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Title

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ABSTRACT
Burn injuries are accidental health issues with which majority of the people is suffering nowadays. A simple burn with chemical might be that extend of dangerous that could take life of any human being or other animal. Chemical burn injuries like acid buns are quiet common in various countries. This study is aimed to evaluate the impact of multiple plant extracts with chitosan on wound healing activity. Asian countries including Bangladesh, India are rich in natural resources and medicinal plants useful in the treatment of burn wound. To compare the healing activity of multiple plant preparations (Azadirecta indica, Curcuma Longa, Aloe barbadensis) with a combination of Chitosan nanoparticle on body weight, burn wound size, bacterial colony count, WBC count and body temperature in Sulphuric acid (H_2SO_4) burned rabbit. 20 male Newzeland White male rabbits were divided into five different groups and each group containing four rabbits for 16 days. Group T_0 served as negative control; Group T_1 wereH_2SO_4 burned rabbit treated with Silver Sulphadiazine drug tropically; Group T_2 were H_2SO_4 burned rabbit treated with 1:1 Silver Sulphadiazine and Chitosan nanoparticle topically; Group T_3 were H_2SO_4 burned rabbit treated with 1:1:1 Neem leaves extracts, turmeric roots extracts & Aloe vera gel and Group T_4 were H_2SO_4 burned rabbit treated with 1:1 Chitosan Nanoparticles & plant extracts. The effects of extracts and nanoparticle combinations on wound healing, bacterial growth & WBC count were tested by Auto analyzer. Results were analyzed by using one way ANOVA at 1% level of significance. The final body weight of different treatments were ($p < 0.01$) significantly decreased from the initial body weight. The wound size was not recovered at non-treatment group where other treatment groups recovered wound size significantly ($p < 0.01$). The WBC count (cells/µl) was greatly ($p < 0.01$) increased at non-treatment and trade drug without antibiotic group than other treatment groups. The bacterial colony forming units was significantly (<0.01) high at non-treated group than treated groups. No significant effect found at body temperature but non-treated groups body temperature was slightly high while other group's temperature was almost normal and same after 8 days of study to rest of the study period. Chitosan & Silver Sulphadiazine combined drugs has positive healing effects on wound size recovery and bacterial colony reduction. On the other side, Chitosan with traditional plant extracts has also improve rabbits recovery which proves that without antibiotic wound healing is also possible by using combination of nanoparticles or traditional plan extracts with Chitosan combination.

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I. INTRODUCTION

A burn is an injury to the skin or other organic tissue primarily caused by heat or due to radiation, radioactivity, electricity, friction or contact with chemicals (WHO, 2018). It is a trauma causing physiological changes in the tissue creating impairments of form, organ loss and death. Chemical burns result from exposure to various chemical substances commonly found in the home, workplace or external surroundings, because of carelessness or neglect. Chemical substances are knowingly used by people in many areas, primarily in domestic and work environments. Approximately 6 million variations of chemical substances are used by people. Chemical burns constitute 3% of all burns, and 30% of chemical burns result in death. The most common causes of chemical burns are acids such as sulfuric, hydrofluoric, hydrochloric and acetic acid, bases such as sodium and potassium hydroxide and calcium hydroxide, oxidants used in the home such as chlorides and peroxides, and various other substances such as hair dyes and airbag injuries (VanHoy *et al.,* 2022). For inappropriate use of corrosive chemicals in science laboratories of educational institutes can cause serious loss to students. Sometimes acid is thrown intentionally, according to a report of BBC news (2013), It is a crime with a marked gender skew. Experts say that women and girls are victims in 75-80% of cases. Of the female victims, about 30% are under 18. Acid Survivor Trust International (A.S.T.I.) declared that in between 2011-2016, 2078 acid attacks cases found in UK, almost 1000 acid attacking happens in India. In their survey also revealed that in Pakistan 57 notified victim found with corrosive chemical attack. At 2002 in Bangladesh ASTI found 400 patient and after 2002, they assumed almost 100 victim per year in Bangladesh went through acid attack. The most shocking reveal of their research was that, approximately 60% of attack remains unreported worldwide every year.

The prognosis of a chemical burn depends on the type of chemical and the degree of injury. Most small lesions heal well but larger wounds do not generally heal and may become scars. Depending on severities of burn

injuries, several treatment is provided in worldwide like first aid, antibiotics, anti-inflammatory medications, debridement, which involves cleaning or removing dirt and dead tissue etc. Sometimes skin grafting, skin replacements or cosmetic surgery is also been done but it is very costly process which every patients cannot effort (healthline.com). As we knew in burn injuries, after first aid treatment, anti-inflammatory medicines and antibiotics are used in burn injuries mostly for healing but with or without proper healing these medications has some several side effects with long term adverse effects. Non-Steroidal Anti Inflammatory Drug (NSAID) cause GI diseases (Loren Laine *et al.,* 2005), allergic reactions and in rare cases, problems with liver, kidneys or heart and circulation, such as heart failure, heart attacks and strokes (nhs.uk). The most alarming situation with drug discovery in today's world is antibiotic resistance. According to a report of CDC's 2019 on Antibiotic Resistance, 2.8 million antibiotic-resistant infections occur in the U.S. each year. More than 35,000 people die as a result.

Scientists has already aware people for using antibiotic sincerely but for easy availability of antibiotics, this is getting hard for us to reduce the use of antibiotics nowadays. This study aims for reducing the use of antibiotics and other harmful medications in chemical burn cases. Topical treatment like Silver Sulphadiazine (Burnol) is on wound healing is also provided for burn wound healing but for betterment of treatment, the usage of other supportive harmful medications are increasing day by day.

In Asian society, Ayurveda is also known as "Goddess of All Healing" and is considered as one of the most effective traditional system of medicine with many curing and healing properties. Several plant extracts and their phyto constituents are known as a promising alternative for wound healing agents due to the presence of diverse active components, ease of access and minimal side effects. The Ayurvedic literature "Sarangdhar Samhita" has also highlighted the concept of polyherbalism in which products with combined extracts of plants are considered more effective rather than individual ones. Medicinal plants with antimicrobial, antioxidant and anti-inflammatory properties have mitigated the wound healing process. Polyherbalism results in cheaper medication by reducing the duration of therapy or individual cost for anti-inflammatory and antimicrobial medications. The incidences of new & relapsing infectious disease and antibiotic resistance has greatly increased the susceptibility of delayed healing. The selected herbs for this study aimed different pharmacological targets involved in the wound healing like suppression of the production of inflammatory cytokines and inflammatory transduction cascades, reduction of oxidative factors, enhancement of anti-oxidative enzymes and prevention of the microbial growth at wound site. The development of formulation for the wound healing having antimicrobial, antioxidant and anti-inflammatory properties is the need of present times. The present study was therefore carried out to evaluate the traditional use of Neem leaves, Turmeric roots and Aloe Vera gel with combination of Chitosan Nanoparticles in wound healing process scientifically. Chitosan is obtained by partial DE acetylation of the amines of chitin, which yields a copolymer of N-acetyl-glucosamine and N-glucosamine. Its use has been explored in various biomaterial and medical applications. Chitosan has desirable qualities, such as hemostasis, wound healing, bacteriostatic, biocompatibility, and biodegradability properties (Barbosa and Amara *et al.,* 2011). Chitosan appears to have no adverse effects after implantation in tissues and, for this reason, it has been used for a wide range of biomedical applications (National Library of Medicine, 2015). Chitosan may be used to inhibit fibroplasia in wound healing and to promote tissue growth and differentiation in culture. It is commonly accepted that the ideal wound covering should mimic many properties of human skin. It should be adhesive, elastic, durable, occlusive and impermeable to bacteria (Ibrahim A. Alsarra *et al.,* 2009). Because of their biocompatibility, ability to absorb exudates and film forming properties, chitosan products are good candidates for burn and wound management.

Furthermore, the positive roles of natural products (neutraceuticals) for the reduction of damage and management wound and other related complications, were also assessed. The aim of this study was to know the effect of Neem, Turmeric, Aloe Vera and Chitosan Nanoparticle in chemical burn wound healing.

The general objective of this study was to evaluate the chemical burn wound healing activity of Neem, Turmeric, Aloe Vera and Chitosan Nanoparticle with the following specific objectives:

1. To assess the wound healing activity including wound size, scare formation, healing time, body temperature and weight.
2. Determine the number of WBC and bacterial colony.

II. REVIEW OF LITERATURE

Definition of Wound

A wound may be defined as any disruption of the integrity of skin, mucous membrane or organ tissue. A distinction is made between simple wounds that are confined to the skin, and complicated wounds which are deeper and also involve injury to muscles, nerves, and vessels. Wounds can be caused by mechanical, thermal, chemical, and radiogenic trauma (Dtsch Arztebl *et al.,* 2008).

Types of wounds

Closed wounds

A relatively slight blow may damage the skin and underlying soft tissues, as shown by bruising, or contusion, which results from the infiltration of blood into the tissues from ruptured small vessels and by swelling caused by the passage of fluid through the walls of damaged capillaries. As a rule, the hemorrhage ceases abruptly, the blood and fluid are absorbed within a few days, and the part is restored to normal. When larger vessels are injured, much more blood escapes, and it collects in the tissues and forms a mass called a hematoma. A direct, forceful blow may damage any of the underlying tissues; blood vessels, nerves, muscles, bones, joints, or the internal organs may be affected. Damage to the deeper tissues may result from the direct impact of the blow upon a tissue. Other common forms of indirect injury result from twisting, as occurs when a person's foot becomes caught and he or she twists upon it, suffering, if the force is great enough, a sprained or broken ankle or a broken leg or hip; from bending; or from deceleration, a form of injury frequently encountered in automobile and aircraft accidents, where one part of the body is fixed while another is relatively mobile, giving rise, in abrupt stops, to a displacement of the mobile parts, commonly called whiplash. (Encyclopaedia of Britannica).

Open wounds

When the skin—or, in the case of injuries of the base of the skull or the sinuses, the mucous membrane—is broken, a wound is exposed to additional hazards, since the tissues may be invaded by foreign material such as bacteria, dirt, and fragments of clothing, which may give rise to serious local or general complications from infection. Furthermore, if the break in the skin is large, the resulting exposure of the wounded tissues to the drying and cooling effects of the air may increase the damage caused by the wounding agent itself (Britanica, 2019).

Skin, being sturdy and elastic and well supplied with blood, tolerates injury well and recovers quickly. The subcutaneous fatty tissues are more delicate and more easily deprived of their blood supply. Muscle, likewise, is sensitive to the damaging effect of shrapnel, being readily torn and unable to survive diminished blood supply for any appreciable time. Muscle, when damaged, is particularly prone to infection.

An injury to bone in an open wound is always serious, for any broken fragment detached from its blood supply will not survive if infection occurs, and it will remain as a foreign body in the wound to cause further complications. Even if the bone is cleanly broken and there are no loose fragments, infection may enter the raw surfaces of the fracture with disastrous results.

Clearly the seriousness of a wound is greatly increased if there is injury to a joint, a nerve, a major blood vessel, or an internal organ (Encyclopaedia of Britannica).

Contamination of a wound may occur at the moment of wounding or at any time thereafter until healing is complete. The effects of various nonbacterial contaminants vary considerably. In general, the critical factor for nonbacterial contaminants is the extent of the contamination. In the case of bacterial contaminants, the type of contaminant is of greater importance. Infection caused by virulent bacteria nourished by dead tissue and organic foreign material in the wound may take several forms, of which the three most important are: gas gangrene, the most dreaded, arising almost exclusively in damaged muscle tissue and spreading with alarming rapidity to cause death if unchecked by surgical or medical treatment; infections caused by organisms such as *Streptococcus* and *Staphylococcus* and the coliform bacteria, in which the local production of pus is a prominent feature accompanying a general reaction that may be severe; and tetanus, an often fatal infection that becomes evident some days after the wound has occurred, frequently without any marked local manifestations but characterized by generalized muscle spasms (Augustyn *et al.*, 2019).

Chemical burn wound

A chemical wound, also called a chemical burn, is damage to the body from a caustic chemical, which is a very strong acid or base that can burn or corrode. Contact with these chemicals can damage the skin, eyes, and lungs (if breathed), or, if swallowed, the inside of the body might damage. It is important to take care of these wounds correctly (Intermountain Healthcare, 2018).

There are many chemicals that can cause burns. Although they are generally acidic and basic in nature, there are more than one million known chemical compounds, of which 300 have been declared by the National Fire Protection Society as highly hazardous chemical substances. Chemical burns account for about 10.7% of all burn injuries and 30% of deaths because of chemical burns (IWJ, 2019).

Symptoms

The symptoms of a chemical wound depend on what part of the body is injured.

- **Skin.** A mild burn on the skin (a first-degree burn) will be red, swollen, and painful. A second-degree burn can cause blisters or make the skin look shiny. With a third-degree burn or worse, the skin might look rough

and white, yellow, or charred black. If the burn is bad, the person may be numb and not feel pain because the nerves are damaged.

- **Eyes.** If a caustic chemical gets in the **eyes**, the person will not be able to see normally and will have pain.
- **Lungs.** Someone who breathes a caustic chemical will cough and may have trouble breathing.
- **Mouth and stomach.** Someone who eats or drinks a caustic chemical will have pain in the throat and stomach. The person may have nausea and may vomit the chemical back up, which can burn the throat and mouth again.

A person who has had a long exposure to a large amount of the chemical will have worse symptoms than someone who had a brief exposure to a small amount of the chemical.

Chemicals can often be classified as acid, alkali, organic, and inorganic compounds. Acids act by denaturing and coagulating proteins. Alkaline burns cause deeper burns than acid burns. Alkaline compounds saponification on the surface epithelium of the skin and laxity causes necrosis. Organic solutions cause injury by dissolving the lipid membrane, leading to disruption of physiological processes. Inorganic solutions cause injury through denaturation mechanisms (Akelma & Karahan *et. al.,* 2019).

Types of burns

- First-Degree or superficial burns are identified by pain, redness, minor swelling and an absence of blistering.
- Second-Degree burns produce a slight thickness of the skin and may include blistering, indicating damage has been done to the underlying layers of skin.
- Third-Degree burns feature leathery, waxen skin and are commonly accompanied by numbness due to full damage to the dermis and surrounding nerves.
- Fourth-degree burns have extended past the skin layers and into the flesh, causing charring and irreparable damage.

Acid burn wound

Acid products include toilet cleaners, battery acid, bleach, chemicals used in industry for crystal etching, and chemicals that are added to gas. Acid solids and liquids can cause injury, depending on the type, the strength, and the length of time the acid is in contact with the body. The damage is usually kept to the area of contact and does not usually cause damage deep in the tissue (Staff *et al.,* 2020)

Potential health effects of acid

Main routes of exposure: Inhalation, Skin contact, Eye contact.

- **Inhalation:** Not expected to be an inhalation hazard unless heated or misted. Can cause severe irritation of the nose and throat. Can cause life-threatening accumulation of fluid in the lungs (pulmonary edema). Symptoms may include coughing, shortness of breath, difficult breathing and tightness in the chest. Long-term damage may result from a severe short-term exposure.
- **Skin contact:** Corrosive contact can cause pain, redness, burns, and blistering. Permanent scarring can result. A severe exposure can cause death.
- **Eye contact:** Corrosive contact causes severe burns with redness, swelling, pain and blurred vision. Permanent damage including blindness can result.
- **Ingestion:** Can burn the lips, tongue, throat and stomach. Symptoms may include nausea, vomiting, stomach cramps and diarrhea. Permanent damage can result. Can cause death.
- **Effects of long-term (Chronic) exposure:** At low concentrations: Can cause dry, red, cracked skin (dermatitis) following skin contact. At high concentrations: May wear away tooth enamel when breathed in. May harm the respiratory system. Can irritate and inflame the airways.
- **Carcinogenicity:** Not known to cause cancer. Strong inorganic mists containing sulfuric acid are carcinogenic to humans. Has been associated with: cancer of the larynx, lung cancer.
- **Teratogenicity / Embryotoxicity:** Not known to harm the unborn child.
- **Reproductive Toxicity:** Not known to be a reproductive hazard.
- **Mutagenicity:** Not known to be a mutagen.

Sulphuric acid

Sulphuric acid is produced from sulphur. Sulphur dioxide is first obtained by the burning of the molten sulphur in presence of air. Sulphur dioxide is then converted to sulphur trioxide in presence of vanadium pentoxide catalyst. The sulphur trioxide thus obtained is absorbed in recycling concentrated sulphuric acid in an absorption tower. The plants installed earlier and the smaller units of sulphuric acid plants use a single absorption process which has conversion efficiency of 96–98%. New large sulphuric acid production plants now–a–days utilize double conversion double absorption (DCDA) process. DCDA process can realize above 99% conversion

efficiency. When concentrated sulfuric acid contacts the skin, the resulting chemical reaction releases heat that brings sustained thermal damage to the skin (Yin S. *et al,* 2017). The sustained heat dehydrates local tissues and cells, and the consequential skin coagulation and necrosis may produce eschars and damage the microvascular system (Jelenko C. *et al,* 1974).

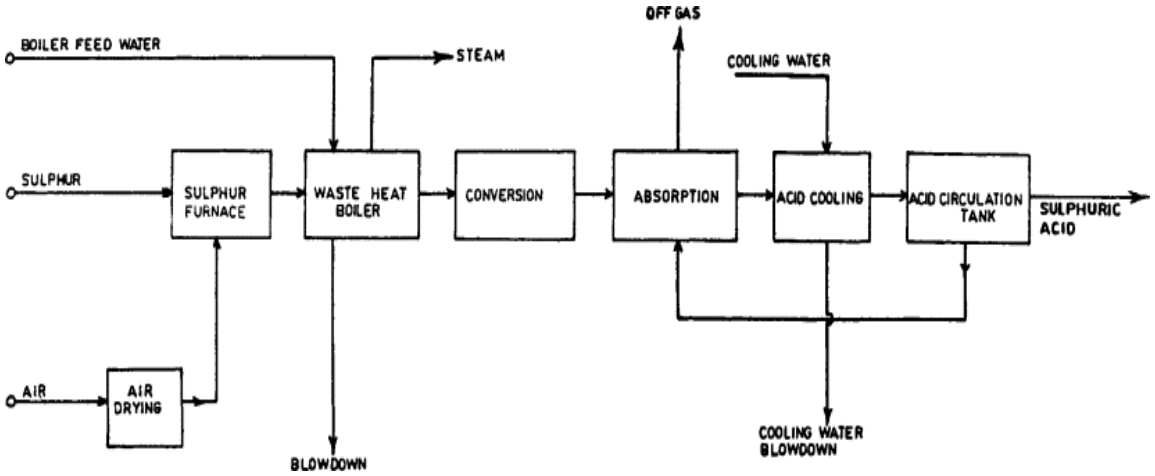

Fig 1: The manufacturing process for sulphuric acid by the single absorption process

Topical burn wound

Cutaneous burns challenge global health care systems with high patient morbidity and mortality rates. One recent study of adults admitted for 2 to 60 days to a US burn center reported that more than 7.9% of burn patients experienced at least 1 hospital-acquired infection (HAI), extending the hospital length of stay and increasing the likelihood of complications and death. Of these HAIs, most (35.8%) were skin and soft tissue infections, followed by respiratory (24.4%), bloodstream (18.1%), and urinary tract (17.8%) infections. A burn covering more than 5% of total body surface area (TBSA) multiplied HAI risk by 3, with higher risk as the burned TBSA increased. Other factors increasing HAI risk included inhalation injury, flame burn, patient age (≥ 60 years), and comorbidities (ie, diabetes, heart failure, myocardial infarction, renal disease, or peripheral arterial disease).Topical 1% silver sulfadiazine cream (SSD), introduced into burn care in the mid-20th century by Dr. Charles Fox, improved global burned patient survival rates and outcomes by reducing the likelihood of burn-related infection. Research in later decades explored other topical treatments capable of reducing the incidence of burn-related infections.

Despite modern advances in surgical techniques and biomedical technologies, management of facial burns remains challenging and post-burn scar formation may be inevitable. The approach to treating a burn wound begins with accurately diagnosing the depth and size of the burn, which is of critical importance for deciding the timing and approach to management. Superficial (first-degree) burns heal without the need for surgery and typically do not lead to adverse scar formation or hyperpigmentation as the entire dermis is preserved. Superficial partial thickness (second-degree) burns typically heal spontaneously within days to weeks and may result in scarring. Deep partial-thickness burns and full thickness (third-degree) burns usually require excision with reconstruction (often skin grafting) and are more prone to developing HTS. It is generally accepted that any burn that is not expected to heal on its own within 3 weeks should undergo excision and grafting as early as is feasible. This is due to the increasing risk of HTS that doubles from 20 to 40% between 2 and 3 weeks, as demonstrated by Cubison at2006 in a study of pediatric scald burns. Even with precise operative technique, proper excision and grafting can still result in undesirable HTS that may not be cosmetically acceptable (Berman *et al.,* 2008); Preventive therapies to reduce the formation of widespread HTS is fundamental to burn care and can significantly alter the outcome depending on the efficacy of treatment, timing of treatment, and duration of treatment. Silicone sheets, tapes, and gel formulations physically cover the healing area and are widely used as a general approach to scar mitigation (Meier and Nanney, 2006; Bleasdale *et al.,* 2015). Over the past decade, a variety of advanced burn dressings have been introduced, many of them containing silver compounds for their long-standing antimicrobial effects. Other topical antibiotic agents and therapeutic occlusive or exposure dressings are all commonly used to facilitate healing and to prevent scar formation, although these non-molecularly targeted therapies have resulted in variable clinical outcomes (Leon-Villapalos *et al.,* 2008; Block *et al.,* 2015).

Although exuberant fibro-proliferation in the burn area starts as part of the normal wound healing, variations in cellular responses can lead to excessive scar-producing processes (Gurtner *et al.,* 2008; Penn *et al.,* 2012). Recent advances in our understanding of the cellular events and molecular signaling pathways underlying fibrotic scar development have led us to pursue investigational approaches to specifically targeted therapies.

Rational manipulation of specific biological targets may hold promise for more effective and successful outcomes toward post-burn scar mitigation. Therapeutic agents involving growth factors, cytokine and other immune modulators combined with new drug delivery technologies are being evaluated for their scar-improving properties (Asadullah *et al.,* 2003; Rd Mag, 2011; So *et al.,* 2011). More recently, cellular mechanotransduction modulators that can be applied topically have emerged as novel pharmacological agents that can accelerate wound healing while decreasing fibrotic scar formation (Gurtner *et al.,* 2011; Wong *et al.,* 2011b; Ma *et al.,* 2018). In this review, we discuss current therapeutic modalities used for craniofacial burn wound and scar management and emerging experimental therapies that exhibit strong potential for clinical application.

Topical burn wound treatment

Antimicrobial agents

To prevent infection, silver preparations such as silver sulfadiazine (SSD) and silver-containing dressings are commonly used for the treatment of second and third-degree burns. However, these agents are discouraged in the face due to their adverse effects on mucosal membranes of the eye, nose, and mouth. Topical ointments such as bacitracin, polymyxin, and mupirocin are used instead (Leon-Villapalos *et al.,* 2008; Maillard and Hartemann, 2013). Silver nitrate is another topical therapy, however, studies have reported that silver nitrate directly reduces fibroblast proliferation, and it is therefore not recommended for prolonged or excessive use (Maillard and Hartemann, 2013). For the neck, SSD is often used in combination with cerium nitrate, another topical antiseptic agent that augments its antimicrobial effects (Garner and Heppell, 2005; Leon-Villapalos *et al.,* 2008). Topical silver therapies have been associated with silver deposition in the liver and kidney, and therefore should be used with caution in pediatric and elderly patients, as well as in large surface area burns (Orsini and Plastic Surgery Educational Foundation Technology Assessment Committee, 2006; Leon-Villapalos *et al.,* 2008; Friedstat and Klein, 2009). Additionally, use of silver-based therapies can result in leukocytopenia, so white blood cell count monitoring should be considered for patients requiring prolonged therapy (Choban and Marshall, 1987). Antimicrobials should be discontinued once epithelialization has completed so that anti-scar therapy can begin.

Debriding agents

Early debridement allowing for healthy tissue regrowth is the cornerstone of burn wound care and is vital to the overall outcome of burn patients. The practice of excising burn eschar in the facial area, however, remains controversial, given its robust blood supply which is favorable to spontaneous healing within an acceptable period. For debridement, surgical excision and/or proteolytic enzymes that digest necrotic tissues can be used. Surgical excision may be performed sharply using a Weck, Goulian, or Watson blade. Alternatively, mechanical debridement may also be performed using hydro-powered devices such as the Versajet (Smith and Nephew, London, United Kingdom), or ultrasonic debridement devices. Bromelain-based agents are most used for enzymatic debridement, however, this method can slow healing time and cause significant pain. For these reasons, use of enzymatic agents has been generally avoided for facial burns thus far.

Growth factors and cytokines

Growth factors and cytokines play a key role in wound healing, including the promotion of proliferation and migration of various cell types, recruitment of circulating inflammatory and progenitor cells, and stimulation of angiogenesis and ECM production (Ching *et al.,* 2011). Topical treatment with growth factors and cytokines has demonstrated positive results in preclinical and clinical trials, especially in the setting of partial thickness burns. Growth factors with positive human clinical data include fibroblast growth factor (bFGF), recombinant human epidermal growth factor (EGF), and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Fu *et al.,* 1998; Zhang *et al.,* 2009; Guo *et al.,* 2010). Other growth factors such as platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) have demonstrated positive effects on burn healing in preclinical models but have yet to be demonstrated to be efficacious in human patients (Ching *et al.,* 2011). A detailed discussion of transforming growth factor-beta (TGF-β), interleukins, and novel mechanomodulatory agents is provided later in this review.

Therapeutic wound dressings

Burn wound dressings are made from a variety of materials including silicone, chitin, alginate, hydro-polymers, and polyurethane foam. Dressings containing therapeutic agents such as aforementioned silver compounds are also used. Use of hyaluronic acid-based wound dressings has been reported to be safe and effective for partial-thickness facial burns. Silicone sheet dressings have been shown to soften post-burn scars, prevent contracture and increase skin and joint mobility. These effects are attributed to greater tissue hydration, which results in improved scar texture and color (Leon-Villapalos *et al.,* 2008; Bleasdale *et al.,* 2015).

Biologic and biosynthetic skin substitutes

Dermal substitutes are bio-matrices that provide a protective layer over open wounds, protecting against infection, reducing pain and stimulating a healing response to allow for eventual skin grafting (Shahrokhi *et al.,* 2014). As in other parts of the body, these skin substitutes constitute an important mechanism for covering deep burn wounds in the face, typically in the setting of large TBSA% burn wounds or in cases where there are limited donor sites available for autografting. The first product introduced into the market was Integra (Integra® Life Science Corporation, Plainsboro, NJ, United States). Originally developed by Harvard and MIT scientists, Integra® is an artificial acellular bilayer composed of a bovine collagen type I and chondroitin-6-sulfate dermis, with a semipermeable silicone membrane "epidermis." The silicone membrane is left in place for at least 2 weeks, allowing for sufficient neovascularization to take place to allow for skin grafting over the dermal substitute (Davison-Kotler *et al.,* 2018).

Acellular dermal matrices (ADMs) benefit from providing natural dermal porosities to support tissue regeneration and neovascularization while providing wound protection (Chua *et al.,* 2016).

For example, Alloderm® (Allergan, Dublin, Ireland) is a human cadaveric allograft which is chemically decellularized using mild non-denaturing detergents, while the collagen matrix is preserved. Bioengineered allograft materials can also contain cellular components, such as Apligraf® (Organogenesis, Inc., Canton, MA, United States), a cellular bilayer substitute containing bovine collagen type I dermis, as well as allogenic neonatal keratinocyes and fibroblasts (Davison-Kotler *et al.,* 2018). A recent clinical trial found that Apligraf placed over meshed autografted burn wounds resulted in improved pigmentation, tissue pliability, vascularization and scar appearance, as opposed to regular meshed autografted wounds (Waymack *et al.,* 2000). Although the host immune response to allograft precludes its use as a permanent skin replacement, achieving temporary wound coverage with allograft stimulates the release of a variety of bioactive substances that accelerate wound closure (Katz and Taichman, 1994). Clinical trials have shown that skin allografts can accelerate the healing of burn wounds as well as chronic wounds in the lower extremity (Nunez-Gutierrez *et al.,* 1996; Gurtner *et al.,* 2020). It has been shown that the viability of skin allografts correlates with take rate (Bravo *et al.,* 2000; Castagnoli *et al.,* 2003), thus effective techniques for preservation of skin grafts are critical to maintain high quality grafts. In light of the high demand for allograft skin especially for the treatment of major burns, allograft preservation in skin banks has become an established practice. Cryopreservation is the preferred method of cadaveric skin preservation, since it better maintains the physicochemical properties and viability of fresh human skin compared to glycerol (Aggarwal *et al.,* 1985; Cinamon *et al.,* 1993; Pirnay *et al.,* 2012). Recently developed human cryopreserved meshed split-thickness skin allografts such as TheraSkin® (Misonix, Farmingdale, NY, United States) have shown promising results in the treatment of chronic wounds in clinical trials (DiDomenico *et al.,* 2011; Gurtner *et al.,* 2020) and demonstrated regenerative properties in pre-clinical studies (Henn *et al.,* 2020), suggesting a potential future therapeutic role in burn care as well.

Human amnion/chorion can be a source of cellular and acellular biologic scaffolds. Dehydrated human amnion/chorion membrane (dHACM) allografts such as EpiBurn or EpiFix, can be used to protect the wound while promoting vascular angiogenesis and healing (Reilly *et al.,* 2017). Similarly, decellularized human amnion membrane (hAM) contains a favorable immunogenic profile, has antimicrobial and anti-fibrotic properties (Robson and Krizek, 1973; Maral *et al.,* 1999), and can reduce the frequency of dressing changes, which is particularly useful in pediatric patients (Branski *et al.,* 2008). Over the past 20 years, there has been an increasing body of literature describing hAM processing methods and clinical use (Herndon and Branski, 2017). Challenges related to its relative fragility and higher cost have thus far limited widespread clinical use (Quinby *et al.,* 1982).

Finally, xenografts offer an alternative method for obtaining wound coverage with the added benefit of improved cost and availability, as compared to allograft materials. Compared to human ADM, xenograft ADM has demonstrated strong protein homology and equal biocompatibility (Ge *et al.,* 2009). Examples of xenografts include: SurgiMend (TEI Biosciences, Boston, MA, United States) acellular bovine dermis; Strattice (LifeCell, Branchburn, NJ, United States) acellular porcine dermis; and Permacol (Covidien, Dublin, Ireland) acellular porcine dermis.

Acid burn wound management of Bangladesh

Acid burn injuries in Bangladesh primarily occur as a result of intentional attacks although there are incidences of accidental acid burns in industry, on the street, and at home. A total of 126 patients with acid burns, 95 from attacks and 31 from accidents, were studied from July 2004 to December 2012. A diagnosis of acid burn was made from history, physical examination and in some cases from chemical analysis of the patients' clothing. Alkali burns were excluded from the study. In the burn unit of Dhaka Medical College Hospital, they applied a slightly different protocol for management of acid burns, beginning with plain water irrigation of the wound, which effectively reduced burn depth and the requirement of surgical treatment. Application of hydrocolloid dressing for 48-72 h helped with the assessment of depth and the course of treatment. Early excision and grafting gives good results but resultant acid trickling creates a marble cake-like appearance of the wound separated by

the vital skin. Excision with a scalpel and direct stitching of the wounds are often a good option. Observation of patients on follow-up revealed that wounds showed a tendency for hypertrophy. Application of pressure garments and other scar treatments were given in all cases unless the burn was highly superficial (Kishore and Loren *et. al.*, 2014).

Most of the chemical burn cases in Bangladesh, doctors dressed the patients in regular basis and prescribed any broad spectrum antibiotic to avoid further infections. Preparations of 1% Silver Sulphadiazine might also be used in minor burn cases.

Traditional use of medicinal plants in wound healing

For more than 5000 years, Egyptians, indigenous peoples of Africa, Asia, Romans, and the Americas have used medicinal plants as first-line therapy for inflammation, burns, ulcers, and surgical wounds. They contain many natural bioactive compounds that help fasten the process of wound healing and regenerate tissue at the wound site (A Sharma *et al*, 2021).

Neem (*Azadirachta indica*)

In wound dressing, it was well known that Neem leaves works as anti-ulcer, antifungal, antibacterial, antiviral, anticancer, and antioxidant (A Sharma *et al.*, 2021). Neem leaves (*Azadirachta indica*) have active ingredients such as nimbidin and sodium nimbidate which possess anti-inflammatory, antibacterial, antifungal and antiviral properties that help in healing process and also contains an excellent nutrition which plays/playing a vital role information of collagen and formation of new capillaries. An experiment was conducted in Pharmacology Lab of Universitas Padjadjaran on October 2012. Twenty seven rats were grouped randomly into 3 groups and 1.5cm of excision wound was created. Negative control group was treated with a topical application of saline solution (sodium chloride 0.9%), treatment group with a topical application of neem leaves extract and positive control group had been treated with a topical application of povidone-iodine for 15 days. There was a significant reduction in the longest diameter of wound in group of neem leaves extract.

Turmeric (*Curcuma longa*)

Curcumin has been used as a remedy and as a food seasoning for many years, being an active agent found in the *Curcuma longa* root and a member of the ginger tribe. Curcumin is used by conventional Ayurvedic medicine practitioners to treat asthma, respiratory diseases, liver disorders, diabetes, and skin injury (A Sharma *et al.,* 2021). Curcumin has been commonly used for decades by different ethnic groups and are among the most widely studied nutraceuticals. A highly pleiotropically molecule has been shown to interact at transcription, translation, and post-translation levels with key cellular pathways (RR Kotha *et al.*, 2019). Turmeric root stimulates fibroblast proliferation, the development of granulation tissue and the deposition of collagen in the healing of cutaneous wounds (YH Yen *et al.,* 2018).

Aloe (*Aloe barbadensis*)

Aloe vera comprises of many natural bioactive compounds, including basic and complex such as glycosides, water-soluble polysaccharides, saponins, pyrocatechol, anthraquinones, acemannan, phytol and oleic acid (Salehi *et al.,* 2018). Aloe vera leaves show greater antimicrobial activity than that of alcohol and aqueous extracts. *Aloe vera* tends to be more susceptible to gram-positive bacterial species than gram-negative species (Arbab *et al.,* 2021). Saponins, acemannan, and anthraquinone derivatives are compounds with a proven antimicrobial activity. Topically applied Aloe vera gel signaling pathways has been documented to significantly reduce the time for wound closure (Sanchez *et al.,* 2020).

Nanoparticles with wound healing activities

Nanotechnology is a rapidly expanding multidisciplinary scientific field, which combines the disciplines of material science and engineering. Nanoparticles (NPs), usually ranging in dimension from 1-100 nanometers (nm), have properties unique from their bulk equivalent. They possess unique physicochemical, optical and biological properties, which can be manipulated suitable for desired applications. Since ancient times, elements such as silver, gold, copper and titanium were used to treat a number of human conditions. More recently, researchers have developed insight in and awareness of nanoparticles and how these could be used for drug delivery, diagnostic and imaging, biosensor, and cosmetic purposes. Several nanomaterials for biological applications have been intensively investigated during the last several decades. These have included liposomes, dendrites, quantum dots, fullerenes, carbon nanotubes, graphene, iron and titanium oxide, and gold and silver nanoparticles. Recently NP-based delivery of ions, such as calcium and oxygen has been used to promote angiogenesis. The application of nanomaterial based scaffold with controlled delivery of calcium ions or oxygen would promote differentiation of ADSC to endothelial cells and angiogenesis. Nanoparticles can be incorporated into biomaterials and scaffolds to create nanocomposite smart materials, which can aid wound healing through

their antimicrobial, selective anti- and pro-inflammatory, and pro-angiogenic properties (Naderi *et al.*, 2018). They can be used as gene delivery vectors altering intracellular gene expression and protein synthesis related to the wound healing process. In addition, they can affect the wound healing process by influencing collagen deposition and realignment.

Recent nanoparticles with antimicrobial properties

In addition to AgNPs, several other nanoparticles have been demonstrated to possess antimicrobial properties. Copper (Cu), graphene oxide, graphene, titanium oxide (TiO2), fibrin, polycationic NPs, and zinc oxide (ZnO) are amongst these nanoparticles often combined with biocompatible scaffolds as wound dressings. Photothermal treatment with the aid of NPs has also been described in the literature (22, 84), highlighting the efficacy of these constructs as antibiotic-free antimicrobial remedies promoting wound healing.

Nanoparticles and angiogenesis

Angiogenesis, the formation of new blood vessels, plays a vital role in several physiological and pathological processes in the body. Angiogenesis is imperative for wound repair because new vessels provide nutrients and oxygen to support the actively proliferating cells.

Wound healing capability of Chitosan nanoparticle

Chitosan is obtained by partial deacetylation of the amines of chitin, which yields a copolymer of N-acetyl-glucosamine and N-glucosamine (Barbosa *et al.*, 2011). Its use has been explored in various biomaterial and medical applications. Chitosan has desirable qualities, such as hemostasis, wound healing, bacteriostatic, biocompatibility, and biodegradability properties (Dai *et al.*, 2011). Chitosan appears to have no adverse effects after implantation in tissues and, for this reason, it has been used for a wide range of biomedical applications (Rosario Pignatello *et al.*, 2011). Tianhong Dai, Masamitsu Tanaka, Ying-Ying Huang, and Michael R Hamblin also stated in the same research article at 2011 that Chitosan may be used to inhibit fibroplasia in wound healing and to promote tissue growth and differentiation in culture. It is commonly accepted that the ideal wound covering should mimic many properties of human skin. It should be adhesive, elastic, durable, occlusive and impermeable to bacteria. Because of their biocompatibility, ability to absorb exudates, and film forming properties, chitosan products are good candidates for burn and wound management.

III. MATERIALS AND METHODS

This study was conducted at the Department of Pharmacy, Varendra University, Rajshahi, to evaluate chemical burn wound healing activities of traditional plant extracts with nanoparticles.

Preparation of house

The experimental shed was swept and washed with tap water followed by disinfection and air drying. All utensils required for the experiment such as feeder, water bottle, cotton, needle etc. were collected and the shed was properly designed with adequate ventilation.

Collection of rabbit

Around nearly 2.5 months old 20 male rabbits (Newzeland White) was purchased and collected from the Local Rabbit farm at Rajshahi.

Experimental animals grouping

20 male rabbits of near about 2.5 months were chosen to carry out this research project. These rabbits were divided into five groups containing 4 rabbits in each. The groups were designing as the following:

T_0= (Chemically burned without Treatment): Administered without any kind of treatment, this group of rabbits were burned and only provided foods and nutrition equally to other groups.

T_1= (Chemically burned and treated with 1% Silver Sulphadiazine): After burning with chemical, this group of rabbits were treated with marketed preparation of 1% Silver Sulphadiazine (Burna).

T_2= (Chemically Burned and Treated with 1% Silver Sulphadiazine & Chitosan): 10gm pure chitosan was mixed with marketed preparation of 10 gm 1% Silver Sulphadiazine (M.M. Mihai *et al.*, 2019) cream and topically used in burned area of rabbits in two times daily.

T_3= (Chemically burned and treated with Plant extracts): *Aloe vere*, Neem and Turmeric extracts were mixed in 2:1:1 (A. Shedoeva *et al.*, 2019) and prepared a gel to use topically in wound area two times per day.

T_4= (Chemically burned and treated with Plant Extracts and Chitosan NP): Chitosan was mixed up with *Aloe vere*, Neem and Turmeric extracts in 2:1:1:1 (International Journal of Nanomedicine 2018:13) ratio to prepare a gel free from foreign particles and used two times per day in wound area.

Fig 2: Experimental Animals

Feeding and watering of rabbit

Rabbits was feeded with different types of spinach like Water Spinach, Malabar Spinach, grass and guava, which was purchased from local market of Rajshahi & purified water was always available in the experimental shed. The rabbits were provided foods and purified water in a randomized way two times and were kept in mild sunshine for one hours in a day during the whole study period.

Fig 3: Dividing foods for experimental animals group

Creating burn wound in rabbits

The animals were properly feeded and taken care for two days and observed before induction of chemical burn wound. Thigh area of the rabbits were shaved and before burning with acid.1 molar solution of 98% Sulphuric Acid (H_2SO_4) was used to create burn wound with a dropper of 0.5 ml. It was sincerely observed to not more than 0.5 ml acid should dropped in any rabbits during creating burns.

Materials & chemicals required

- Distilled Water
- Silver Sulphadiazine ointment (Burna)
- Chitosan Nanoparticle
- Acetic Acid Solution
- Sodium Methyl Paraben
- Nutrient Agar Media
- Beaker, mixer and stirrer
- Cotton
- Autoclave
- Incubator

- Petridis

Chitosan was purchased from the Sigma-Aldrich in Germany, Silver Sulphadiazine USP 1% solution was purchased from local Pharmacy, manufactured by Square Pharmaceuticals Ltd. *Aloe vera*, Neem and Turmeric were purchased from the local market in Rajshahi. While distilled water, Acetic Acid solution, Sodium Methyl Paraben, Nutrient Aggar Media and other elements were available in the laboratory.

Fig 5: Burna Cream

Fig 6: Chitosan Nanoparticle

Preparations containing plant materials and Chitosan NP

Plant Extracts with Chitosan Nanoparticle was prepared according to the ionotropic gelation method (Aktas *et al.,* 2005) by dissolving 6.66 g chitosan in 3.33 ml acetic acid aqueous solution to obtain concentration of 2 g/ml and subsequently dissolve 10 ml Neem extracts and 10 ml Turmeric extracts to the solution. Stir for 15 minutes and finally add 20 ml *Aloe vera* gel with 50 mg Sodium Methyl Paraben (Preservative) to complete the 50 ml solution for use. After preparing the solution, preserve this at air tight bottle to assure its quality and take care of room temperature below 25℃ and dry place.

Collection and preparation of extract

Neem leaves, Turmeric roots, Aloe Vera were purchased from the local market of Rajshahi at a reasonable price.

Turmeric root extract

The sample preparation was followed by Rajesh *et al.,* (2017) with some modification. Turmeric root was collected from the local market in Rajshahi. The roots were washed carefully with distilled water to remove any extraneous material. The roots were shade dried at room temperature and the grounded to course powder using electric grinder. Then the ethanol is used for the process of extraction and stored in refrigerator until use.

Fig 7: Turmeric root extract

Neem leaves extract

The sample preparation was followed by (Ahmad *et al.*, 2012) with some change. The cleaning of Neem leaves was made using distilled water before cutting them into small pieces and then dried at room temperature. The dried sample was then pulverized into fine powder in an electric grinder extracted (by maceration method in 70% methanol) and concentrated (using rotary evaporator) under reduced pressure which was then stored at 4°C in refrigerator until use (Ahmad *et al.*, 2010) .

Fig 8: Neem leaves extracts

Aloe vera gel preparation

The procedure was followed by (Healthline.com, 2019) with some modification. Aloe vera was collected from the local market in Rajshahi. The fresh leaves of Aloe washed carefully with distilled water to remove any extraneous material. Using a small spoon, scooped it into your blender carefully not to include any pieces of the aloe vera skin. Blend the gel until its frothy and liquefied, which should only take a few seconds.

Fig 9: Filtration Process during extraction

Topical use of Neem, Turmeric, Aloe vera with Chitosan NP

Preparations of Turmeric roots, Neem leaves and Aloe vera gel with chitosan NP were used tropically to different treatment groups to the experimental rabbit.

The usage of tropical medication were done two times per day timely for each individual rabbit.

Recording of different parameters

Recording of wound size, scar formation and healing time

For time to time wound size (length & width) were measured by using centimeter scale in 4 days interval till day 16. Any formation of scare was also noted carefully as scare was the sign of healing.

Determination of body weight

Body weight was taken on day 1st and 16th day of treatment (During treatment) using Comfort Electronic Balance.

Determination of body temperature

A thermometer was placed to the rectal area of the rabbit for one minutes to measure body temperature of finding any changes within 4 days interval to the complete study period.

Determination of bacterial colony count

To learn about infection formation, bacterial colony count was done in a regular basis using plate count. Cell was extracted from wound area using a sterile loop and cultured. After bacterial culture, cfu/ml was counted by manual plate count method (sciencing, com, 2018). The standard plate count method consists of diluting a sample with sterile saline or phosphate buffer diluent until the bacteria are dilute enough to count accurately. That is, the final plates in the series should have between 30 and 300 colonies. Fewer than 30 colonies are not acceptable for statistical reasons (too few may not be representative of the sample), and more than 300 colonies on a plate are likely to produce colonies too close to each other to be distinguished as distinct colony-forming units **(**CFU's**).** Thus, the number of colonies should give the number of bacteria that can grow under the incubation conditions employed (Biology Libre Texts, 2021).

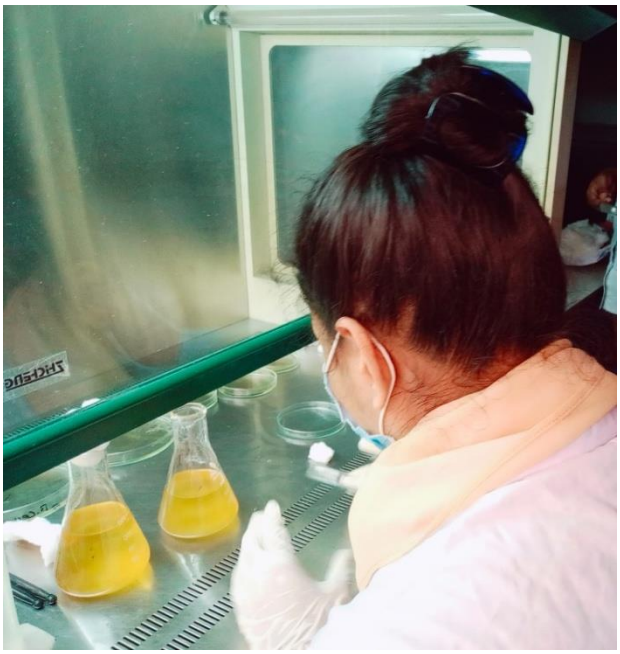

Fig 12: Bacterial culture for CFU count

Determination of WBC enumeration

WBC was counted by using Hemacytometer (Bright-Line™ Hemacytometer, Z359629) with Manual Hemocytometer Counts method. Rabbit blood were ejected from the ear vain and manually determined WBC count regularly. It involves diluting blood in a diluent that lyses the red cells to remove them from view. A hemocytometer is charged with the diluted blood and nuclei are counted in the appropriate areas of the grid using a light microscope. The basic formula for Manual Hemocytometer Counts method is,

$$\text{Cells/µl} = \frac{\text{No. of cells in 1 large square} \times \text{Dilution factor}}{\text{Volume of factor (0.1)}}$$

Dilution factor = reciprocal of dilution (20)

Volume factor= (width × length × height) = 0.1

Statistical analysis

The results of various biochemical and immunological parametrs were expressed as ±SEM. Data analysis of the Statistics were done using SPSS version 22 and Microsoft Excel. Statistically significant differences between group means were determined by analysis of variance (ANOVA).

IV. RESULT

In the table 1 the result represents the wound size level in square centimeter**,** the present study revealed that the wound size were significantly not recovered in the non-treated group, T_0 (3.10 ± 0.00^{b}), trade drug and Chitosan NP combination treatment group, T_2 (0.15 ± 0.05^{a}) and traditional plant extracts with Chitosan combination, T_4 (0.21 ± 0.00^{a}) from the Trade drug without antibiotic treated group, T_1 (0.55 ± 0.05^{a}) and only plant extracts treatment group T_3 ($0.65\pm0.05^{a)}$.

Table 1: Effects of nanoparticle (Chitosan) and traditional plant materials (Neem, Turmeric & Aloe vera) on wound area measurement (cm^2) recovery of rabbit

Treatment	Day 1	Day 4	Day 8	Day 12	Day 16
T_o	3.50 ± 0.30^{ab}	3.55 ± 0.15^{a}	3.40 ± 0.10^{c}	3.30 ± 0.00^{b}	3.10 ± 0.00^{b}
T_1	3.52 ± 0.15^{b}	3.20 ± 030^{a}	2.65 ± 0.55^{bc}	1.50 ± 0.20^{a}	0.55 ± 0.05^{a}
T_2	3.53 ± 0.05^{a}	2.00 ± 0.00^{a}	1.20 ± 0.00^{ab}	0.35 ± 0.00^{a}	0.15 ± 0.05^{a}
T_3	3.55 ± 0.05^{b}	3.20 ± 0.60^{a}	1.65 ± 0.15^{ab}	1.20 ± 0.00^{a}	0.65 ± 0.05^{a}
T_4	3.50 ± 0.20^{ab}	2.00 ± 0.28^{b}	1.80 ± 0.30^{a}	0.75 ± 0.005^{a}	0.21 ± 0.00^{a}
P-Value	0.007***	0.030**	0.007***	0.000***	0.000***

**Refers it's significant at 1% level

*** Refers it's significant at 5% level

Mean for wound recover size with different superscript within the rows were significantly different at $p<0.01$.

N.B: T_0= chemically burned but not treated. T_1= chemically burned and only treated with trade drug without antibiotic. T_2= chemically burned and treated with trade medicine & Chitosan NP combination. T_3= chemically burned and treated with combination of Neem, Turmeric & Aloe vera extraction. T_4= chemically burned and treated with plant extraction & Chitosan NP combination.

Here ** means significant at 1% level. Figures indicate the Mean ± SE (standard error); NS means not significant

In the figure 5, it was showed the wound condition of rabbits. At group T_0 or non-treatment group, wound was not been recovered after 16 days of study. At Burna (trade drug) treated group T_1, wound healing was also not that progressive but at Chitosan and Burna treated group, T_2, wound healing activity was quiet impressive & giving a hope that without antibiotic chemical burn wound can also be healed. At only traditional plant extracts treated group T_3, wound was also healed but not as T_2 and T_4 (Combination of traditional plant extracts & Chitosan NP) group.

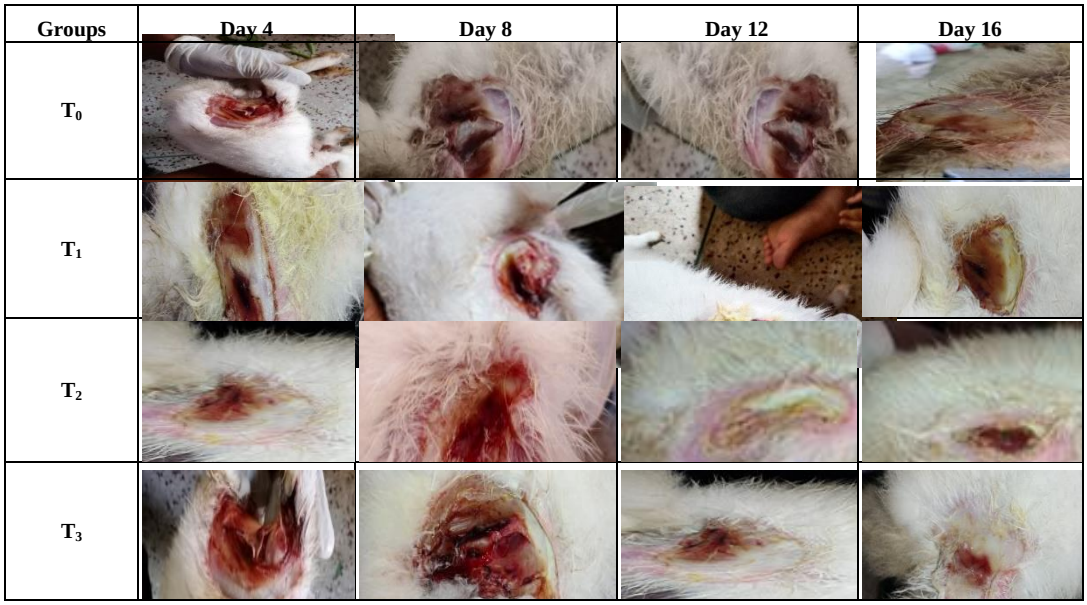

Fig 11: Comparison of chemical burn wound size and scare formation of on different study groups

The table 2 shows that the initial body weight Body weights of different groups were almost same. But the final body weights of these groups were varied significantly after 16 days of study. The present study indicated that the final body weight of chemical burned but not treated, T_0 (3.89.50±6.5) & chemically burned and only treated with trade drug, T_1 (3.90.00±5.5) was significantly decreased from the treatment of Chemically burned and treated with trade drug & Chitosan combination, T_2 (3.95.50±4.5), traditional plant extracts with Chitosan combination, T_4 (3.92.503±3.5) and Traditional plant extracts, T_3 (3.91.00±51.0).

Table 2: Effects of nanoparticle (Chitosan) and traditional plant materials (Neem, Turmeric & Aloe vera) on body weight (Kg) of rabbit

Experimental groups of rabbit	Body weight(kg)	
	Initial body weight	Final body weight
T_0	3.95.00±5.0^a	3.89.50±6.5^a
T_1	3.95.50±12.5^a	3.90.00±5.5^a
T_2	3.94.00±15.0^a	3.95.50±4.5^b
T_3	3.92.00±10.0^a	3.91.00±51.0^a
T_4	3.93.00±9.0^a	3.92.503±3.5^a
P value	0.484*	0.00**

Mean for Initial body weight with different superscripts were not significant but Final body weight with different superscript in the columns were significantly different at $p<0.01$.

N.B: T_0= chemically burned but not treated. T_1= chemically burned and only treated with trade drug without antibiotic. T_2= chemically burned and treated with trade medicine & Chitosan NP combination. T_3= chemically burned and treated with combination of Neem, Turmeric & Aloe vera extraction. T_4= chemically burned and treated with plant extraction & Chitosan NP combination.

Here ** means significant at 1% level. Figures indicate the Mean ± SE (standard error); NS means not significant

In the table 3, the result represents the body temperature (◦F), the present study revealed that the body temperature was slightly increased in non-treated group, T_0 (103.00±1.0^a). All other groups body temperature is almost same and normal after 16 days of study.

Table 3: Effects of nanoparticle (Chitosan) and traditional plant materials (Neem, Turmeric & Aloe vera) on Body temperature (°F) of rabbit

Treatment	Day 0	Day 4	Day 8	Day 12	Day 16
T_o	102.50±0.50^a	105.00±0.50^a	103.00±1.5^a	103.50±0.50^a	103.00±1.0^a
T_1	103.00±0.00^a	104.50±0.00^a	102.50±0.50^a	102.50±1.5^a	102.50±0.50^a
T_2	101.70±1.5^a	103.00±1.00^a	102.20±1.5^a	101.50±1.5^a	102.50±1.5^a
T_3	102.90±1.00^a	104.50±0.50^a	103.00±0.00^a	102.00±0.00^a	102.00±0.00^a
T_4	103.20±0.50^a	103.50±0.50^a	102.50±0.50^a	102.00±0.50^a	103.00±1.25^a
P-Value	0.038**	0.043**	0.042**	0.040**	0.040**

**Refers it's significant at 1% level

Mean for body temperature with different superscript within the rows were significantly different at $p<0.001$.

N.B: T_0= chemically burned but not treated. T_1= chemically burned and only treated with trade drug without antibiotic. T_2= chemically burned and treated with trade medicine & Chitosan NP combination. T_3= chemically burned and treated with combination of Neem, Turmeric & Aloe vera extraction. T_4= chemically burned and treated with plant extraction & Chitosan NP combination.

Here ** means significant at 1% level. Figures indicate the Mean ± SE (standard error); NS means not significant.

In the table 4, it shows that the bacterial colony counts (cfu/ml), the present study revealed that the WBC count were significantly increased in the non-treated group, T_0 (160.0 ± 40^{b}) from treatment of Chemically burned and treated with trade drug & Chitosan combination, T_2 (30.5 ± 22.5^{ab}), traditional plant extracts with Chitosan combination, T_4 (38.5 ± 6.5^{a}) and Traditional plant extracts, T_3 (48.5 ± 3.5^{ab}) and chemically burned and only treated with trade drug, T_1 (87.0 ± 11^{ab}).

Table 4: Effects of nanoparticle (Chitosan) and traditional plant materials (Neem, Turmeric & Aloe vera) on Bacterial colony forming units of rabbit

Bacterial Colony Count (cfu/ml)

Treatment	Day 4	Day 8	Day 12	Day 16
T_o	232.5 ± 45.5^{a}	212.5 ± 7.5^{b}	202.5 ± 2.5^{bc}	160.0 ± 40^{b}
T_1	189.5 ± 0.50^{ab}	150.0 ± 5^{b}	106.0 ± 4^{c}	87.0 ± 11^{ab}
T_2	122.0 ± 17.0^{b}	108.0 ± 10^{a}	96.0 ± 6^{a}	30.5 ± 22.5^{ab}
T_3	179.5 ± 1.5^{a}	173.5 ± 1.5^{a}	103.5 ± 9.5^{ab}	48.5 ± 3.5^{ab}
T_4	179.5 ± 22.5^{a}	135.0 ± 3^{b}	98.0 ± 2^{abc}	38.5 ± 6.5^{a}
P-Value	0.008***	0.001***	0.012***	0.048**

**Refers it's significant at 1% level

***Refers it's significant at 5% level

Mean for bacterial colony forming unit with different superscript within the rows were significantly different at $p<0.001$.

N.B: T_0= chemically burned but not treated. T_1= chemically burned and only treated with trade drug without antibiotic. T_2= chemically burned and treated with trade medicine & Chitosan NP combination. T_3= chemically burned and treated with combination of *Neem*, Turmeric & Aloe vera extraction. T_4= chemically burned and treated with plant extraction & Chitosan NP combination.

Here ** means significant at 1% level. Figures indicate the Mean ± SE (standard error); NS means not significant.

In the table 5, it shows that the WBC count cells/µl, the present study revealed that the WBC count were significantly increased in the non-treated group, T_0 (10700.00 ± 610.0^{c}) & chemically burned and only treated with trade drug, T_1 (10000.00 ± 950.0^{c}) from the treatment of Chemically burned and treated with trade drug & Chitosan combination, T_2 (7700.00 ± 250.0^{b}), traditional plant extracts with Chitosan combination, T_4 (8100.00 ± 600.0^{a}) and Traditional plant extracts, T_3 (8600.00 ± 800.0^{a})

Table 5: Effects of nanoparticle (Chitosan) and traditional plant materials (Neem, Turmeric & Aloe vera) on WBC counts of rabbit

WBC Count (cells/µl)

Treatment	Day 0	Day 4	Day 8	Day 12	Day 16
T_o	8500.00 ± 0.00^{b}	12000.00 ± 600.0^{d}	12500.00 ± 900.0^{d}	11500.00 ± 650.0^{c}	10700.00 ± 610.0^{c}
T_1	8800.00 ± 700.0^{b}	12000.00 ± 100.0^{c}	11700.00 ± 900.0^{c}	10500.00 ± 800.0^{bc}	10000.00 ± 950.0^{c}
T_2	8600.00 ± 0.00^{a}	10000.00 ± 50.0^{a}	8000.00 ± 250.0^{a}	7700.00 ± 550.0^{a}	7700.00 ± 250.0^{b}
T_3	7950.00 ± 50.0^{a}	11000.00 ± 0.00^{b}	10600.00 ± 500.0^{b}	9800.00 ± 1050.0^{c}	8600.00 ± 800.0^{a}
T_4	8300.00 ± 500.0^{a}	10500.00 ± 950^{a}	9000.00 ± 750.0^{ab}	8800.00 ± 950.0^{ab}	8100.00 ± 600.0^{a}
P-Value	0.040**	0.048**	0.031**	0.023**	0.010**

**Refers it's significant at 1% level

Mean for WBC count with different superscript within the rows were significantly different at $p<0.001$.

N.B: T_0= chemically burned but not treated. T_1= chemically burned and only treated with trade drug without antibiotic. T_2= chemically burned and treated with trade medicine & Chitosan NP combination. T_3= chemically burned and treated with combination of Neem, Turmeric & Aloe vera extraction. T_4= chemically burned and treated with plant extraction & Chitosan NP combination.

Here ** means significant at 1% level. Figures indicate the Mean ± SE (standard error); NS means not significant

V. DISCUSSION

The experiment was conducted to determine the comparative efficacy of chemical burn wound healing activity of Chitosan with traditional plants (Neem, Turmeric, Aloe vera) on body weight, wound size recovery,

bacterial colony count, WBC count and body temperature in Sulphuric Acid burned rabbit. It was also compared the different plant drugs combination on body weight, wound size recovery, Bacterial colony count, WBC count and body temperature in Sulphuric Acid burned rabbit. To perform the experiment, 20 rabbits were randomly divided into five equal groups named T_0, T_1, T_2, T_3, T_4 and each group containing 4 rabbits. Sulphuric acid burn was created by using 98% concentrated 0.5 ml acid at each group (T_1, T_2, T_3 and T_4) of rabbits. Group T_0 rabbit were kept as non-treatment group without giving any treatment. Group T_1 rabbit were kept as burned without giving any antibacterial agent but only established topically used trade drug (1% Silver Sulphadiazine). Group T_2 rabbits were treated with both 1% Silver Sulphadiazine and Chitosan nanoparticle. Groups of rabbit, T_3 were treated with combined traditional plant extracts topically & T_4 group was treated with both plant extracts & Chitosan nanoparticle combination. The study period was 16 days long.

Wound area measurements

The present study revealed that the wound size level in square centimeter**,** the present study revealed that the wound size were significantly not recovered in the non-treated group, T_0 (3.10 ± 0.00^b), trade drug and Chitosan NP combination treatment group, T_2 (0.15 ± 0.05^a) and traditional plant extracts with Chitosan combination, T_4 (0.21 ± 0.00^a) from the Trade drug without antibiotic treated group, T_1 (0.55 ± 0.05^a) and only plant extracts treatment group T_3 ($0.65\pm0.05^{a)}$.

The present study indicated that the wound size were significantly not recovered in the non-treatment group (T_0), compared to the other study groups. This result showed that the wound size recovery were significantly ($P<0.01$) better in the treatments of Burna (1% Silver Sulphadiazine) and Chitosan NP combination group and Chitosan and Plant extracts (Neem, Turmeric, Aloe vera) combined group to the other treatment groups.

These observation similar to the findings of Hooi Leong Loo *et al.,* 2022 who reported that Chitosan nanoparticle has significant effects on burn wound healing by faster drug delivery. Similar observations were made by Tianhong Dai *et al.,* 2012 who reviewed that Chitosan has antimicrobial activity and burn wound healing activity.

Traditional plant drugs were used from almost 2000 years ago for injuries. Mughisa Munir *et al.,* 2021 assessed that herbal gel containing an *Azadirachta indica* leaf extract has a potential effects on wound healing. He found the complete recover of wound on rabbit model by using herbal preparations of *Aloe vera* gel and Neem leafs and both combination.

In this study it was revealed that the combination of Chitosan and Burna combined group (T_2) is more effective than other study groups. Chitosan and plant extracts combined groups (T_4) also give a positive result in healing but it is proved in the study that burn wound without treatment (T_0) can be more harmful and it could be spread.

Body weight

The present study indicated that the final body weight of chemical burned but not treated, T_0 ($3.89.50\pm6.5$) & chemically burned and only treated with trade drug, T_1 ($3.90.00\pm5.5$) was significantly decreased from the treatment of Chemically burned and treated with trade drug & Chitosan combination, T_2 ($3.95.50\pm4.5$), traditional plant extracts with Chitosan combination, T_4 ($3.92.50\pm3.5$) and Traditional plant extracts, T_3 ($3.91.00\pm51.0$).

In the present study, it was found that the final body weight of each group decreased after 16 days of study except group T_2 which were treated by Burna (1% Silver Sulphadizaine) at Chitosan combination. Non-Treatment group rabbits (T_0) body weight decreased much-more than other study groups. Burn and trauma decreased body weight proved by a similar study (Zhe-Wei Fei *et al.,* 2013). They found that the body weight decreased almost 0.4 Kg after three days of burn injury. The results were also supported Neerasingh *et al* 1989 who reported that the body weight was lowered by 5 to 25% in non-treatment group but was higher in treated with Silver Sulphadiazine nanoparticle group of mice by 6.60 to 30%.The present study revealed that the body weights were decreased after burn injuries.

Body Temperature

In this study, we observed that the body temperature (◦F), the present study revealed that the body temperature was slightly increased in non-treated group, T_0 (103.00 ± 1.0^a). All other groups body temperature is almost same and normal after 16 days of study. But immediate after burn, body temperature increased in between groups at Day 4 and it recovered with time. Gore DC *et al.,* 2003 and M.G. Jeschk *et al.,* 2008 revealed that A high body temperature has been described as the most common sign of systemic response to injury and virtually all burn patients have elevated core body temperatures and even a full haemogram may reveal leukocytosis. It triggers WBC cell for recovery injuries. Similar result was also found by A.E. Mavrogordato *et al.,* 2008, He showed that after burn injuries body temperature increase 1° - 2° and it reflects the body's inability to dissipate

heat. In our study body temperature between rabbit groups increased significantly after day 4 and it recovered within day 8. After 16 days of study all group of rabbits body temperature found almost normal.

Bacterial load

This study revealed that bacterial colony counts (cfu/ml), the present study revealed that the WBC count were significantly increased in the non-treated group, T_0 (160.0 ± 40^{b}) from treatment of Chemically burned and treated with trade drug & Chitosan combination, T_2 (30.5 ± 22.5^{ab}), traditional plant extracts with Chitosan combination, T_4 (38.5 ± 6.5^{a}) and Traditional plant extracts, T_3 (48.5 ± 3.5^{ab}) and chemically burned and only treated with trade drug, T_1 (87.0 ± 11^{ab}).

Andres *et al.* investigated the interaction between chitin or chitosan powder and various kinds of pathogenic microorganisms. The deacetylation yields were 35, 60 and 80% ± 10%. In another study, No *et al.* compared the antibacterial activities of chitosans and chitosan oligomers against both Gram-negative and Gram-positive bacteria. About traditional plant extracts, Pereira RF *et al.*, 2013 investigated that Aloe vera as anti-inflammatory, antibacterial, antiseptic and its reliability to inducing collagen synthesis during the wound healing, its gel form is thought to be used for the treatment of skin disorders. Taye *et al.*, 2011; Avdeshetal. *et al.*, 2012 showed that The strong antibacterial activity against bacterial strains suggests that traditional plants can be used as a treatment for wound-causing bacteria and viruses. Abdul Rahman & Abdullah Hassan Humaid at 2018, studied that Ethanol turmeric extract showed inhibitory effects for *S. aureus* only. The MIC value of turmeric extract with S. aureus was 0.75 µg/mL. Combination of these three plant extracts and also with combined to Chitosan nanoparticle showed an excellent antimicrobial activity on chemical burn wound.

WBC count

In this study result also indicated that the WBC count cells/µl, the present study revealed that the WBC count were significantly increased in the non-treated group, T_0 (10700.00 ± 610.0^{c}) & chemically burned and only treated with trade drug, T_1 (10000.00 ± 950.0^{c}) from the treatment of Chemically burned and treated with trade drug & Chitosan combination, T_2 (7700.00 ± 250.0^{b}), traditional plant extracts with Chitosan combination, T_4 (8100.00 ± 600.0^{a}) and Traditional plant extracts, T_3 (8600.00 ± 800.0^{a}).

The findings of the present study showed that the lower WBC was significantly found in $T_2$and T_4groupsafter 16 days of study which were treated with combination of Chitosan nanoparticles with Silver Silphadiazine trade drug and traditional plant extract. Compared to non-treated group (T_0) and only Silver Sulphadiazine trade drug group without antibiotic (T_1), WBC count was quiet normal in T_2, T_4 and T_3 (Neem, Turmeric, *Aloe vera* combination) groups after 16 days of study period. Increasing WBC indicates that wound recovery is not that in progression it's still fighting for recovery.

VI. CONCLUSION

It can be concluded that Chitosan nanoparticle showed an effective wound healing activity with combination of Silver Sulphadiazine trade drug and traditional plan extracts. Combination of Chitosan & Silver Sulphadiazine drug showed a better activity on bacterial load while only Silver Sulphadiazine without any oral antibacterial drug could not prove that antibacterial efficacy on chemical burn wound. It can be recommended that combination of Chitosan nanoparticle with Silver Sulphadiazine nanoparticle can be used topically in chemical burn wound for preventing bacterial infection caused by wound. On the other side, live body weight decreased in the non-treated group of rabbit much more than other groups. Further study can be done to calculate the minimum effective dose, toxic dose and lethal dose for patent the traditional plant extracts extract and Chitosan nanoparticle.

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