

Wound healing activity of nanoparticles with traditional plants in chemically burned rabbit model

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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 burns 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 (*Azadiractaindica*, *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 Newzealand 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 were H_2SO_4 burned rabbit treated with Silver Sulphadiazine drug topically; 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/ μ l) 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.

Keywords: Chemical burn, Wound healing, Chitosan, Nanoparticle, Neem, Turmeric, Aloe vera

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. 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 (VanHoyet *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.

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. 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. 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 present study was 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. MATERIALS AND METHODS

This study was conducted at the Department of Physiology and Pharmacology, Hajee Mohammad Danesh Science and Technology University, Dinajpur to evaluate chemical burn wound healing activities of traditional plant extracts with nanoparticles.

2.1 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.

2.2 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.

2.3 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:

T₀= (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.

T₁= (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).

T₂= (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.

T₃= (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.

T₄= (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 1: Experimental Animals

2.4 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 2: Dividing foods for experimental animals group

2.5 Experimental layout of study

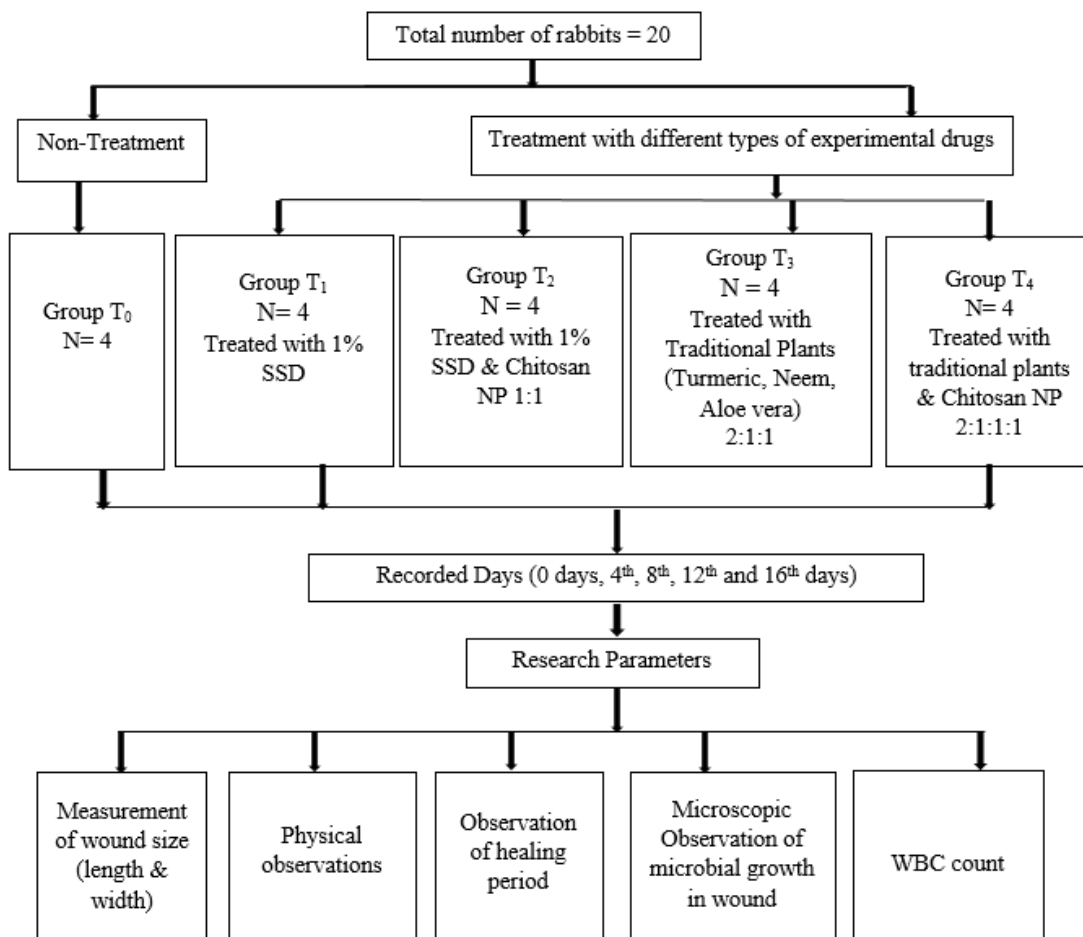


Fig 3: Experimental layout of study

2.6 Creating burn wound in rabbits

The animals were properly fed 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 be dropped in any rabbits during creating burns.

2.7 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 4: Burna Cream



Fig 5: Chitosan Nanoparticle

2.8 Preparations containing plant materials and Chitosan NP

Plant Extracts with Chitosan Nanoparticle was prepared according to the ionotropic gelation method (Aktaset *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 veragel* 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°C and dry place.

2.9 Collection and preparation of extract

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

2.9.1 Turmeric root extraction

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.

2.9.2 Neem leaves extraction

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).

2.9.3 Aloe vera gel preparation

The procedure was followed by (Healthline.com, 2019) with some modification. Aloe vera was collected from the local market in Dinajpur. 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.

2.10 Topical use of Neem, Turmeric, Aloe verawith Chitosan NP

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

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

2.11 Recording of different parameters

2.11.1 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.

2.11.2 Determination of body weight

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

2.11.3 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.

3.11.4 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

3.11.5 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 vein 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,

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

Dilution factor = reciprocal of dilution (20)

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

3.12 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).

III. 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, T₀ (3.10±0.00^b), trade drug and Chitosan NP combination treatment group, T₂ (0.15±0.05^a) and traditional plant extracts with Chitosan combination, T₄ (0.21±0.00^a) from the Trade drug without antibiotic treated group, T₁ (0.55±0.05^a) and only plant extracts treatment group T₃ (0.65±0.05^a).

Table 1: Effects of nanoparticle (Chitosan) and traditional plant materials (Neem, Turmeric & Aloe vera) on wound area measurement (cm²) recovery of rabbit

Treatment	Day 1	Day 4	Day 8	Day 12	Day 16
T ₀	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±0.30 ^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: T₀= chemically burned but not treated. T₁= chemically burned and only treated with trade drug without antibiotic. T₂= chemically burned and treated with trade medicine & Chitosan NP combination. T₃= chemically burned and treated with combination of Neem, Turmeric & Aloe vera extraction. T₄= 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 T₀ or non-treatment group, wound was not been recovered after 16 days of study. At Burna (trade drug) treated group T₁, wound healing was also not that progressive but at Chitosan and Burna treated group, T₂, 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 T₃, wound was also healed but not as T₂ and T₄ (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, T₀ (3.89.50±6.5) & chemically burned and only treated with trade drug, T₁ (3.90.00±5.5) was significantly decreased from the treatment of Chemically burned and treated with trade drug & Chitosan combination, T₂ (3.95.50±4.5), traditional plant extracts with Chitosan combination, T₄ (3.92.503±3.5) and Traditional plant extracts, T₃ (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: T₀= chemically burned but not treated. T₁= chemically burned and only treated with trade drug without antibiotic. T₂= chemically burned and treated with trade medicine & Chitosan NP combination. T₃= chemically burned and treated with combination of Neem, Turmeric & Aloe vera extraction. T₄= 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, T₀ (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
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T ₀	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: T₀= chemically burned but not treated. T₁= chemically burned and only treated with trade drug without antibiotic. T₂= chemically burned and treated with trade medicine & Chitosan NP combination. T₃= chemically burned and treated with combination of *Neem*, Turmeric & Aloe vera extraction. T₄= 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, T₀ (160.0±40^b) from treatment of Chemically burned and treated with trade drug & Chitosan combination, T₂ (30.5±22.5^{ab}), traditional plant extracts with Chitosan combination, T₄ (38.5±6.5^a) and Traditional plant extracts, T₃ (48.5±3.5^{ab}) and chemically burned and only treated with trade drug, T₁ (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 (cfu/ml)

Treatment	Day 4	Day 8	Day 12	Day 16
T ₀	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: T₀= chemically burned but not treated. T₁= chemically burned and only treated with trade drug without antibiotic. T₂= chemically burned and treated with trade medicine & Chitosan NP combination. T₃= chemically burned and treated with combination of *Neem*, Turmeric & Aloe vera extraction. T₄= 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, T₀ (10700.00±610.0^c) & chemically burned and only treated with trade drug, T₁ (10000.00±950.0^c) from the treatment of Chemically burned and treated with trade drug & Chitosan combination, T₂ (7700.00±250.0^b), traditional plant extracts with Chitosan combination, T₄ (8100.00±600.0^a) and Traditional plant extracts, T₃ (8600.00±800.0^a)

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

Treatment	Day 0	Day 4	Day 8	Day 12	Day 16
T ₀	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: T₀= chemically burned but not treated. T₁= chemically burned and only treated with trade drug without antibiotic. T₂= chemically burned and treated with trade medicine & Chitosan NP combination. T₃= chemically burned and treated with combination of Neem, Turmeric & Aloe vera extraction. T₄= 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

IV. 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 T₀, T₁, T₂, T₃, T₄ and each group containing 4 rabbits. Sulphuric acid burn was created by using 98% concentrated 0.5 ml acid at each group (T₁, T₂, T₃ and T₄) of rabbits. Group T₀ rabbit were kept as non-treatment group without giving any treatment. Group T₁ rabbit were kept as burned without giving any antibacterial agent but only established topically used trade drug (1% Silver Sulphadiazine). Group T₂ rabbits were treated with both 1% Silver Sulphadiazine and Chitosan nanoparticle. Groups of rabbit, T₃ were treated with combined traditional plant extracts topically & T₄ group was treated with both plant extracts & Chitosan nanoparticle combination. The study period was 16 days long.

5.1 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, T₀ (3.10±0.00^b), trade drug and Chitosan NP combination treatment group, T₂ (0.15±0.05^a) and traditional plant extracts with Chitosan combination, T₄ (0.21±0.00^a) from the Trade drug without antibiotic treated group, T₁ (0.55±0.05^a) and only plant extracts treatment group T₃ (0.65±0.05^a).

The present study indicated that the wound size were significantly not recovered in the non-treatment group (T₀), 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. MughisaMuniret *et al.*, 2021 assessed that herbal gel containing an *Azadirachtaindica* 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 (T₂) is more effective than other study groups. Chitosan and plant extracts combined groups (T₄) also give a positive result in healing but it is proved in the study that burn wound without treatment (T₀) can be more harmful and it could be spread.

5.2 Body weight

The present study indicated that the final body weight of chemical burned but not treated, T₀ (3.89.50±6.5) & chemically burned and only treated with trade drug, T₁ (3.90.00±5.5) was significantly decreased from the treatment of Chemically burned and treated with trade drug & Chitosan combination, T₂ (3.95.50±4.5), traditional plant extracts with Chitosan combination, T₄ (3.92.50±3.5) and Traditional plant extracts, T₃ (3.91.00±51.0).

In the present study, it was found that the final body weight of each group decreased after 16 days of study except group T₂ which were treated by Burna (1% Silver Sulphadiazine) at Chitosan combination. Non-Treatment group rabbits (T₀) 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 Neerasinghet *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.

5.3 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, T₀ (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.

5.4 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, T₀ (160.0±40^b) from treatment of Chemically burned and treated with trade drug & Chitosan combination, T₂ (30.5±22.5^{ab}), traditional plant extracts with Chitosan combination, T₄ (38.5±6.5^a) and Traditional plant extracts, T₃ (48.5±3.5^{ab}) and chemically burned and only treated with trade drug, T₁ (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.

5.5 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, T₀ (10700.00±610.0^c) & chemically burned and only treated with trade drug, T₁ (10000.00±950.0^c) from the treatment of Chemically burned and treated with trade drug & Chitosan combination, T₂ (7700.00±250.0^b), traditional plant extracts with Chitosan combination, T₄ (8100.00±600.0^b) and Traditional plant extracts, T₃ (8600.00±800.0^a).

The findings of the present study showed that the lower WBC was significantly found in T₂ and T₄ groups after 16 days of study which were treated with combination of Chitosan nanoparticles with Silver Sulphadiazine trade drug and traditional plant extract. Compared to non-treated group (T₀) and only Silver Sulphadiazine trade drug group without antibiotic (T₁), WBC count was quiet normal in T₂, T₄ and T₃ (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.

V. CONCLUSION

It can be concluded that Chitosan nanoparticle showed an effective wound healing activity with combination of Silver Sulphadiazine trade drug and traditional plant 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.

VI. REFERENCES

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