which call for hydrocolloids that can dry completely in a brief period of time. Utilizing gum manufacturing ice dairy products

give positive outcomes. Ice cream mixtures need to include guar gum at a concentration of 0.3% (95). Similar to carob gum, its efficacy can be enhanced by mixing it with other stabilizers. Ice cream's body, texture, chewiness, and resilience to heat shock are all enhanced by guar gum. Compared to full-fat yoghurt, low-fat yoghurt with guar gum that has been slightly dissolved (2–6% concentration) has improved textural and rheological properties.

### 2.4.3 Processed meat products

Both hot and cold water can be held in large quantities by guar gum. In order to produce sausages and other packed meat products, it is effective as a binder and lubricant. In processed meat products, it serves specialised functions such as regulating syneresis, preventing transfer of fat during preservation, regulating the thickness of the state of fluid during manufacturing and chilling, and avoiding water buildup inside the can while still being preserved. Additionally, guar gum increases the rheological control and blending constancy of egg yolk emulsions.

### 2.4.4 Bakery products

Guar gum improves the machinability of cake and biscuit dough, making it easier to remove the dough from the mould and cut it without it disintegrating. When employed at 1% in doughnut batter, it has advantageous film-forming and adhesive characteristics that inhibit fats and oils from entering. In frozen pie fillings, guar gum and starch have been shown to aid in reducing dryness, shrinkage, and breakage. It was included to the wheat batter, and outcome was a significantly larger baked loaf. Guar gum also reduces starch retro gradation, which stops chapatti from ageing at room temperature as well as in the refrigerator.

### 2.4.5 Salad dressings and Sauces

It can be employed as a thickening agent in salad dressings at 0.2-0.8% of total weight due to its capacity to dissolve in cold water and stability with acidic environment emulsification. By making the water phase more viscous and reducing the rate at which the water and oil phases separate, it serves as an emulsion stabilizer in salad dressings. It has been demonstrated that guar gum can effectively replace tragacanth as a thickening agent

in condiment and pickle pastes. Guar gum enhances tomato ketchup's stability compared to other hydrogels, stability such as carboxymethylcellulose, Pectin, gum acacia, and alginate. Guar gum is a unique thickener for tomato ketchup because it reduces serum loss and improves flow characteristics when applied.

## 2.5 Health Benefits

Animal studies have been undertaken to see if guar gum has any negative or helpful effects. Clostridium butyricum totally degrades guar in the large intestine. Guar gum doesn't affect animals until it is administered in high doses (approximately 10–15 percent by weight). The animal's growth will be inhibited as a result of the high dosage's impaired digestion and reduced feed intake. It is believed that the increased viscosity of the duodenal contents triggered by guar gum ingestion at increasing concentrations is the primary reason for the negative consequences. Guar gum can therefore only be utilized 0.5–1.0% at small quantities for its beneficial properties. Above this level, there are undesirable side effects, including increased viscosity, decreased protein efficacy, and increased fat ingestion.

The high viscosity of guar gum would not only inhibit with the nourishing qualities of the meal but also with the structural, chemical, and textural features of the food item., which the consumer would not accept when used at a higher concentration, above 1.0 percent. Guar gum that has been slightly dissolved shortens the polymer's chain and lowers its molecular weight, which creates a new soluble fibre with a fundamentally comparable chemical structure to natural guar gum. It has a range of clinical nutrition applications involving dietary fibre intake. It takes care of every issue raised by guar gum's extremely high viscosity.

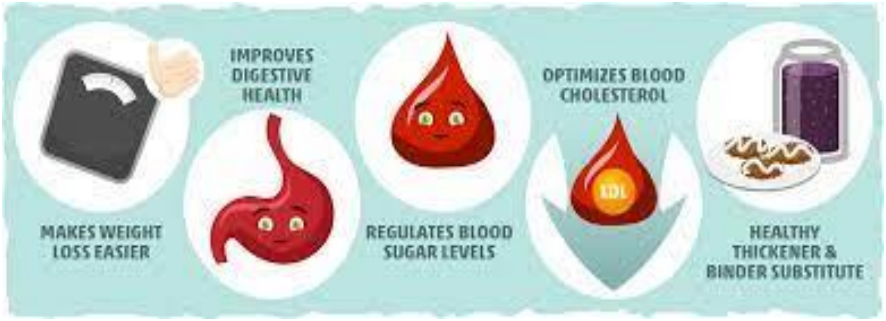



**Figure 2.3**: Health Benefits of guar gum

Hydrolyzed guar gum can be utilize to boost the nutrients of numerous food products, including drinks, while preserving the food's nutritional value and flavor. Adding PHGG to the diet lessens the frequency of diarrhea, the need for laxatives, and the signs of spastic bowel syndrome. Water-soluble, non-gelling fibre is the most efficient method of irritable bowel syndrome therapy. Spatic bowel syndrome symptoms were lessened by incompletely hydrolyzed guar gum in both the diarrhea and constipation types because of its non-gelling and water soluble qualities.

Guar gum reduces fat and glucose levels due to its ability to produce gels. Additionally, it helps with weight loss and obesity prevention. Due to the soluble fibre in guar gum's ability to form gels, slow gastric emptying leads to higher satiety. Supplementing with guar gum decreases hunger, desire, and the impulse to eat. By boosting bile acid excretion in the faeces and reducing enter hepatic bile acid, guar gum decreases cholesterol levels. This may promote the making of spleen acids from fat and, therefore, lower levels of hepatic free cholesterol.

# CHAPTER 3
# MATERIALS AND METHODS

## 3.1 Plant powder formation

Guar gum is grown in many districts of Pakistan. The powder will be taken from Pakistan Gum Industries.

## 3.2 Aqueous Extract Preparation

The aqueous plant extract of guar gum plant was prepared by dissolving 10g of plant powder in 100 ml water then after 2 days the solution was passed through the Wittman filter paper. The filtered water contain water soluble fractions were evaporated through petri dish (96).

**Figure 3.1:** Aqueous Extract Preparation

## 3.3 Culturing of HeLa Cell Line

HepG2 cells were cultured once they had reached 70–80% confluence in culture. For splitting, the cells affixed to the culturing flask's walls were washed with 1X phosphate buffer saline (PBS), and then they were exposed to 0.05% trypsin-EDTA until the cells detached from the flask's surface. By looking at the flask under an inverted microscope, the cells' detachment was verified. Fetal Bovine Serum (FBS) was added in a few drops, and the flask was thoroughly combined by swirling. The mixture was centrifuged; to do so, it was placed in a 15-ml tube and spun at 2,000 rpm for 5 minutes. After centrifugation, the pellet was re-suspended without the supernatant. By looking at the flask under an

inverted microscope, the cells' detachment was verified. Fetal Bovine Serum (FBS) was added in a few drops, and the flask was thoroughly combined by swirling. The mixture was centrifuged; to do so, it was placed in a 15-ml tube and spun at 2,000 rpm for 5 minutes. After centrifugation, the pellet was re-suspended without the supernatant (101). Cells were replated in 96 well plates where treatment was given to them.

Stock solution of guar gum extract was prepared as 100mg/ml. cells were given the treatment as 1ul, 5ul and 10 ul of this stock. Treatment was given for 24 hours ans then MTT assay was performed.

## 3.4 Cell viability assays

To calculate cell viability, 3-(4, 5-dimethylthiazol-2- yl) -2, 5-diphenyltetrazolium bromide (MTT) assay was done utilizing a 96-well plate (110). Cells were washed out with phosphate buffer saline (PBS) after 24 hours of treatment with different doses of hermal extracts powder, and then were incubated for 3-4 hours with 100µl of DMEM and 25µl MTT solution. After 4 hours, the formazan crystals were dissolved in 10% sodium dodecyl sulphate (SDS) and the absorbance at 570 nm was measured (102). The percentage viability was estimated using the following formula:

% Cell viability = (Experimental (OD570)/ Control (OD570))× 100

## 3.5 Animal model

Albino rats, weighing between 160 and 200 g, were raised in the animal house of the "Molecular Medicine Research Centre (CRIMM) of the Molecular Biology and Biotechnology Institute (IMBB)" at the University of Lahore, Pakistan. The Pakistani ethical review committee gave its approval for the use of this animal in research.

**Figure 3.2:** Animal Model

## 3.6 Grouping of Rats

Albino rats were bought and six groups, each with n=3 were made. One group of mice were not given an injury while the other 5 groups were given acid burn injury.

## Table 3.1 Grouping of Rats

| No. | Group Name | Group Description |
|---|---|---|
| 1 | **Normal (N)** | Normal rats with no injury |
| 2 | **Injured (I)** | Completely injured with HCl and left untreated |
| 3 | **Placebo 1 (Plac 1)** | 50 mg/ml Normal saline (placebo) |
| 4 | **Treatment 1 (T1)** | 100 mg/ml Normal Saline |
| 5 | **Placebo 2 (Plac 2)** | 50 mg/ml Guar gum Extract |
| 6 | **Treatment 2 (T2)** | 100 mg/ml Guar gum aqueous extract |

## 3.7 Acid Burn Injury in Rat

Some rats were employed as controls, and some were assigned to the burn group. All animals were given 90 mg/ml of ketamine to put them to sleep before having their dorsum's

longitudinally and transversally shaved. Controls were then placed in separate cages for recuperation. The other animals were then given acid-soaked filter paper, which caused burns. The acid strips were in contact with the shaved animal skin to produce third-degree burns. The burns were at 4 places on body of each animal. After the burns, the animals were received plant powder mix in its food and place in cages for recovery. A summary of mortality rates was made at 3, 7, 15, and 25 days. After this time, animals that were still alive were sacrificed using fatal amounts of plant powders (103, 104).

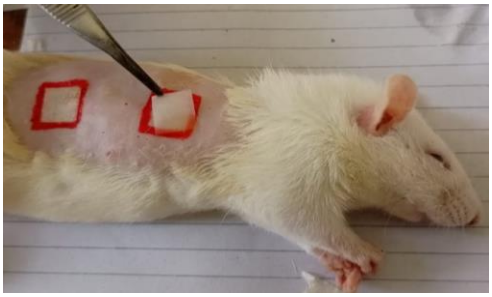


**Figure 3.3:** Acid Burn Injury in Rats

## 3.8 Treatment

The groups of rats were treated with different concentrations of Guar gum aqueous extract that were 50 mg/ml and 100 mg/ml and remaining two groups were given normal saline of 50 mg/ml and 100 mg/ml concentrations.

**Figure 3.4:** Treatment of Rats by giving guar gum extract

## 3.9 Wound Healing Index Measurement

Wound healing analysis was performed on days 5, 10, and 15 to measure the reduction of damaged areas. In short, it was a sheet of transparent plastic that was placed in the wound of anesthetized rats, placed on the ventral side down. The damaged area was marked with using a pointer to the mouse. The percentage reduction in wound size was taken using the current wound values (105). Average wound size was of 0.9957 $cm^2$. Five-second wounds were yellowish with an illegible erythematous border. The 10- and 20-second wounds were clearly defined, consistently brown, and had an erythematous ring. Average tissue injury depths for periods of 5, 10, and 20 seconds were 1.30 mm, 2.35 mm, and 2.60 mm, respectively. Full-thickness damage was the result of a 5-second burn. Burns lasting between 10 and 20 seconds caused full-thickness damage, affecting nearby skeletal tissue (106).

## Skin sample collection of Rats

After 1 week all the rats were dissected and their skin were collected and stored for histopathology tests.

**Figure 3.5:** Skin sample collection after 7 days

### 3.10 Sandwich Elisa

A sandwich ELISA for annexin V and VEGF was conducted. The microtiter plate with 100 µl of the appropriate coating antibody was coated, at a concentration between 1-10 µg/ml in coating buffer. Blocking solution of 150 µl was added to each. It was incubated for 60 minutes at 37°C and was washed 4 times in wash buffer with phosphate buffer saline (PBS). The 100 µl of suitably bovine serum albumin (BSA) was added to prevent antigens and antibodies from binding non-specifically to the wells. Then each well was filled with a 100 ul sample (serum obtained from the blood of treated rats) and then again were incubated for 90 minutes at 37°C and were washed 3 times in wash buffer before being incubated for 2 hours at 37°C with a horse reddish peroxidase (HRP) conjugated donkey anti-rabbit secondary antibody. And after washing 100 µl of diluted chromogenic solution 3,3,5,5 TMB (Tetramethylbenzidine) was added to each well. Later on 0.18 M sulphuric acid was added to stop the solution and at 450 nm, desired color change was attained (107, 108).

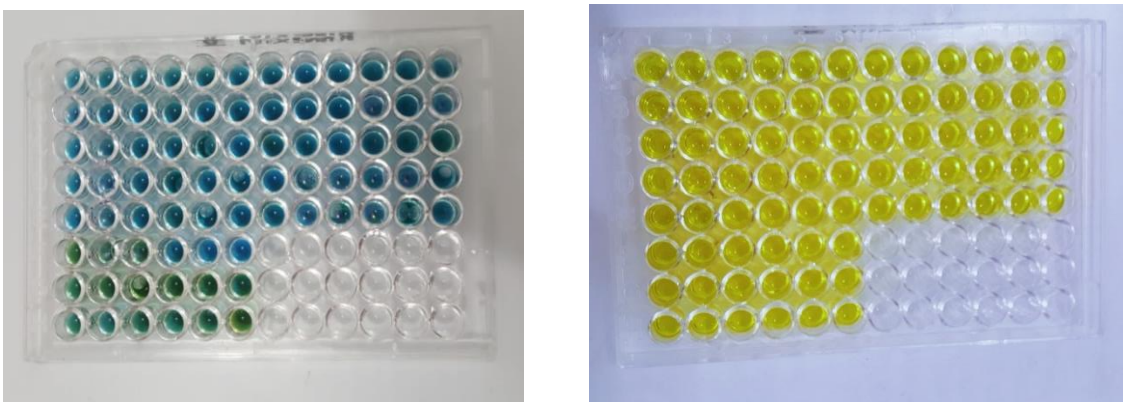


**Figure 3.6:** Addition of TMB to ELISA plate (left), Adding of $H_2SO_4$ to stop the reaction (right)

## 3.11 Estimation of Antioxidants:

### 3.11.1 Catalase Estimation

Using the Sinha approach, the activity of catalase was observed in 1972. The following ingredients were added to 0.1 ml of cell culture medium: 1.0 ml of phosphate buffer (10 mM, pH 7.0), 0.4 ml of H2O2 (0.2 M), and (Sigma Aldrich, USA). The addition of 2.0 ml

of reagent for dichromate acetic acid stopped the reaction. Material were heated in a water bath for ten minutes, then cooled. The absorption at 530 nm was then measured (97).

### 3.11.2 Glutathione Estimation

Using the Beutler et al. approach, quantity of glutathione (GSH) in the media for cell growth was calculated (1963). 2.0 ml of disodium hydrogen phosphate buffer (0.3 M), 0.25 ml of 5, 50-dithiobis-(2-nitrobenzoic acid) or DTNB (0.001 M), and 0.5 ml of cell culture media were additioned to a test tube (Invitrogen Inc., USA). After 15 minutes of incubation, the mixture was measured for absorbance using a spectrophotometer at 412 nm.

Using the Beutler et al. approach, the reduced glutathione (GSH) in the media for cell growth was calculated (1963). For this, 2.0 ml of disodium hydrogen phosphate buffer (0.3 M), 0.25 ml of 5, 50-dithiobis-(2-nitrobenzoic acid) or DTNB (0.001 M), and 0.5 ml of cell culture media from groups were added to a test tube (Invitrogen Inc., USA). After 15 minutes of incubation, the mixture was measured for absorbance using a spectrophotometer at 412 nm (97).

### 3.11.3 Estimation of SOD

With the help of Kakkar et al.'s approach, the estimated SOD (superoxide dismutase) activity was evaluated in 1984. A process was initiated by adding 0.2 ml of nicotinamide adenine dinucleotide (NADH) to 0.1 ml of cell culture media, 1.2 ml of sodium pyrophosphate buffer (52 mM, pH 8.3), 0.1 ml of phenazine methosulphate (PMS) (186 lM) from Santa Cruz Biotechnology in the United States, and 0.3 ml of (750 lM). The reaction was halted by adding 0.1 ml of glacial acetic acid during a 90-second incubation period at 30 °C. With 4.0 ml of n-butanol, the reaction mixture was agitated briskly. After being incubated for ten minutes, the mixture was then centrifuged for five minutes at 2,000 rpm.. Its absorbance at 560 nm was measured in the upper butanol layer (97).

## 3.12 Histological evaluation of wound skin samples

0.5 cm2 of skin samples from the injured area were removed 7 days after transplantation and fixed in formalin (Sigma, USA). Dehydrated skin samples were embedded in wax and

removed with a microtome (5 um). The skin piece was stained, and the skin architecture was examined during the H&E procedure (109).

## 3.13 Analytical Statistics

GraphPad Prism software was used to quantitatively analyse the numerical data produced by the various experimental groups and references will be added with the help of ENDNOTE.

# CHAPTER 4
# RESULTS

## 4.1 MTT Assay

This test is conducted on rats to determine the anticancer activity of the guar gum plant. The graphical data shows that one group of rats received various doses of guar gum while the other group was left untreated. A one-way ANOVA was used to estimate the results.

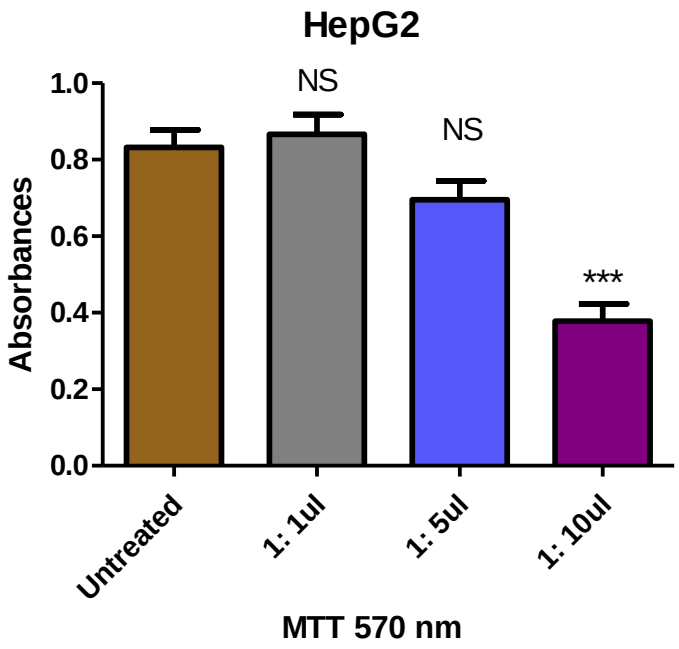



**Figure 4.1:** MTT assay levels in treated group of rats with selected doses of Guar gum and in comparison to untreated group of rat. *** symbols indicates that there is high statistical significance in the results ($P < 0.001$). The mean ±SEM is used to express the values.

**Table 4.1.** Graphical result values of MTT assay expressed in mean ±SEM

| | **Untreated** | **1: 1 ul** | **1: 5 ul** | **1: 10 ul** |
|---|---|---|---|---|
| **MTT** | 0.83 ± 0.046 | 0.87±0.052 | 0.70±0.048 | 0.38±0.045 |

## 4.2 Wound Index

Wound shrinking was observed in the treated groups of rats at different stages and time intervals.

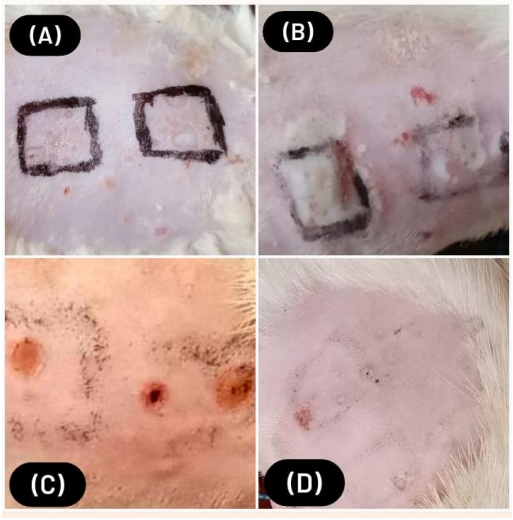



**Figure 4.2:** (A) Time of injury (B) After 2 Days (C) After 4 Days (D) After 7 Days, healing process

The data from graph demonstrates that the group of rats treated with guargum powder exhibits a substantial increase in wound contraction and healing when compared to the wounded groups, and the outcomes were calculated using a one-way ANOVA in GraphPad.

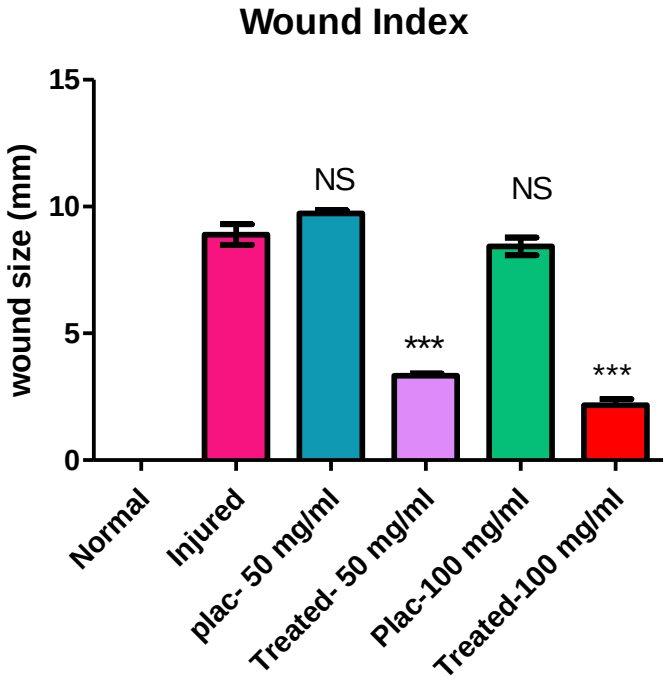



**Figure 4.3:** Levels of wound Index in treated groups of rats with selected doses of guar gum in comoarision of injured group of rats. The *** symbol denotes high statistical significance in results ($P < 0.05$). The mean ± SEM is used to express the values.

**Table 4.2** Wound Index Levels (mean ±SEM) values.

| | **Normal** | **Injured** | **Placebo 50 mg/ml** | **Treated 100 mg/ml** | **Placebo 100 mg/ml** | **Treated 100 mg/ml** |
|---|---|---|---|---|---|---|
| **WOUND INDEX** | 0.00±0.00 | 8.9±0.40 | 9.7±0.12 | 3.3±0.088 | 8.4±0.35 | 2.2±0.24 |

## 4.3 ELISA VEGF

The graphical data represents that the group of rats treated with guar gum powder in comparison to injured groups shows a significant value of VEGF marker and results were estimated by applying one-way ANOVA.

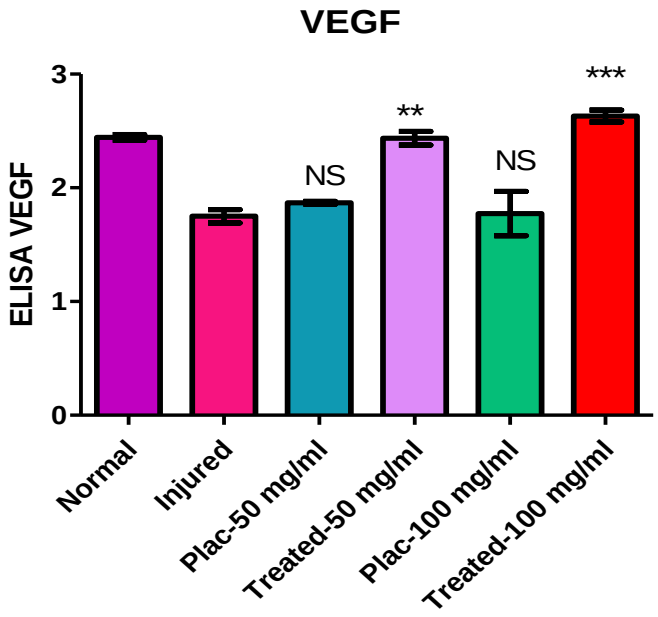



**Figure 4.4:** VEGF levels in treated group of rats with selected doses of guar gum powder and in comparison to injured group of rats. *** indicates that the results are highly significant ($P < 0.001$). The symbol ** denotes statistical significant in results ($P < 0.05$). The mean ±SEM is used to express the values.

**Table 4.3.** VEGF level measured via ELISA (mean ±SEM) values.

| Normal | Injured | Placebo 50 mg/ml | Treated 50 mg/ml | Placebo 100 mg/ml | Treated 100 mg/ml |
|---|---|---|---|---|---|
| 2.4±0.025 | 1.8±0.059 | 1.9±0.011 | 2.4±0.061 | 1.8±0.20 | 2.6±0.052 |

## 4.4 ELISA ANNEXIN V

The graphical data represents that the group of rats treated with guar gum powder in comparison to injured groups shows a significant value of ANNEXIN V marker and results were estimated by applying one-way ANOVA.

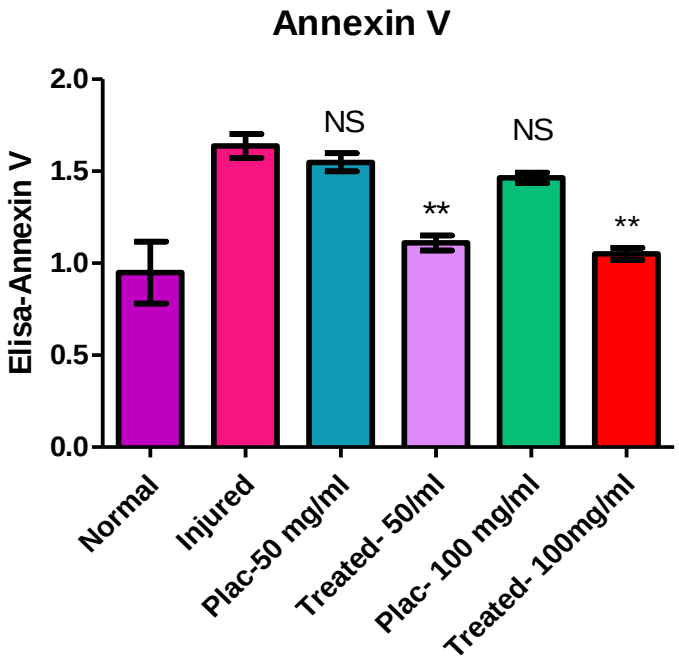



**Figure 4.5:** Annexin V levels in treated group of rats with selected doses of guar gum powder in comparison to injured group of rats. ** indicates that the results are significant ($P < 0.001$). The mean ±SEM is used to express the values.

**Table 4.4** Annexin level measured via ELISA (mean ±SEM) values.

| Normal | Injured | Placebo 50 mg/ml | Treated 50 mg/ml | Placebo 100 mg/ml | Treated 100 mg/ml |
|---|---|---|---|---|---|
| 0.95±0.17 | 1.6±0.065 | 1.5±0.049 | 1.1±0.041 | 1.5±0.029 | 1.1±0.033 |

## 4.5 Antioxidant Analysis

### 4.5.1 Estimation of APOX

APOX levels in the guar gum plant are measured using this assay. The graphical data shows that the rats in the guar gum powder treatment group were compared to the wounded groups, and a one-way ANOVA was used to assess the results.

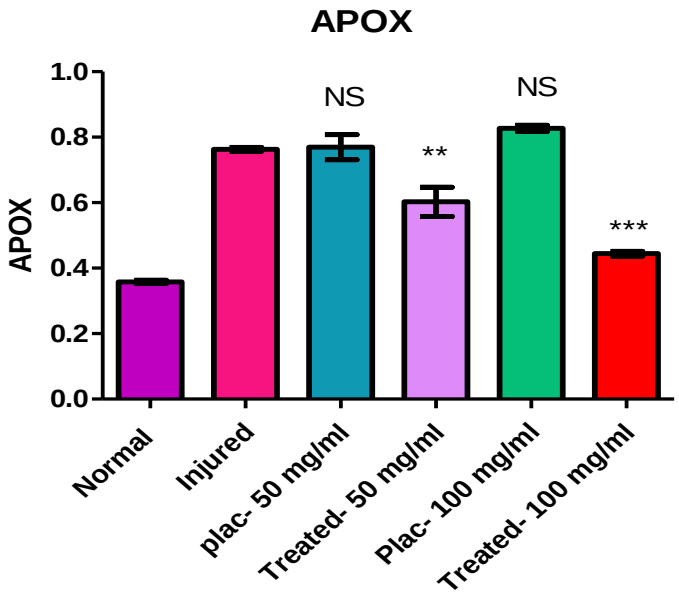



**Figure 4.5.1:** APOX levels in treated group of rats with selected doses of guar gum powder and in comparison to injured group of rats. *** indicates that there is highly statistical significance in the results ($P < 0.001$). The mean ±SEM is used to express the values.

**Table 4.5.1** APOX levels (mean ±SEM) values.

| | **Normal** | **Injured** | **Placebo 50 mg/ml** | **Treated 50 mg/ml** | **Placebo 100 mg/ml** | **Treated 100 mg/ml** |
|---|---|---|---|---|---|---|
| **APOX** | 0.36±0.0052 | 0.76±0.055 | 0.77±0.038 | 0.60±0.044 | 0.83±0.0095 | 0.44±0.0072 |

### 4.5.2 Estimation of GSH

GSH levels in the guar gum plant are measured using this assay. The graphical data shows that the rats in the guar gum powder treatment group were compared to the wounded groups, and a one-way ANOVA was used to assess the results.

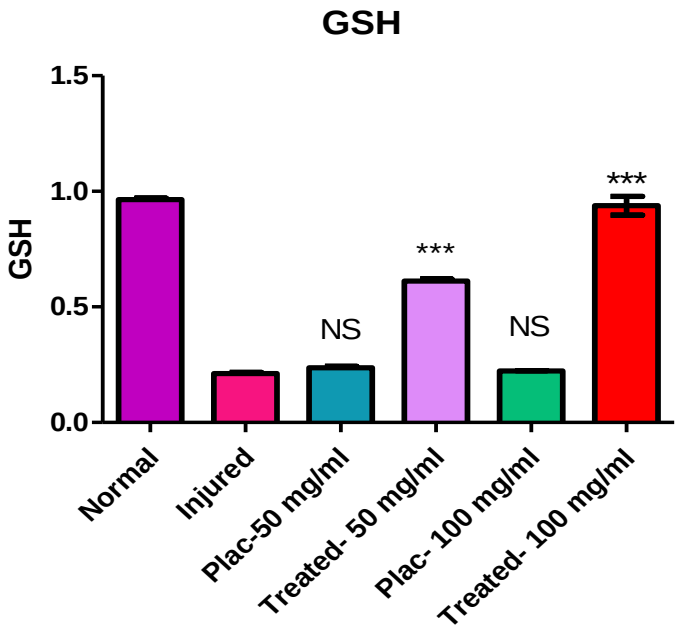



**Figure 4.5.2:** GSH levels in injury vs. treated group of rats with selected doses of guar gum powder. *** indicates that the results are highly significant ($P < 0.001$). The mean ±SEM is used to express the values.

**Table 4.5.2** GSH levels (mean ±SEM) values.

| | Normal | Injured | Placebo 50 mg/ml | Treated 50 mg/ml | Placebo 100 mg/ml | Treated 100 mg/ml |
|---|---|---|---|---|---|---|
| **GSH** | 0.96±0.0058 | 0.21±0.044 | 0.24±0.0064 | 0.61±0.0098 | 0.22±0.00033 | 0.94±0.041 |

### 4.5.3 Estimation of CAT

CAT levels in the guar gum plant are determined by this technique. The graphical data shows that the rats in the guar gum powder treatment group were compared to the wounded groups, and a one-way ANOVA was used to assess the results.

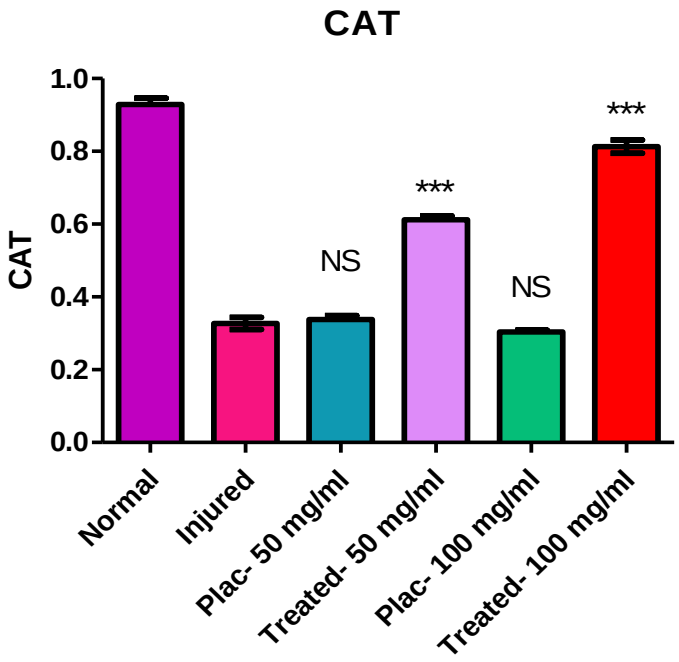



**Figure 4.5.3:** CAT levels in treated group of rats with selected doses of guar gum powder and in comparison to injured group of rats. *** indicates that the results are highly significant ($P < 0.001$). The mean ±SEM is used to express the values.

**Table 4.5.3** CAT levels (mean ±SEM) values.

| | Normal | Injured | Placebo 50 mg/ml | Treated 50 mg/ml | Placebo 100 mg/ml | Treated 100 mg/ml |
|---|---|---|---|---|---|---|
| **CAT** | 0.93±0.017 | 0.33±0.017 | 0.34±0.011 | 0.61±0.011 | 0.30±0.0049 | 0.81±0.018 |

### 4.5.4 Estimation of SOD

SOD levels in the guar gum plant are determined by this technique. The graphical data shows that the rats in the guar gum powder treatment group were compared to the wounded groups and a one-way ANOVA was used to assess the results.

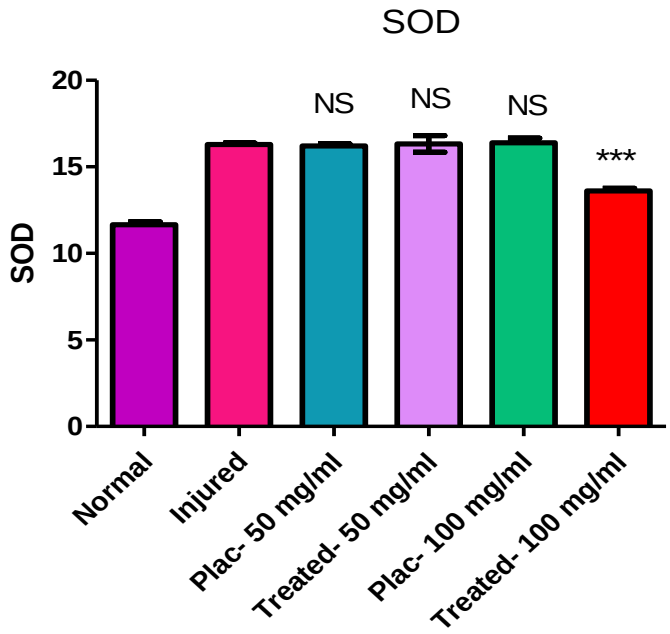



**Figure 4.5.4:** SOD levels in treated groups of rats with selected doses of guar gum plant. *** Symbols indicates that the results are highly significant (P < 0.001). The mean ±SEM is used to express the values.

**Table 4.5.4** SOD levels (mean ±SEM) values.

| | **Normal** | **Injured** | **Placebo 50 mg/ml** | **Treated 50 mg/ml** | **Placebo 100 mg/ml** | **Treated 100 mg/ml** |
|---|---|---|---|---|---|---|
| **SOD** | 12±0.18 | 16±0.096 | 16±0.12 | 16±0.48 | 16±0.25 | 14±0.14 |

## 4.6 HISTOPATHOLOGY

### 4.6.1 GROUP 1: NORMAL GROUP

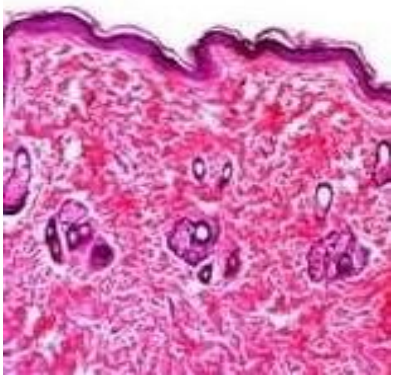


**Figure 4.6.1:** Normal Skin

In this diagram, the two main layers of skin are the epidermis and dermis, and deep beneath the dermis lies a subcutaneous fascia known as the hypodermis. The epidermis consists of four to five layers of cells, three of which are less numerous and the bulk of which are keratinocytes. The thickness of the skin is depicted in the normal group result in the above picture, indicating that the glands of the skin are healthy histologically.

### 4.6.2 GROUP 2: INJURY GROUP

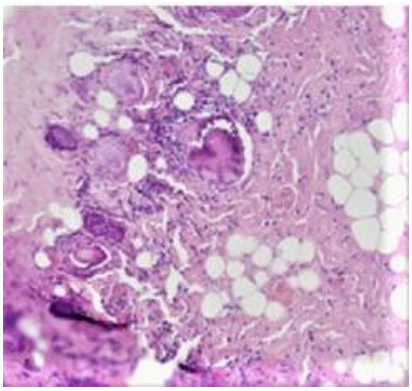


**Figure 4.6.2:** Injury Group

A burn wound is depicted in the diagram, which is characterized by an inflammatory reaction and rapid oedema formation. Thickness of skin is compromised. Infiltration of inflammatory cells can be seen.

### 4.6.3 GROUP 3: PLACEBO GROUP 50 mg/ml

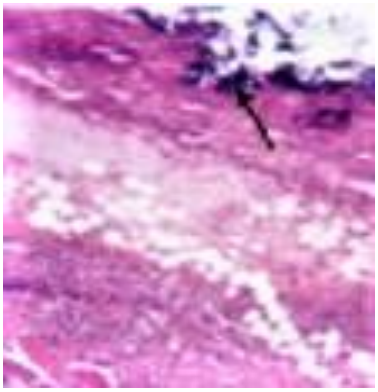


**Figure 4.6.3:** Placebo group 50 mg/ml

A burn wound is depicted in the diagram, which is characterized by an inflammatory reaction and rapid edema formation. Thickness of skin is compromised. Infiltration of inflammatory cells can be seen. Histopathological results of Placebo 1 rat with 50mg/ml swild Guar gum powder dose depicts that architecture of skin was broken down and infiltrations of inflammatory cells was also observed.

### 4.6.4 GROUP 4: PLACEBO GROUP 100 mg/ml

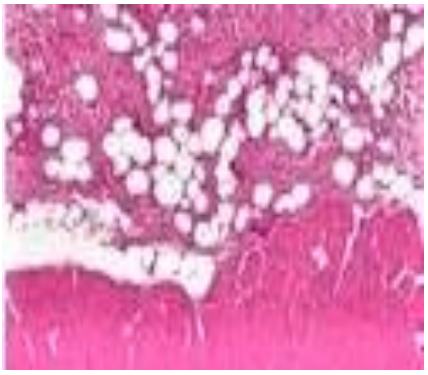


**Figure 4.6.4:** Placebo group 100 mg/ml

A burn wound is depicted in the diagram, which is characterized by an inflammatory reaction and rapid oedema formation. Thickness of skin is compromised. Infiltration of inflammatory cells can be seen. Histopathological results of Placebo 2 rat with 100mg/ml guar gum powder dose depicts that architecture of skin was broken down and infiltrations of inflammatory cells were also observed.

### 4.6.5 GROUP 5: TREATMENT GROUP 50 mg/ml OF GUARGUM POWDER

This group shows that infiltration cell amount become less which is characterized by healing process and hence recovery occurs.

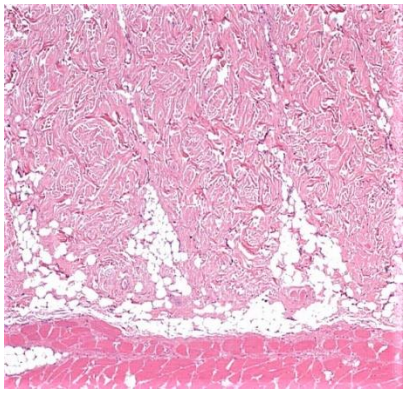


**Figure 4.6.5:** Treated group 50 mg/ml

### 4.6.6 GROUP 6: TREATMENT GROUP 100mg/ml OF GUARGUM POWDER

As a treatment for injuries, 100 mg/ml of Guar gum powder was given. In above diagram it can be seen that the morphology is very close to normal. Skin architecture is smooth. The release of different factors by dendritic cells accelerates early cell proliferation, ensuring rapid burn healing. As a result, medications that improve dendritic cell function are considered medicines for burn wound care.

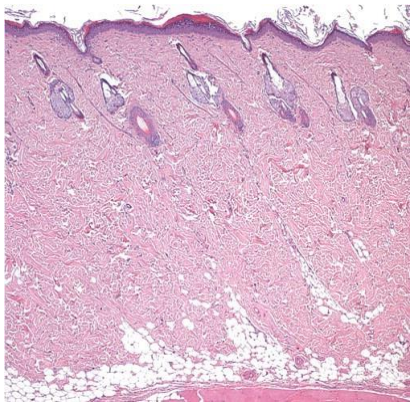


**Figure 4.6.6:** Treated group 100 mg/ml

# CHAPTER 5
# DISCUSSION

Physical injuries that cause an opening or breaking in the skin, such as surgery or acute traumas, are referred to as "wounds." Patients experience serious health issues as a result of subsequent infections, which has led to important advancements in wound care. Antibacterial and antibiotic characteristics are essential for a speedier wound healing process when creating a nanocomposite film for a dressing (110).

In order to restore disrupted anatomical stability, accelerate healing, lower the risk of infection, and restore the skin's functional state, wounds must be healed properly (111). Inflammation, cell proliferation, and cell migration are the sequence of events that lead to the repair of wounded tissues, including the regeneration and replacement stages. Immediately the following damage, the inflammation stage starts with vasoconstriction, which promotes homeostasis and releases inflammation mediators. This stage's primary goal is to stop blood flow to the wound, not to restore injured tissue.

Current synthetic wound-healing drugs prevent infection while also reducing the risk of side effects and not accelerating the healing process (112). Treatments based on natural substances may have a number of benefits, such as biocompatibility, efficacy, and simplicity of withdrawal from natural bases. In this study, we concentrate on employing injectable, natural, and ecological hydrocolloid combined with wound-healing compounds for in vivo wound healing studies (113).

Guar gum has cancer-preventive qualities. Plant materials and isolated components have been used to demonstrate anti-diabetic, anticonvulsant, antioxidant, anti-inflammatory, immuno modulatory, analgesic, antimicrobial, antiviral, antihypertensive, anticancer, antihyperglycemic, ant nociceptive, gastro protective, and wound healing activities in vitro and in vivo (114) . Wound index levels was measured in treated group of rats with selected doses of guar gum powder and in comparison to injured group of rats. The symbol *** denotes high statistical significance in results ($P < 0.05$). MTT assay was conducted, which shows significant results which depicts that guar gum has anticancer properties. The ELISA assay was conducted, VEGF levels with doses of guar gum powder in treated group of rats compare to injured group shows, *** indicates that the results are highly significant (P <

0.001). The symbol * denotes statistical significant in results ($P < 0.05$). While the Annexin levels in treated group of rats with selected doses of guar gum powder and in comparison to injured group of rats shows ** which indicates that the results are significant ($P < 0.001$).

Several Antioxidants were used which cause burning of skin and reactions starts, hence skin under stress was observed. APOX and GSH levels in treated group of rats with selected doses of guar gum powder and in comparison to injured group of rats, ** indicates that there is statistical significance in the results ($P < 0.001$). The CAT levels and SOD levels in treated group of rats with selected doses of guar gum powder and in comparison to injured group of rats. *** indicates that the results are highly significant ($P < 0.001$). Histopathology results shows that guar gum plant has wound healing ability.

# Conclusion

On different analyses, Guar gum extracts showed potential Anticancer and wound-healing properties and based on our treatment and current study, we found that guar gum powder also has the wound healing ability in albino rats. At different doses, it shows different results, at a dose of 50 mg/ml the results are good and at a dose of 100 mg/ml the results are best and highly significant. The specific dose of extracts, indicated maximum wound healing potential by increases the level of vascular endothelial growth factor (VEGF). The confirmation of anticancer and wound healing properties of Guar gum extracts open a new prospect for further testing to treat acid burn injuries with potential enhanced protectively properties.

# CHAPTER 6
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