Original Research Article

Effect of Turmeric(*Curcuma longa*) Extract with Zinc Oxide Nanoparticles Application on Burn Wound

ABSTRACT

An experiment was conducted at the Department of Physiology and Pharmacology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh to see the clinical effectiveness of turmeric extract with zinc oxide (ZnO) nanoparticles in a hot iron burn wound in the rabbit model. Data were collected on the healing rate, healing time, and White blood cells (WBC) count. Sixteen male New Zealand White rabbits were divided into four groups where group T0 served as the non-treated, T1 as burn healing using turmeric extract, T2 burn heals with turmeric extract and ZnO nanoparticles; and T3 burn heals with Bactrocin®. The results showed that the T2 treatment had the best (93±0.707%), T3 better (88.5±1.04%), T1 good (86.5±1.55%) performance, and while the T0 (67.5±0.86%) gave the worst performance. The T3 (26.5±0.60 days) and T2 treatments had the greatest results (24.5±0.6455 days) and required the fewest days of healing compared to T0 which takes a maximum of days (35.5±0.64). The study revealed that the WBC count was maximum in the non-treated group (at 0,1st, 2nd, 3rd, and 4th week) which is (5600±31.06, 9000±36.47, 8000±38.41, 7500±36.11 and 6000±43.11 cell/ul respectively). The T2 treatments showed lower WBC count during the entire studied period (0, 1st and 4th days) and T3 at (3rd week and 4th week). Overall, the results of the study showed that the use of turmeric extract and ZnO nanoparticles led to faster burn wound healing with a shorter time required, proving that treating burn wounds in rabbits without the use of the commercial drug is both feasible and recommended for practice.

Keywords: *Curcuma longa, ZnO nanoparticles, Commercial drug, Wound, Rabbit*

1. INTRODUCTION

Any anatomical or physiological disruption in skin structure that results in skin cell destruction is referred to as a wound [1]. As a result, wound healing is an intricate and multifaceted process that starts as soon as the skin is lost and involves the reaction and interaction of cells and mediators to restore natural skin capacity. Three overlapping and ongoing stages of wound healing include remodeling, inflammation, and proliferation [2]. In the first stage of homeostasis, vascular constriction, and clot formation brought on by fibrin and platelets stop bleeding right away after damage [3, 4]. Burn injuries cause maternal and infant mortality and morbidity. Factors that affect this morbidity and mortality, include the depth and size of the burn [5]. Due to the role of flame fires and attempted suicides, practical activities such as education to decrease this awful event and its physical and emotional complications from responsible governors still seem mandatory. Due to pollution and infections at the wound site, the main worry nowadays is the delayed and poor healing of burn injuries throughout the world [6]. Over the years, a variety of medicinal herbs have also been utilized for the care and healing of wounds [7].

Turmeric (*Curcuma longa*) is a member of the ginger family (*Zingiberaceae*). It has been used since antiquity as a dye and condiment. Turmeric, sometimes called Indian saffron or the golden spice, is a tall plant that grows in Asia and Central America. In Asian countries, it has been consumed daily for centuries and no toxicity has been reported [8]. Its bright yellow color of it is derived primarily from the fat-soluble, polyphenolic pigments known as curcuminoids. Curcumin (diferuloylmethane), the principal curcuminoid found in C. longa, is generally considered its most active constituent. Other curcuminoids found in turmeric include bisdemethoxycurcumin and demethoxycurcumin [9]. Curcumin is the active ingredient in turmeric, and it has powerful biological properties. Turmeric has also been reported to exhibit properties like an antiseptic, cure for skin diseases, wound healing, respiratory distress, cure for poisoning, eliminate waste products from the body, and remedy for the treatment of sprains and swellings caused by injury [10]. Several biological activities of curcumin have been identified, including anti-inflammatory, anticarcinogenic, anti-infectious, antioxidant, anti-apoptotic, and wound healing activities [11]. To enhance its bioavailability, curcumin has been incorporated into different formulations using hydrogel, nanoparticles, micelles, hyaluronic/oleic acid-loaded, and glucosylation of the hydrophobic molecule in pre-clinical studies. It has shown potential as a wound-healing agent. Turmeric has been getting attention recently because of its antioxidant abilities. The antioxidant effect of turmeric appears to be so powerful that it may protect the liver from being damaged by toxins.

Scientists are interested in a range of biomedical applications since nanotechnology is a fast-expanding and complex study field [12]. The fields of industry, health, food, biomedicine, and cosmetics are only a few of the areas of life where nanoparticles have been introduced. The standard definition of a nanoparticle or ultrafine particle is a material particle with a diameter of one to one hundred nanometers (nm). It can also refer to fibers and tubes that are smaller than 100 nm in only two directions, or bigger particles up to 500 nm in size. Nanoparticles are being used in a variety of areas of life, including the biomedical, cosmetics, food, and industrial sectors. The most intriguing inorganic metal oxides are ZnO-NPs because they are easy to prepare, affordable to produce, and clean [13].

Numerous studies have been done to show how well zinc works to heal wounds. It has been demonstrated that preparations containing zinc accelerate the healing of wounds and reduce bacterial infection at the tissue site. Additionally, they helped the wound's granulation tissue to develop [14]. ZnO is an inorganic substance that demonstrates exceptional qualities such as semiconductor, broad radiation absorption, piezoelectric, pyroelectric, and high catalytic activity. Scientists' interest in a range of biomedical applications has been spurred by the rapidly expanding and demanding field of nanotechnology [15]. Recently developed antimicrobial nanoparticles have proven to be quite helpful, especially for wound healing [16]. It has lessened acute toxicity in addition to overcoming bacterial resistance. It has also decreased the price of treatment when compared to conventional antibiotics [16]. [17] found that nanoparticles have longer-lasting therapeutic benefits than conventional antibiotics because they persist in the bloodstream for longer periods [17]. Hardly any research has been conducted to determine the effectiveness of turmeric with zinc nanoparticles in the rabbit. In light of the above-mentioned qualities, therapeutic applications of turmeric extract, and ZnO nanoparticles, the following experiment was conducted on rabbits to know the clinical efficacy of turmeric with ZnO nanoparticles on burn wound healing activity based on healing rate, healing time, and WBC count.

2. MATERIALS AND METHODS

2.1 Experimental period and location

The experiment was carried out in the animal research laboratory of the department of Physiology and Pharmacology, HSTU, Dinajpur, between June 5 and July 17, 2022.

2.2 Collection of rabbit

At Rangpur, 16 male New Zealand White rabbits were collected when they were around 2.5 months old.

2.3 Experimental treatments

Sixteen male rabbits of near about 2.5 months were chosen and divided into four groups i.e. T_0= non-treated group, T_1= Hot iron burn and treated with turmeric extract, T_2= Hot iron burn and treated with turmeric extract and ZnO-NPs and T_3= Hot iron burn and treated with commercial drug (Bactrocin®). The temperature of hot iron burn was 100^0c. ZnO nanoparticles (Brand: Adnano Technologies Private Limited) were obtained in a usable form from the market. Bactrocin ointment is indicated for the topical treatment of impetigo (skin diseases) due to *Staphylococcus aureus* and *Streptococcus pyogenes*.

2.4 Collection and preparation of extract

The HSTU campus is where the turmeric was collected. The dried turmeric sample was then grind into a fine powder using an electric grinder, extracted using a maceration process in 70% methanol, and concentrated using a rotary evaporator at decreased pressure. The finished product was then kept at 4°C in a refrigerator until it was needed. 5g of turmeric extract were given as treatment to each group. Daily two time's 5 g turmeric was used in each group. The sample preparation was followed by [18].

2.5 Combined used of turmeric extract and ZnO-NPs

The gel was made by combining 4g turmeric and 1g ZnO. Then, this mixture was utilized in the hot iron burn wound positions twice daily [19].

2.6 Creating hot iron burn wounds in rabbits

A single, incredibly little hair measuring 0.20 mm remained on the skin. The required area is first shaved with a surgical blade. The rabbits' left thigh muscle is wound with a round, 2.2 cm-diameter iron that has been heated on a gas burner for three seconds and wound was created. After that, fresh water was used to treat the newly developed wound. This method resulted in the burning of 16 rabbits in all. There has only been one rabbit injury.

2.6 Data Collection

2.6.1 Recording of wound healing time

Any formation of the scare was also noted carefully as the sign of healing at the time interval of 7 days for each treatment group. The experimented room was in a dry, clean, well-ventilated area at room temperatures between about 25°C or up to 30°C, depending on climatic conditions.

2.6.2 Determination of healing rate

Every treatment group's recovery rate was assessed after a 7-day break. It was computed by dividing the time needed to completely close the wound by the largest average wound margin distance from the wound center. The following formula was used to determine the healing rate at weekly intervals:

Wound Closure (%) = [(Area of the wound on day 0 – area of the wound on indicated day)/Area of the wound on day 0]*100 [20].

2.6.3 Determination of healing time

From the day the wound was first created until the research's end day count, the rabbit's recovery time was determined. The recovery time was calculated for each treatment group on a weekly basis.

2.6.4 Determination of WBC enumeration

WBC counts were carried out by Automated Mindray BC-10 Haematology Analyzer and done manually at Green diagnostic center, Dinajpur. The WBC was counted for each treatment group every week.

2.7 Statistical analysis

Microsoft Excel and SPSS version 22 were used to calculate the findings of several biochemical parameters. Analysis of variance was used to identify variations between group means that were statistically significant (ANOVA).

3. RESULTS AND DISCUSSION

3.1 Wound Healing Rate (%)

The healing rate of the burn-injured rabbits was shown in Table 1 to have steadily increased from the first to the fourth week of the study period and to have significantly varied between the experimental treatments ($P<0.01$). The T2 treatment group (turmeric extract with ZnO nanoparticles) showed the highest healing rate (93±0.707), followed by the T3 treatment group (88.5±1.04), the T1 treatment group (86.5±1.55), and the T0 treatment group (67.5±0.86)

(Figure 1). Among the investigated therapies, the T2 (turmeric extract with ZnO nanoparticle) treated group displayed the best results in terms of healing rate throughout the entire study (1st week to 4th week). Previous investigations have shown that the well-known antibacterial compound ZnO is effective at very low bacterial concentrations. The biosynthesized ZnO from turmeric plant extract has also been shown to have antibacterial properties due to its tiny size, which offers a high surface area and increases interaction between the NPs and the bacterial cells. This is in line with [21], which showed that biosynthesis was effective against a variety of bacteria and fungi, including *Staphylococcus aureus, Serratia marcescens, Proteus mirabilis*, and *Citrobacter freundii*. These improved healing rates compared to other treatments.

Table 1: Effects of turmeric(*Curcuma longa*) extract, turmeric extract with ZnO nanoparticles and Bactrocin® on hot iron burn wound healing rate (%) of rabbit weekly

Treatment	1st Week	2nd Week	3rd Week	4th Week
T_0	22.75±0.94[a]	39.5±1.04[a]	53.75±1.25[a]	67.5±0.86[a]
T_1	32.25±2.28[b]	46.25±2.17[b]	66.5±0.64[b]	86.5±1.55[b]
T_2	44.25±1.49[c]	67.75±0.94[d]	84.75±1.25[d]	93±0.707[c]
T_3	40.25±2.32[c]	61.25±1.49[c]	78.75±1.18[c]	88.5±1.04[b]
P-Value	0.000***	0.000***	0.000***	0.000***

*** Significant at 1% level, Mean for wound recover size with different superscript within the rows were significantly different at $p<0.01$.

Here, T_0= Control/non-treated group, T_1= Hot iron burn and treated with turmeric extract, T_2= Hot iron burn and treated with turmeric extract and ZnO-NPs, T_3= Hot iron burn and treated with Bactrocin®

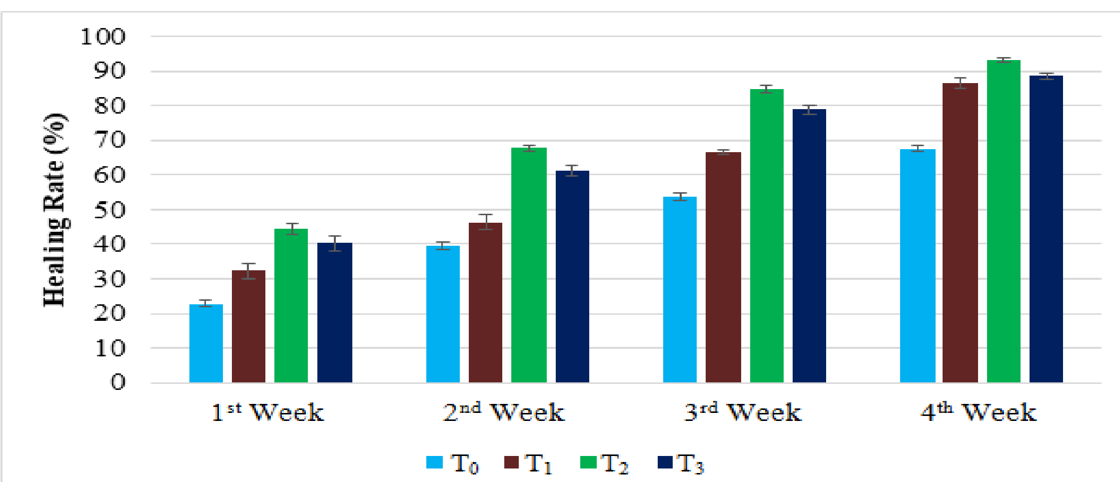

Figure 1: Effects of turmeric(*Curcuma longa)* extract, turmeric extract with ZnO-NPs and Bactrocin® on hot iron burn wound healing rate (%) of rabbit

Here, T_0= Control/non-treated group, T_1= Hot iron burn and treated with turmeric extract, T_2= Hot iron burn and treated with turmeric extract and ZnO-NPs, T_3= Hot iron burn and treated with Bactrocin®

3.2 Healing Time

Table 2 showed the healing time requirements (days) of the different treatments during the study period. The result of the study showed significant variation among the experimental treatments (P<0.01). The T3 (commercial medicine Bactrocin) treatment (26.5±0.60a) and T2 (Turmeric extract) treatments had the best results (24.5±0.6455a) and required the fewest days of healing compared to other treatments for the rabbit's artificially hot iron burn wound. The T0 (control) treatment takes a maximum of days (35.5±0.64c) than other treatments to heal the artificially burn wound of the rabbit. On the other hand, the control group takes the maximum number of days to settle the artificial burn wound of the rabbits (Figure 2). The glycolysis process, which includes converting carbohydrates into energy, is one potential method of interaction between Zn^+ and plant phytochemicals. These interactions result in the fastest and highest healing of the rabbit's burn wound [22]. The mechanism of action of turmeric extract in wounds is by inhibiting the enzymes cyclooxygenase (COX-2) and lipooxygenase (LOX), which play a role in the inflammatory process, accelerating tissue re-epithelialization, cell proliferation, and collagen synthesis. It improves the healing process rapidly.

Table 2: Effects of turmeric extract, turmeric extract with ZnO nanoparticles and Bactrocin® on hot iron burn wound healing time of rabbit at 4th week

Treatment	Healing Time (Days)
T_0	$35.5±0.64^c$
T_1	$29.25±1.10^{ab}$
T_2	$24.5±0.6455^a$
T_3	$26.5±0.60^a$
P-Value	0.000***

*** Significant at 1% level, Mean for wound recover size with different superscript within the rows were significantly different at p<0.01.

Here, T_0= Control/non-treated group, T_1= Hot iron burn and treated with turmeric extract, T_2= Hot iron burn and treated with turmeric extract and ZnO-NPs, T_3= Hot iron burn and treated with Bactrocin®

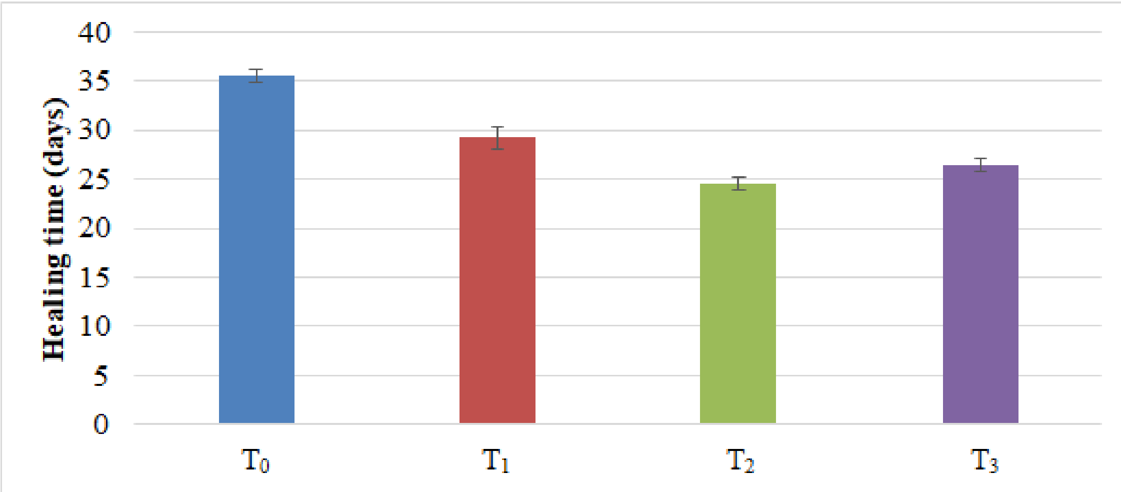

Figure 2: Effects of turmeric extract, turmeric extract with ZnO nanoparticles and Bactrocin® on hot iron burnd wound healing time of rabbit at 4th week

Here, T_0= Control/non-treated group, T_1= Hot iron burn and treated with turmeric extract, T_2= Hot iron burn and treated with turmeric extract and ZnO-NPs, T_3= Hot iron burn and treated with Bactrocin®

3.3 WBC count

The WBC Count cells/ul of the various researched treatments, which are considerably influenced by the various experimented groups, are shown in Table 3 (P<0.01). The WBC count (cells/ul) was significantly higher in the control group (P<0.01), followed by the T1 (single Turmeric extract) treatment. The present study revealed that the WBC count was greatly increased in the non-treated treated group (at 0,1st, 2nd, 3rd, and 4th week) which is (5600±31.06d, 9000±36.47d, 8000±38.41c, 7500±36.11d and 6000±43.11d respectively). The T2 treatments showed lower WBC count during the entire studied period (0, 7, and 28 days) and T3 at (the 3rd week and 4th week) (Figure 3). Administration of curcumin resulted in decreases in the size of the burn wounds and a reduction in inflammation [23]. Turmeric also provides antioxidant effects by restoring an imbalance between the production of ROS (free radicals) and antioxidant activity. In the proliferative phase, curcumin can produce collagen synthesis of fibroblast migration, vascular density, and epithelialization.

Table 3: Effects of turmeric extract, turmeric extract with ZnO nanoparticles and Bactrocin® on WBC count (cell/ul) of rabbit at weekly interval

Treatment	Day 0	1st week	2nd week	3rd week	4th week
T_0	5600±31.06^{d}	9000±36.47^{d}	8000±38.41^{c}	7500±36.11^{d}	6000±43.11^{d}
T_1	4800±36.44^{c}	8800±39.41^{c}	7700±39.21^{b}	6800±37.32^{c}	5600±39.45^{c}

T_2	4000±39.14[a]	8000±40.11[a]	7200±41.22[a]	6500±32.44[b]	5400±38.33[b]
T_3	4500±34.36[a]	8200±35.21[a]	7300±32.41[a]	6000±37.22a	5000±34.33[a]
P-Value	0.000***	0.000***	0.000***	0.000***	0.000***

*** Significant at 1% level, Mean for wound recover size with different superscript within the rows were significantly different at p<0.01.

Here, T_0= Control/non-treated group, T_1= Hot iron burn and treated with turmeric extract, T_2= Hot iron burn and treated with turmeric extract and ZnO-NPs, T_3= Hot iron burn and treated with Bactrocin®

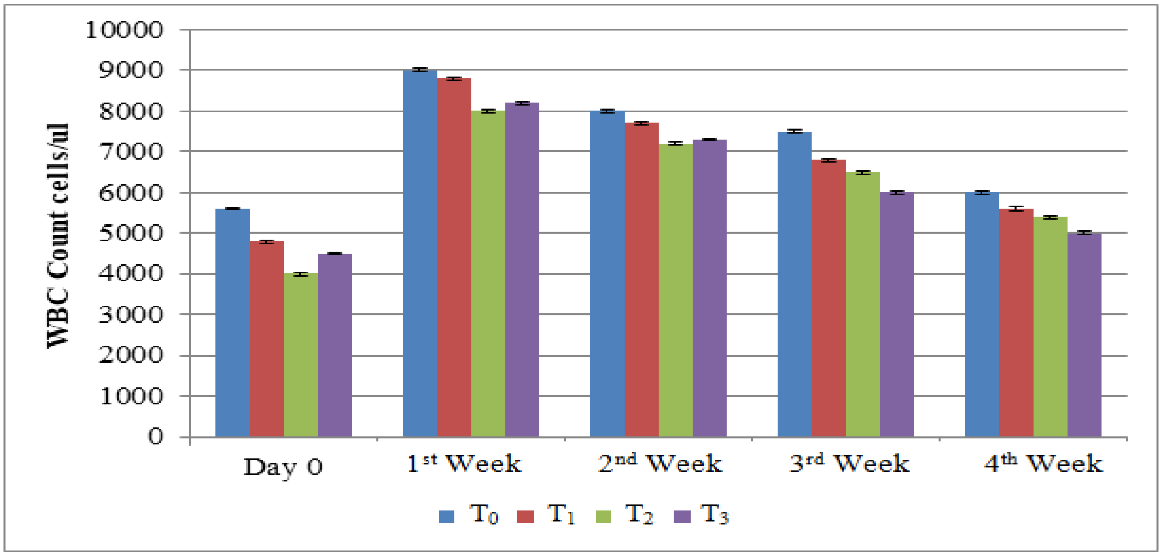

Figure 3: Effects of turmeric extract, turmeric extract with ZnO nanoparticles and Bactrocin® on WBC count (cell/ul) of rabbit at weekly interval

Here, T_0= Control/non-treated group, T_1= Hot iron burn and treated with turmeric extract, T_2= Hot iron burn and treated with turmeric extract and ZnO-NPs, T_3= Hot iron burn and treated with Bactrocin®

4. CONCLUSIONS

The results of the study showed that applying ZnO nanoparticles and turmeric extract to burn wounds in rabbits resulted in quicker wound healing with a shorter recovery period as combining curcumin with zinc oxide nanoparticles may enhance the healing of burn wounds through a synergistic effect. Curcumin's anti-inflammatory, antioxidant, and collagen-boosting qualities may work in conjunction with zinc oxide nanoparticles' antibacterial and collagen-boosting qualities to promote quicker and more complete wound healing. So it demonstrating that treating burn wounds without the use of antibiotics is both feasible and advised.

REFERENCES

1. George Broughton II, Janis JE, Attinger CE. The basic science of wound healing. Plastic and reconstructive surgery. 2006 Jun 1;117(7S):12S-34S.
2. Lugović-Mihić L, Duvančić T, Vučić M, Situm M, Kolić M, Mihić J. SDRIFE (baboon syndrome) due to paracetamol: case report. Acta Dermatovenerol Croat. 2013 Aug 23;21:113-7.
3. Reinke JM, Sorg H. Wound repair and regeneration. European surgical research. 2012;49(1):35-43.
4. Allacker K, De Troyer F, Velner R, Verhoeven M, Fremouw M. Integraged sustainability assessment of dwellings in the Belgian context. SASBE proceedings, Smart and Sustainable Built Environment. 2009 Jun 1.
5. Pasalar M, Mohammadi AA, Rajaeefard AR, Neghab M, Tolidie HR, Mehrabani D. Epidemiology of burns during pregnancy in southern Iran: Effect on maternal and fetal outcomes. World Appl Sci J. 2013;28(2):153-8.
6. Senthil M, Brown M, Xu DZ, Lu Q, Feketeova E, Deitch EA. Gut-lymph hypothesis of systemic inflammatory response syndrome/multiple-organ dysfunction syndrome: validating studies in a porcine model. Journal of Trauma and Acute Care Surgery. 2006 May 1;60(5):958-67.
7. Shahbaz MA, Goschütz M, Kaiser SA. Endoscopic anisole-LIF imaging of flame propagation and temperature fluctuations in a production SI engine. In8th European Combustion Meeting 2017 Apr 18 (pp. 18-21).
8. Ammon CJ. The isolation of receiver effects from teleseismic P waveforms. Bull. Seismol. Soc. Am. 1991 Dec 1;81(6):2504-10.
9. Akram M, Shahab-Uddin AA, Usmanghani KH, Hannan AB, Mohiuddin E, Asif M. Curcuma longa and curcumin: a review article. Rom J Biol Plant Biol. 2010;55(2):65-70.
10. Srimal RC. Turmeric: a brief review of medicinal properties. Fitoterapia (Milano). 1997;68(6):483-93.
11. Yucel AF, Kanter M, Pergel A, Erboga M, Guzel A. The role of curcumin on intestinal oxidative stress, cell proliferation and apoptosis after ischemia/reperfusion injury in rats. Journal of Molecular Histology. 2011 Dec;42:579-87.
12. Akbar S, Tauseef I, Subhan F, Sultana N, Khan I, Ahmed U, Haleem KS. An overview of the plant-mediated synthesis of zinc oxide nanoparticles and their antimicrobial potential. Inorganic and Nano-Metal Chemistry. 2020 Apr 2;50(4):257-71.

13. Jayaseelan C, Rahuman AA, Kirthi AV, Marimuthu S, Santhoshkumar T, Bagavan A, Gaurav K, Karthik L, Rao KB. Novel microbial route to synthesize ZnO nanoparticles using Aeromonas hydrophila and their activity against pathogenic bacteria and fungi. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2012 May 1;90:78-84.
14. Rostan EF, DeBuys HV, Madey DL, Pinnell SR. Evidence supporting zinc as an important antioxidant for skin. International journal of dermatology. 2002 Sep;41(9):606-11.
15. Faisal S, Khan MA, Jan H, Shah SA, Shah S, Rizwan M, Akbar MT. Edible mushroom (Flammulina velutipes) as biosource for silver nanoparticles: from synthesis to diverse biomedical and environmental applications. Nanotechnology. 2020 Nov 18;32(6):065101.
16. Saleem H, Zaidi SJ, Ismail AF, Goh PS. Advances of nanomaterials for air pollution remediation and their impacts on the environment. Chemosphere. 2022 Jan 1;287:132083.
17. Seo K, Kim I, Jung S, Jo M, Park S, Park J, Shin J, Biju KP, Kong J, Lee K, Lee B. Analog memory and spike-timing-dependent plasticity characteristics of a nanoscale titanium oxide bilayer resistive switching device. Nanotechnology. 2011 May 16;22(25):254023.
18. Mehedi M. Wound healing activity of nanoparticles with traditional plants in chemically burn rabbit model. M.S. Thesis. Department of Physiology and Pharmacology, Hajee Mohammad Danesh Science and Technology University, Dinajpur. 2021.
19. Bhutta ZA, Ashar A, Mahfooz A, Khan JA, Saleem MI, Rashid A, Aqib AI, Kulyar MF, Sarwar I, Shoaib M, Nawaz S. Enhanced wound healing activity of nano ZnO and nano Curcuma longa in third-degree burn. Applied Nanoscience. 2021 Apr;11:1267-78.
20. Sato H, Ebisawa K, Takanari K, Yagi S, Toriyama K, Yamawaki-Ogata A, Kamei Y. Skin-derived precursor cells promote wound healing in diabetic mice. Annals of plastic surgery. 2015 Jan 1;74(1):114-20.
21. Salam HA, Rajiv P, Kamaraj M, Jagadeeswaran P, Gunalan S, Sivaraj R. Plants: green route for nanoparticle synthesis. Int Res J Biol Sci. 2012;1(5):85-90.
22. Agarwal S, Sorkin M, Levi B. Heterotopic ossification and hypertrophic scars. Clinics in plastic surgery. 2017 Oct 1;44(4):749-55.

23. Mehrabani D, Farjam M, Geramizadeh B, Tanideh N, Amini M, Panjehshahin MR. The healing effect of curcumin on burn wounds in rat. World journal of plastic surgery. 2015 Jan;4(1):29.