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Effect of N-Acetylcysteine Oral Administration on Cutaneous Wound Healing

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ABSTRACT

Thermal injury is prevalent and a burdensome critical care problem due to inadequate safety measures. A severe burn is associated with release of inflammatory mediators including reactive oxygen and nitrogen species (ROS, RNS), which are implicated in pathophysiological events in burn patients. Excessive productions of free radicals are harmful and implicated in tissue damage and multiple organ failure. Supplementation of antioxidants has proven beneficial in decreasing free radicals in burns. The aim of the present study was to investigate the effect of N-acetyl cysteine (NAC) on biochemical alterations during the post-burn stage in scalded rats. Twenty-four male Sprague-Dawley rats were anesthetized and dorsum was shaved. Second-degree skin burn was induced by immersing an iron mold (1.0 cm^2 in diameter) in boiling (100°C) water for 5 min and placed on the back of the rats for 20 sec without applying pressure. Rats were divided into three groups; control group, burn injury group, burn injury + NAC group. Wound size and histopathological changes of epidermis were evaluated for the various groups. Hydroxyproline was estimated in wound tissue. Wounded skin tissues were cut off and stored at −20°C for PCR and ELISA analysis. The therapeutic effects of a NAC supplementation for a short period of time on rat skin wound healing were investigated. The NAC treatment promoted an improvement of wound healing by reducing the oxidative stress, improving wound closure, reducing tissue inflammation with no significant change in blood parameters. There were no significant differences in weekly and final body weights and no significant changes observed in hematological and biochemical markers. A significant increase in collagen synthesis was observed in the NAC treated group with up-regulation of VEGF, IGF-1, PCNA, SDF-1 and CXCR-4 and down regulation of caspase3, P53 and CRP. The study showed that NAC is totally safe to use on rats. Hence for the future it is suggested that more experiments should be conducted for use in humans in conjugation medication for healing burn wounds.

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Key words
Burns, N-acetyl cysteine, Oxidative stress, Rats wound healing

INTRODUCTION

Thermal burn injury remains a great challenge to clinicians. Thermal burns cause an injury to the different layers of the skin (Ye and De, 2017). The type of burn and the severity of the burn depend on the number of skin layers affected. The pathophysiology of thermal burn causes damage to skin anatomy leading to its functional impairment (Greenhalgh, 2019). Thermal injury in human and animals models may be complicated by dysfunction of organs distant from the original burn wound (Kaddoura *et al*., 2017). The physiopathological events following thermal injury are not limited to the skin surface but its effects also disturb the cell structure and functions mediating acute inflammatory reaction and cause tissue swelling (Lateef *et al*., 2019).

Thermal injury also induces the activation of hypermetabolism causing damage to cell antioxidant system and results in increased production of proinflammatory cytokines, as well as the reactive oxygen species (ROS), leads to secondary complication like oxidative stress. Oxidative stress is a phenomenon caused by an imbalance between production and detoxification of ROS in cells (Nielson *et al*., 2017; Auger *et al*., 2017). Antioxidant system acts as a defense barrier for the cells. Burn patients may face the challenge of oxidative stress induced injury along with burn injury. For the assurance of burn patient health there is a need to design a strategy which strengthens the antioxidant defense system.

The impairments associated with burn injury are usually treated, either by medication or surgical procedure, or combination of both. During the wound healing process ROS are produced by inflammatory cells. In this context, antioxidant therapies are suggested to accelerate wound

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healing by controlling oxidative stress. N-acetyl cysteine (NAC), is a precursor of glutathione (GSH) which is an endogenous antioxidant. Antioxidant therapy for wound healing is promising, however few animal and clinical studies are available. NAC confers several beneficial effects in human and animal models, and is being investigated in acetaminophen toxicity, cystic fibrosis, diabetes, perioperative atrial fibrillation, acute cholestasis-induced renal failure, chronic obstructive pulmonary disease, and acute smoke inhalation injury. Keeping in view of therapeutic nature of NAC, it is hypothesized that NAC would also accelerate wound healing. So, the main purpose of the study was to determine the effect of NAC in burn wound healing.

MATERIALS AND METHODS

Experimental animals

The study included 24 Sprague-Dawley male rats, each having a weight of 250±30 g. Rats were housed in individually ventilated cages with 12/12-h light/dark cycles, food and water ad libitum. The rats were treated according to the ethical guidelines of the Institutional Animal Ethical Committee Institute of Molecular Biology and Biotechnology, Lahore Pakistan. Institutional Animal Ethical Committee approved all experimental procedures. All the procedures were performed in accordance with the approved guidelines of the Institutional Animal Ethical Committee of the University of Lahore (Ethics certificate no. IMBB/UOL/19/696).

Preparation of burn wound model

The rats were anesthetized by intraperitoneal administration of ketamine (50 mg/kg) and xylazine (10 mg/kg). After anesthesia the dorsa of the rats were shaved and swabbed with 70% ethanol for asepsis. Second-degree skin burn was induced by immersing an iron mold (1.0 cm^2 in diameter) in boiling (100°C) water for 5 min and placed on the back of the rats for 20 seconds without applying pressure. After burn the excision of the burn skin wound was made in dorsal skin to create excisional burn wound model on the same day. Animals were housed individually in clean cages. Rats were individually caged and received a single dose of paracetamol (75 mg/kg b.w.) immediately after surgery.

Experimental procedure

The rats were divided into three groups each of four rats: Group 1 (Control) were without any injury. Group 2 (burn injury group) animals were given injury as described, above and then normal saline orally (p.o.) gavage method for 7 consecutive days. Group 3 (Burn injury + NAC treated Group) were given burn injury and then given N-acetylcysteine (NAC, Sigma Aldrich, USA) at 150mgkg−1 by gavage method for 7 consecutive days. After 14 days of treatment, animals were euthanized and tissues were harvested and processed for biochemical measurements.

Measurement of wound contraction

An excision wound margin was traced on a sheet of sterile autoclaved transparent paper and then placed on 1 mm^2 graph paper on the day of wounding and subsequently at a gap period of 3 days till 14th day. Changes in wound area were measured and results were expressed as percentage (%) of the original wound area by using the following formula: (Initial wound size-specific day wound size)/ Initial wound size x 100. Wounds were considered completely healed when moist granulation tissue was no longer apparent and the wound was covered with new epithelium.

Histopathological studies

Wound tissue specimens from control, burn and treatment groups were taken after 14 days of absolute healing excision, and after tissue processing paraffin sections of 5 µm thickness were cut and stained with hematoxylin and eosin. Sections were qualitatively assessed under the light microscope and observed in respect of fibroblast proliferation, collagen formation, angiogenesis and epithelialization.

Hydroxyproline assay

The wound area was excised at the size of 1.0 cm x 1.0 cm and analyzed to estimate the amount of hydroxyproline in wound tissue. Hydroxyproline content was determined according to our previously published protocol (Ali *et al*., 2021).

Collection of blood and hematological studies

Five ml of blood were drawn from heart after given anesthesia from all groups for hematological and biochemical study. Blood from each sample was divided into two portion; first drawn in EDTA vacutainer tube and second in without EDTA vacutainer tube. All blood samples were sent to diagnostic lab immediately for analysis after collection. The serum and plasma samples were frozen at -20°C for biochemical analysis. The whole blood samples were analyzed using an automatized blood analyzer (Urite, China) for hemoglobin (Hb), red blood cell count (RBC), white blood cell count (WBC), platelets, and lymphocytes.

Serum biochemical analyses

All biochemical analysis was performed at Center

for Research in Molecular Medicine (CRIMM), The University of Lahore. The following biochemical parameters were analyzed: Blood chemistry (glucose, lactate dehydrogenase, sodium, potassium, calcium, chloride, and phosphate): Lipid profile (total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL): Liver profile (alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, albumin, and bilirubin): Renal profile (urea, and creatinine) were performed according to the manufacturer's protocol of reagent kits purchased from bio diagnostics.

Measurement of antioxidant enzyme activities

Wound tissue was rinsed with ice-cold saline, and homogenized using a homogenizer. The homogenate was processed on ice at a concentration of 10% in potassium phosphate buffer (50mM, pH 7.8) containing mammalian protease inhibitors. The homogenates were then centrifuged for 30 min at 4,500 rpm and 4°C. This homogenate were used to determine the activities of glutathione reductase (GSH), catalase (CAT), and superoxide dismutase (SOD), and level of malondialdehyde (MDA), according to procedure previously described (Ali *et al*., 2017).

PCR analysis

Total RNA were isolated from skin samples using TRIzol reagent (Invitrogen, USA) and were quantified with ND-1000 spectrophotometer (NanoDrop Technologies, USA). cDNA was synthesized from RNA (1µg) using Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase kit (Invitrogen Corporation, USA). Reverse transcriptase PCR (RT-PCR) analysis of groups was carried out for *Caspase-3, p53, HIF-1, VEGF-A, IGF-1, PCNA* and *β-actin* was performed, *β-actin* was used as an internal control. The RT-PCR was carried out using a GeneAmp PCR system 9700 (Applied Biosystem) for 35 cycles. Gene bands were quantified by using ImageJ software, according to the instructions given in user guide. The sequences (5' to 3') and product lengths (bp) for the primer pairs are mentioned in (Table I).

Indirect ELISA

Indirect ELISA for VEGF, IGF-1, SDF-1, CXCR-4, CRP and β-actin was performed according to our previously published protocol (Ali *et al*., 2021). Description of Primary and secondary antibodies used are as follows: anti-VEGF rabbit antibody, anti-IGF-1 rabbit antibody, anti-SDF-1 rabbit antibody, anti-CXCR-4 rabbit antibody, anti-CRP rabbit antibody, anti-IGF-1 rabbit antibody, and anti-β-actin mouse antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Followed by 1h incubation with HRP-conjugated anti-Rabbit and anti-Mouse IgG (Rockland, Limerick, PA).

Table I. Blood parameters of rats before and after exposure to treatment.

Parameters	Control	Burn	Burn+NAC
Blood parameter			
WBC count 10^9/l	12.34±4.00	10.94±4.13	12.10±6.05
RBC count 10^{12}/l	7.15±0.01	6.19±0.05	7.00±0.05
Haemoglobin g/dl	14.23±0.12	12.50±0.05	13.09±0.08
PLT count 10^9/l	886.8±126.2	745.7±255.4	833.0±267.0
Liver function test			
AST (IU/l)	151.4±5.93	154.3±5.63	152.1±6.78
ALT (IU/l)	71.42±0.44	73.20±0.96	72.30±1.59
Cholesterol mg/dl	59.78±2.89	61.00±3.50	60.70±1.53
Triglycerides mg/dl	66.56±2.89	68.00±2.89	67.18±2.89
Albumin mg/dl	4.36±0.04	4.25±0.41	4.35±0.07
Bilirubin mg/dl	0.056±0.00	0.051±0.07	0.058±0.01
Renal test			
Urea mg/dl	34.89±1.20	37.00±3.18	35.95±2.81
Creatinine mg/dl	0.35±0.02	0.37±0.03	0.35±0.03
Serum electrolyte			
Potassium (mmol/l)	5.09±0.09	8.39±0.56	6.43±0.33
Phosphate (mg/dl)	12.67±0.33	13.10±0.05	12.87±0.18
Sodium (mmol/L)	145.0±1.49	135.0±5.08	148.2±2.73
Calcium (mg/dl)	10.23±0.14	8.767±0.03	9.467±1.45
Chloride (mmol/L)	108.2±0.14	97.86±0.14	108.2±0.14

Statistical analysis

All experimental data was analyzed using Graphpad prism v 7.0 software. (GraphPad Software, La Jolla, CA). Student's unpaired t-test was used to determine statistical difference between any two groups whereas for more than two groups, 1-way or 2-way ANOVA with Bonferroni post-hoc test was used. Each experiment was conducted thrice in triplicate using separate cultures. One symbolic sign for statistical significance denotes $p \leq 0.05$, two signs denote $p \leq 0.01$ and three signs denote $p \leq 0.001$.

RESULTS

Effect of NAC on wound healing

To evaluate the effects of NAC treatment on burn wound closure, wound contraction was measured. An increase in wound-healing activity was observed in the rats treated with NAC, as compared with the burn group (Fig. 1A).

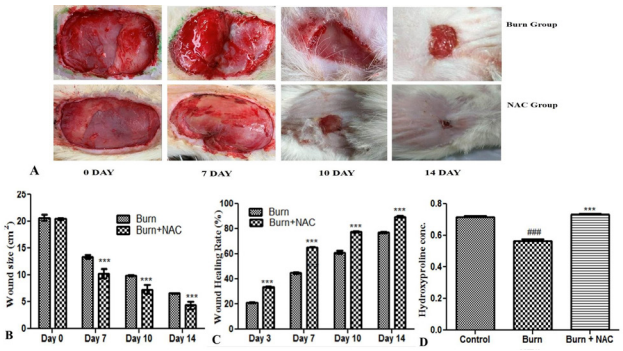


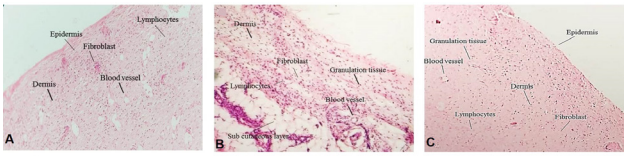
Fig. 1. Effect of NAC on wound healing. **(A)** Wound contraction area in burn, and burn + NAC group. Wound appearance following 14 days of treatment with NAC. **(B-C)** Wound healing area and wound healing rate. **(D)** Effects of NAC on hydroxyproline levels in the burn wound model. Control versus burn group, $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; and $^{\#\#\#}P < 0.001$; Control versus Burn+NAC group, $^{\Phi}P < 0.05$; $^{\Phi\Phi}P < 0.01$; and $^{\Phi\Phi\Phi}P < 0.001$; Burn versus Burn+NAC group, $^{*}P < 0.05$; $^{**}P < 0.01$; and $^{***}P < 0.001$.

Figure 1A and C shows that the wound area was consistently reduced as the time progresses from to day 14. The wound healing rate is significantly increased in NAC treated group, from 33.0% on day 3 to 65.3%, 77.7% and 89.7 % on day 7, 10 and day 14. In comparison, the burn group exhibited 21.0%, 44.85%, 61.29% and 76.9 % contraction on day 3, 7, 10, and 14, respectively.

Increase in hydroxyproline content indicates increased collagen synthesis which in turn leads to enhanced wound healing (Nayak and Pereira, 2006). In our study, hydroxyproline content were found to be significantly increased in the NAC treated group (0.73 ± 0.005 mg/g of tissue) than the burn group (0.56 ± 0.01 mg/g of tissue) which implies more collagen deposition in treated groups than the burn group (Fig. 1D). Significant differences were observed in hydroxyproline concentrations between groups II and III when compared with control group I.

Histological examination of skin tissue

Figure 2 shows histological evidence for wound healing. There is a moderate level of granulation tissue in control group whereas mild in burn and burn+NAC group this indicate formation of new connective tissue, and microscopic blood vessels. Fibroblasts are responsible for the contractile process in wound closure. So presence of too many fibroblasts at wound site indicates that wound are still in the phase of healing. Neutrophils are absent in all treatment groups (Table I and Fig. 2). Neutrophil are helpful in normal wound healing process and recruited to injury site soon after injury. However, in normal skin and healed skin neutrophils are not frequently observed on day 14 the number of inflammatory cells were also reduced significantly, epithelialization level was increased in burn+NAC compared with the burn group.



Groups	Epidermis and Dermis	Granulation Tissue	Lymphocytes	Neutrophils	Fibroblast
Control group	**i)** Epidermis is partially present **ii)** Skin adnexa is present	None	Few	None	Few
Burn group	**i)** Epidermis is not present **ii)** Skin adnexa present	Mild	Many	None	Many
Burn+NAC group	**i)**Epidermis is partially present **ii)** Skin adnexa is present	Moderate	Few	None	Few

Fig. 2. Histological examination of skin tissue. **A,** Control group; **B,** Burn group; **C,** Burn+NAC group; **D,** Summary of histological of features of wound healing.

Hematological and serum biochemical parameters

Table II shows the effect of NCA on hematological parameters, liver function test, renal test and serum electrolytes of rats inflicted with burn injury on skin. We noticed decrease in RBC's count, HB concentration, and increase in the WBC's count in burn group in comparison to control whereas burn+NAC group shows similar results to control group. Liver enzymes activity were analyzed where there was increase in AST, ALT, enzyme activities in burn group as compared with control group and burn+NAC group. In addition, burn group show little variation (decrease) in serum albumin and total bilirubin compared with normal control and burn+NAC group. The level of cholesterol and triglycerides changed non-significantly in all treatment groups. Kidney function analysis show non-significant increase in serum creatinine and urea in burn group comparison to control group and burn+NAC group. Serum electrolytes: Burns also lead to increase in potassium level and phosphate level while sodium, calcium and chlorine level was decreased compared to control group and burn+NAC group.

Oxidative stress markers

Figure 3 shows activities of CAT, GSH, SOD, and MDA measured in skins of rats inflicted with skin burn injury and them treatment with NAC. On the 14 days post-wounding, CAT (0.117 ± 0.004 nm), GSH (0.199 ± 0.004 nm), SOD (0.199 ± 0.005 nm) activities was significantly reduced in burn group when compared to control group CAT (0.550 ± 0.015 nm), GSH (0.580 ± 0.003 nm), and SOD (0.528 ± 0.022 nm). The activity of MDA was increased burn group (0.461 ± 0.016 nm) when compared with control group (0.134 ± 0.002 nm)

(Fig. 3D). Treatment with NAC had significant effect on enzyme activities. Figure 3A showed that treatment of wound with NAC resulted in an increased SOD activity (0.471 ± 0.021 nm). Figure 3B showed that treatment of wound with NAC resulted in an increased GSH activity (0.467 ± 0.004 nm). Figure 3C showed that treatment of wound with NAC resulted in an increased CAT activity (0.353 ± 0.008 nm). Figure 3D showed that treatment of wound with NAC resulted in decreased MDA activity (0.299 ± 0.025 nm).

Table II. Primer sequences.

Gene	Primer sequence	Size (bp)
Caspase 3	5'ACAGAGCTGGACTGCGGTAT3' (F) 5'TGCGGTAGAGTAAGCATACAGG3' (R)	104
P53	5'TTGGATCCATGTTTTGCCAACTGGCC3'(F) 5'TTGAATTCAGGCTCCCCTTTCTTGCG3'(R)	120
HIF-1	5'-CTAGGGATGCAGCACGATCT-3' (F) 5'-AGATGGGAGCTCACGTTGTG-3' (R)	100
VEGF	5'GCCCTGAGTCAAGAGGACAG3' (F) 5'GAGGAGGAGGAGCCATTACC3' (R)	284
IGF-1	5'GCTGAAGCCGTTCATTTAGC3' (F) 5'CCACCCAGTTGCTATTGCTT3' (R	193
PCNA	5'-TGACCCTCACCGATACAACA-3' (F) 5'-CTGTACAGCACAGCCACGTT-3' (R)	123
β actin	5'-GCTGTGTTGTCCCTGTATGC-3' (F) 5'-GAGCGCGTAACCCTCATAGA-3' (R)	102

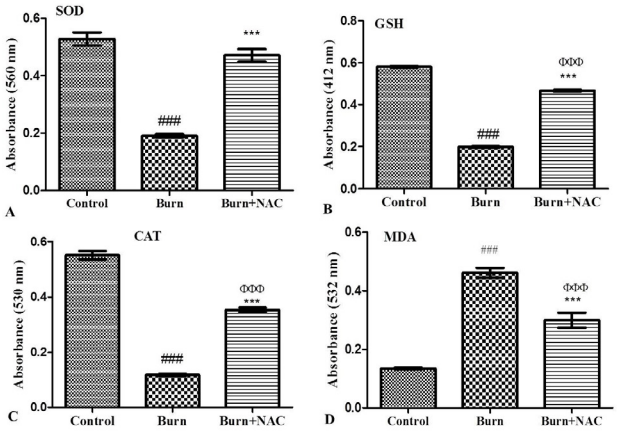


Fig. 3. Assessment of oxidative stress markers in treatment groups. **A,** Superoxide dismutase (SOD); **B,** Reduced glutathione (GSH); **C,** Catalase (CAT); **D,** Malondialdehyde (MD A). Error bars represent ± standard error of mean (SEM). Control versus burn group, $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; and $^{\#\#\#}P < 0.001$; Control versus Burn+NAC group, $^{\Phi}P < 0.05$; $^{\Phi\Phi}P < 0.01$; and $^{\Phi\Phi\Phi}P < 0.001$; Burn versus Burn+NAC group, $^{*}P < 0.05$; $^{**}P < 0.01$; and $^{***}P < 0.001$.

Gene expression profiling

Gene expression profiling was done on the control, burn and NAC-treated groups. The results of this profiling showed decreased levels of *caspase-3, p53* (apoptotic genes), and *HIF-1* (hypoxic gene), and elevated levels of *VEGF* (angiogenesis gene) and *IGF-1* (survival gene) NAC treated group as compared with the burn group (Fig. 4A, B). The wound area was measured by tracing the wound margin, and its surface area was calculated using Image J software (National Institutes of Health, Bethesda, MA, USA).

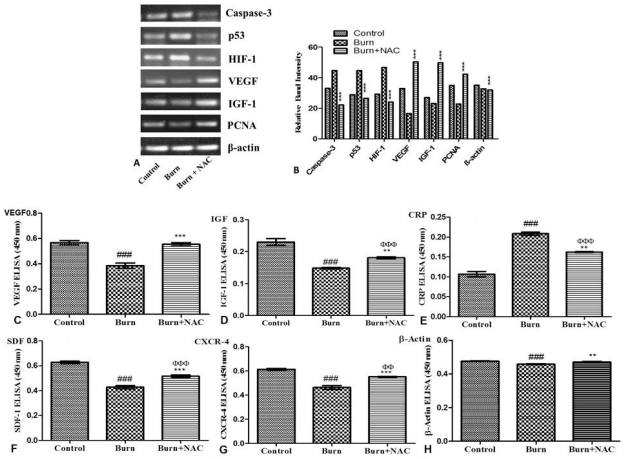


Fig. 4. Effect of NAC on gene-expression analysis of rats inflicted with burn injury. Gene expression of skin preconditioned with NAC groups compared with control and Burn group along with corresponding quantification. Error bars represent ± standard error of mean (SEM). Control versus burn group, $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; and $^{\#\#\#}P < 0.001$; Control versus Burn+NAC group, $^{\Phi}P < 0.05$; $^{\Phi\Phi}P < 0.01$; and $^{\Phi\Phi\Phi}P < 0.001$; Burn versus Burn+NAC group, $^{*}P < 0.05$; $^{**}P < 0.01$; and $^{***}P < 0.001$.

Protein level

To monitor the effect of NAC on wounded skin sample release of growth factors (IGF-1, VEGF), progenitor cell mobilization (SDF-1, CXCR-4), β-actin, and inflammation makers (CRP) were observed. β-actin was used as an internal control. As shown in Figure 4C-H, the levels of IGF-I and VEGF were high in the Burn+NAC group (VEGF=0.554 ± 0.013 nm; IGF=0.180 ± 0.002 nm) compared with burn group (VEGF=0.385 ± 0.020 nm; IGF=0.148 ± 0.021 nm) ($P < 0.05$). Burn+NAC group showed significantly reduce CRP level (0.162 ± 0.001) compared with burn group (0.208 ± 0.003).

The level of progenitor cell mobilization maker in skin wound sample indicated that the level of SDF-1 and CXCR-4 were high in Burn+NAC group (SDF-1= 0.516 ±

0.009 nm; CXCR-4= 0.462 ± 0.015 ± 0.009 nm) compared with burn group (SDF-1= 0.427 ± 0.012 nm; CXCR-4= 0.516 ± 0.009 nm) ($P<0.05$) (Fig. 4F-G). β-actin level remain almost remain same in Burn+NAC (0.471 ± 0.002 nm) and burn group (0.457 ± 0.002 nm) (Fig. 4H). Whereas the level of these markers in control group are as follow; (VEGF=0.567 ± 0.016 nm; IGF=0.230 ± 0.010 nm; SDF-1=0.627 ± 0.009 nm; CXCR-4=0.613 ± 0.007 nm; CRP=0.106 ± 0.006 nm; β-actin=0.476 ± 0.001 nm).

DISCUSSION

Previous reports have attributed the effectiveness of NAC as an antioxidant, as a precursor of glutathione (GSH) synthesis in the body. Wound healing is a complex and highly regulated process that initiates at the time of physical injury and continues till the healing process (Velnar *et al*., 2009). The objective of wound healing is to heal wounds in the shortest time period with least pain that will improve the quality of the patient's life. There are many studies conducted on antioxidant treatment methods to speed up the wound-healing process. Many therapeutic effects of NAC, including mucolytic, antioxidant, anti-aging, and antitumor effects, have been reported (Šalamon *et al*., 2019). In this study the therapeutic effects of a NAC supplementation for a short period of time on skin wound healing in rats were investigated. The NAC treatment promoted an improvement of wound healing by reducing the oxidative stress, improving wound closure with no significant effect on biochemical parameters. In this study, significant wound closure was observed in NAC treated rats. Increased wound contraction in treated rats might be a result of the enhanced activity of fibroblasts in the treated rats as after histological analysis we find few fibroblast as compared to burn groups that indicate that the tissue is still under healing process.

Burn depth in the skin is categorized as superficial, superficial partial-thickness, deep partial-thickness, full-thickness and subdermal burns (Meyerholz *et al*., 2009). In this study, we mainly focus on superficial partial-thickness burns so we analyzed the tissue parameters through recording epidermal morphological and biochemical changes. Microscopic examination of healed tissue sections revealed that the tissue regeneration was much faster in the NAC treated group compared to control wounds. H and E staining results show less infiltration of lymphocytes, enhanced proliferation of fibroblasts as a result of NAC treatment compared with control rats. Significant increase in hydroxyproline content in the NAC treated group revealed the enhanced migration of fibroblast cells and collagen synthesis during the wound healing process. The decreased collagen content in burn rats may be a result of inflammation, which inhibits fibroblast proliferation and leads to delayed healing. Hydroxyproline is a non-essential amino acid found in collagen. Previous studies show that the increased while its decreased level leads to poor wound-healing (Chen *et al*., 2012).

We analyzed the hematological and biochemical changes in the burn and treated animals, levels of these parameters are very important in animal care. These parameters are helpful for making inference about given therapy as these parameters tell us how the body is responding to the thermal injury. Our results show non-significant differences in the treatment group compared with control group which indicated that oral administration of NAC did not alter these parameters.

In thermal burns, excessive production of free radicals recruits inflammatory cells at the injury site which may lead to endothelial dysfunction (Mittal *et al*., 2014). However, this excessive production of free radicals can be minimized with help of antioxidants, such as SOD, catalase and glutathione. Antioxidants reduce oxidative stress and accelerate burn wound healing process (Comino-Sanz *et al*., 2021). So it is necessary to estimate these antioxidants in healed tissue. Significant alterations in the antioxidant profile, combined with elevated levels of MDA, which is a marker of fatty acid oxidation, may lead to impaired wound healing in rats (Bilgen *et al*., 2019). In the present study, the levels of SOD, CAT, GSH and MDA were quantified in healed tissues. The results demonstrated that treatment with NAC significantly increased the activity of SOD, CAT, GSH, and reduced the levels of MDA compared with the burn group.

The process of regeneration is dependent on proliferation of fibroblast and secretion a number of growth factors and cytokines (Zarei and Soleimaninejad, 2018); however, proliferation and secretion of these factors diminishes with oxidative stress (Guo and Dipietro, 2010). Beneficial effect of NAC treatment on wound regeneration was also confirmed by PCR and ELISA results. NAC treatment also increases the expression of IGF-1, VEGF and PCNA as compared to burn group this indicate that NAC favor the tissue regeneration by reducing the oxidative stress. VEGF has been shown to increase vascular permeability, promotes angiogenesis, attracts monocytes to the site of injury and is also involved in neovascularization (DiPietro, 2016). NAC as antioxidant reduce the oxidative stress and it is confirmed by decreased expression of HIF-1, HIF-1, is an important transcription factor in the cellular response to hypoxia (Li *et al*., 2019).

Our results showed a significant increase in SDF-1 and CXCR-4 expression in NAC treated group as compared to burn group. SDF-1/CXCR4 axis is involved in homing, engraftment, neo-vascularization and cell proliferation

(Cencioni *et al*., 2012). The results of the present study are in accordance with other studies that demonstrated significant role of SDF-1/CXCR4 signaling in wound repair and regeneration (Chen *et al*., 2021). Our results also shows that NAC resulted in decreased expression of caspase-3, P53 and CRP.

CONCULSION

We have demonstrated that NAC administration can significantly enhance wound healing. This study showed that NAC is totally safe to be use in rats and in human as it is helpful in wound repair.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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