

## **EFFECT OF N-ACETYL CYSTEINE ON WOUND HEALING**

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### **Summary**

N- acetyl cysteine was evaluated for its wound healing activity in ether anaesthetized albino rats by using incision, excision wound models. Significant increase in skin breaking strength, granuloma breaking strength, wound contraction and decreased in epithelization period was observed. A supportive study made on granuloma tissue to estimate the levels of superoxide-dismutase, catalase, glutathione, vitamin c and lipid peroxidation are recorded and a significant increase in the level of these antioxidant enzymes and decrease in the levels of lipid peroxidation was observed. Enhanced wound healing activity may be due to free radical scavenging action of the NAC and the enhanced level of antioxidant enzymes in granuloma tissue. Better collagenation may be because of improved antioxidant studies.

**Keywords:** Wound healing activity, free radical scavenging activity, N-acetyl cysteine, antioxidants.

### **Introduction**

A wound is a disruption of tissue integrity that is typically associated with loss of substance [1]. Each year nearly 25 million persons are affected with various acute and chronic wound and are in need of intervention. Almost 11 million lacerations are treated annually in emergency departments in the US, and this number is likely growing [2]. Signs of infection include red skin around the wound, discharge containing pus, swelling, warmth, foul odor, and fever [3]. Deeper injuries to the muscle tissue, skeletal tissue or the inner organs are defined as complicated wounds [1]. Proper healing of wounds is essential for the restoration of disrupted anatomical continuity and functional status of the skin.

In acute wounds a temporary increase in oxidants is observed. Thus, the antioxidant defence is based on the gradual detoxification of oxidants and causes a state of redox homeostasis. However, in chronic wounds the detoxification process is hindered due to uncontrolled production of ROS and RNS during the inflammatory phase as these are not quenched by antioxidants [4]. As a result homeostasis is shifted and wounds remain stagnant [5].

In acute as well as in chronic wounds, the enzymatic antioxidants decreases and leads to depletion of non enzymatic antioxidants due to high oxidative stress [6,7,8]. This effect is more pronounced in chronic wounds than in acute wounds. Thus, supplementation of wounds with antioxidants prevent oxidative damage of cells and enhances healing.

As oxidative stress is an integral part of wound healing leading to depletion of enzymatic and nonenzymatic antioxidants. The present study was designed to study the effect of NAC, a natural antioxidant on wound healing by using acute wound models.

N-acetyl-L-cysteine is a precursor of glutathione [9]. The antioxidant supplement, n- acetylcysteine, is a sulfur-based amino acid and precursor of glutathione, a natural antioxidant enzyme produced in the body to fight free-radical activity.

Cysteine is found in beta-keratin, the main protein in nails, skin and hair. It helps maintain a healthy, youthful appearance by encouraging collagen production and skin elasticity. N-acetyl cysteine (NAC) is a form of the amino acid **cysteine** that is most easily absorbed from supplements. NAC is effective in the prevention and/or treatment of cancer, heavy metal poisoning, smoker's cough, bronchitis, heart disease, cystic fibrosis, acetaminophen poisoning, and septic shock. Its detoxifying effects may helps to enhance the benefits of regular exercise by protecting the body from oxidative stress.

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## **Materials and Methods**

### ***Treatment Schedule***

NAC at a dose of 250 mg/kg was dissolved in water and administered daily by oral gavage to the experimental animals with wounds until they were cured.

### ***Wound Healing Activity***

The wound healing activity was evaluated by using excision and incision models.

### ***Excision Wound Model***

An impression was made on the dorsal interscapular region 5mm away from the ears using a circular seal of 2.5 cms diameter [10]. Full thickness skin from the demarked area was excised to get a wound area of approximately 500mm. After achieving haemostasis the animal was placed in its individual cage. On alternate days, the physical attributes of healing namely, wound closure (contraction), epithelization time and scar features were studied in this model.

Wound area was calculated by counting the number of squares of the retraced wound area on a graph paper from the polythene sheet. The degree of wound healing was calculated as percentage closure of the wound area from the original wound using the following formula:

$$\text{Percentage closure} = 1 - A_d / A_o \times 100$$

Where  $A_o$  = wound area on day zero and

$A_d$  = wound area on corresponding days.

### ***Incision Wound Model***

Two parallel straight incisions of 6 cms were made through the entire thickness of skin on either side of the vertebral column with the help of a sharp surgical blade [11] and closed by means of four interrupted sutures with zero black silk thread placed at equidistant points about 1 cm apart. The sutures were removed on eighth post wounding day and breaking strength was determined on the eleventh post wounding day by continuous, constant water flow technique of Lee, 1968. The anaesthetized animal was secured to the operation table in its natural position and lines were drawn on either side of the incision wound. Two allies forceps were firmly applied on the lines, facing each other in the same plane. The forceps on one side is hooked to a metal rod which was fixed firmly to the operation table, while the other to a light polythene container through a string run over a pulley. Then water was allowed to flow under a constant rate in to the polythene container so as to build a gradual force to disrupt the wound. The flow of water is regulated by means of an occlusion clamp on the rubber tubing connected to the water reservoir kept at a suitable height. As soon as the gaping of the wound is observed the water flow was cut off and the volume of water in the polythene container measured and converted to the corresponding weight.

### ***Statistical Analysis***

Results were subjected to student's T test. In all tests, the criterion for statistical significance was  $p < 0.05$ .

### ***Results***

In incision wound model, significant increase was observed in the skin tensile strength of NAC treated group when compared to control animals (Table- 1). In studies using excision wound model, animals treated with NAC showed a significant decrease in epithelization period as evidenced by shorter period for fall of escher as compared to control. The drug extract also facilitated the rate of wound contraction significantly in NAC treated animals when compared to control animals (Table- 2). Studies on antioxidant enzymes revealed that the NAC treated animals of incision wound model showed significant increase in the levels of superoxide dismutase, catalase, vitamin c and glutathione, the powerful antioxidant enzymes of the body that are known to quench superoxide radicals and there is a subsequent decrease in lipid peroxidation levels on 2<sup>nd</sup> day and 7<sup>th</sup> day of treatment (Table- 3 and Table- 4). Studies on antioxidant enzymes revealed that the NAC treated animals of excision wound model showed significant increase in the levels of superoxide dismutase, catalase, vitamin c and glutathione, the powerful antioxidant enzymes of the body that

are known to quench superoxide radicals and there is a subsequent decrease in lipid peroxidation levels on 2<sup>nd</sup> day and 20<sup>th</sup> day of treatment (Table- 5 and Table- 6).

Table-1: Effect Of N-Acetyl Cysteine On Tensile Strength In Incision Wound Model

Group	Tensile strength(g $\pm$ sem)
Control	240 $\pm$ 19.579
Nac	370.75 $\pm$ 7.454***

Values are expressed as mean  $\pm$  SEM; n=5 in each group

\*\*\* (p<0.001) vs control group

Table-2: Effect Of N-Acetyl Cysteine On Excision Wound Model

Percentage of closure of excision wound area					
Treatment	Day 4	Day 8	Day 12	Day 16	Epithelialization in days
S Control	16.92 $\pm$ 1.431	49.6 $\pm$ 3.586	63.94 $\pm$ 1.214	85.54 $\pm$ 1.784	20 $\pm$ 0.3162
Nac	36.8 $\pm$ 1.881***	68.4 $\pm$ 1.631***	91.4 $\pm$ 0.6***	96.1 $\pm$ 1.364***	15 $\pm$ 0.3162***

Values are expressed as mean  $\pm$  SEM; n=5 in each group; \*\*\* (p<0.001) vs control group

TABLE-3: Effect of N-Acetyl Cysteine on Enzymatic, Nonenzymatic antioxidants and on Lipidperoxidation in incision wound model on second day of treatment

GROUP	SOD (IU / ml)	CATALASE ( $\mu$ moles /mg/ min)	GSH (mg/dl)	VIT C (mg / dl)	LIPID PEROXIDATION (n mols / ml/ hr)
INCISION CONTROL	7.6 $\pm$ 1.122	0.4112 $\pm$ 0.005490	624.6 $\pm$ 40.662	0.0438 $\pm$ 0.006829	3060 $\pm$ 337.05
INCISION TREATED	35 $\pm$ 4.370***	1.489 $\pm$ 0.02378***	889.6 $\pm$ 45.476**	1.900 $\pm$ 0.1134***	2820 $\pm$ 525.74

Values are expressed as mean  $\pm$  SEM; n=5 in each group;\*\* Indicates( P<0.01),\*\*\* Indicates (p<0.001) vs control group

Table 4: Effect of N-Acetylcysteine on Enzymatic, Nonenzymatic Antioxidants and on Lipid Peroxidation in incision Wound Model on Seventh day of treatment

Group	SOD (IU /ml)	Catalase ( $\mu$ moles/mg/ min)	GSH (mg/dl)	Vit C (mg / dl)	Lipid Peroxidation (n mols / ml/ hr)
Incision control	17.8 $\pm$ 0.8602	0.4762 $\pm$ 0.02357	716 $\pm$ 29.104	0.565 $\pm$ 0.2003	2960 $\pm$ 587.88
Incision treated	85 $\pm$ 1095***	8.014 $\pm$ 0.4839***	1024 $\pm$ 49.721***	3.86 $\pm$ 0.4501***	1317.2 $\pm$ 50.856*

Values are expressed as mean  $\pm$  SEM; n=5 in each group\* indicates (p < 0.05), \*\* Indicates (P<0.01) & \*\*\* Indicates (p<0.001) vs control group

Table-5: Effect of N-Acetyl Cysteine on Enzymatic, Nonenzymatic Antioxidants and on Lipid Peroxidation in Excision Wound Model on Second day of treatment

GSH (MG/DL)	VIT C (MG / DL)	GROUP	SOD (IU / ML)	CATALASE ( $\mu$ MOLES /MG/ MIN)	LIPID PEROXIDATION (N MOLLS / ML/ HR)
212.60 $\pm$ 22.469	0.04440 $\pm$ 0.004118	EXCISION CONTROL	6.8 $\pm$ 0.8602	0.4674 $\pm$ 0.04519	4520.00 $\pm$ 611.06
569.80 $\pm$ 33.192***	0.5576 $\pm$ 0.02417***	EXCISION TREATED	31.2 $\pm$ 3.089***	1.819 $\pm$ 0.05864***	740.00 $\pm$ 40.000***

Values are expressed as mean  $\pm$  SEM; n=5 in each group; \*\*\* Indicates (p<0.001) vs control group

Table-6: Effect of N-Acetyl Cysteine on Enzymatic, Nonenzymatic Antioxidants and on Lipid Peroxidation in excision wound model on twentieth day of treatment

Group	SOD (IU / ml)	CATALASE ( $\mu$ moles /mg/ min)	GSH (mg/dl)	VIT C (mg / dl)	Lipid Peroxidation (n mols / ml/ hr)
Excision control	18.6 $\pm$ 0.400	0.5054 $\pm$ 0.06313	339.00 $\pm$ 8.637	0.259 $\pm$ 0.02077	3220.00 $\pm$ 571.37
Excision treated	77.4 $\pm$ 4.697***	2.208 $\pm$ 0.3044***	776.5 $\pm$ 12.143***	1.200 $\pm$ 0.1140***	560.00 $\pm$ 81.240***

Values are expressed as mean  $\pm$  SEM; n=5 in each group; \*\*\* Indicates (p<0.001) vs control group

### Discussion

Wound healing is a fundamental response to tissue injury that results in restoration of tissue integrity, which is due to synthesis of connective tissue matrix [12]. Collagen is produced by fibroblasts and assists the wound in gaining the tensile strength during wound repair [13]. Collagen is a major protein of extra cellular matrix and is the component that ultimately contributes to wound strength [12].

Increase in breaking strength of granulation tissue of NAC treated animals indicates the enhanced collagen migration by increased cross linking (Table- 1).

Wound contraction is defined as the centripetal movement of the edges of a full thickness wound in order to facilitate closure of the defect [14]. The progression of wound healing is judged by the periodic assessment of contraction of excision wounds [15]. Wound healing was faster in NAC treated animals when compared to control (Table- 2).

In the present study SOD levels were found to be significantly increased by the NAC treatment when compared to control (Table- 3), (Table- 4), (Table- 5), (Table- 6). During the initial phase of wound healing, immune cells are rushed to the wound site to protect against harmful invaders. They use free radicals to fight bacteria and to dispose of dead tissue. Once the free radicals have accomplished their job however, they must be neutralized so the actual healing process begins. SOD and other antioxidants such as vitamins C and D stop the free radical oxidation process and promote the healing and repair process, itself. Injury depletes SOD and other antioxidants.

Catalase is a haem-containing enzyme that catalyzes the dismutation of hydrogen peroxide into water and oxygen. The enzyme is found in all aerobic eukaryotes and important in the removal of hydrogen peroxide generated in peroxisomes by oxidases, involved in – oxidation of fatty acids [16]. SOD, CAT enzymes are important scavengers of superoxide ion [17]. In the present study catalase levels were significantly increased in NAC treated group when compared to control group (Table- 3), (Table- 4), (Table- 5) and (Table- 6).

In the present study GSH levels were significantly increased by the NAC treatment when compared to control group (Table- ), (Table- 4), (Table- 5), (Table- 6), Glutathione (GSH) is present in mast cells, where it functions as an antioxidant protecting cells from toxic effects of ROS. GSH is a tripeptide comprised of glutamate, cysteine, and glycine (Glu-Cys-Gly), present in mast cells, whose antioxidant function is facilitated by the sulphhydryl group of cysteine, where it functions as an antioxidant protecting cells from toxic effects of ROS. GSH can minimize the effects of oxidative stress and accelerate wound healing by increasing the contraction capacity of fibroblasts and preventing keratinocytes from apoptosis [18].

Online with this in the present study also vitamin C levels were significantly increased in NAC treated group when compared to control (Table- 3), (Table- 4), (Table- 5) and (Table- 6), Vitamin C is crucial for the proper function of the enzyme procollagen hydroxylase which produces collagen, the primary constituent of the granulation tissue that heals a wound and the key component in blood vessel walls.

In the present study lipid peroxidation levels were significantly decreased when compared to control (Table-3), (Table- 4), (Table- 5) and (Table- 6). Lipid peroxidation is complex process occurring in aerobic cells and reflects the interaction between molecular oxygen and polyunsaturated fatty acids. This involves formation and propagation of lipid radicals (L), uptake of oxygen, rearrangement of double bonds, generation of lipid alkoxy (LO), lipid peroxy (LOO) radicals, lipid

hydroperoxides (LOOH) as well as variety of degradation products [19]. By products of lipid peroxidation cause marked alteration in the structural integrity and function of cell membranes.

### **Conclusion**

The results of the present study reveal that NAC fastens the wound healing in incision and excision models by increasing enzymatic antioxidants such as SOD and catalase, which scavenges superoxide radicals and H<sub>2</sub>O<sub>2</sub> respectively. SOD enhances collagen and new tissue to grow and reduces swelling, where as catalase increases the migration and proliferation of keratinocytes by preventing H<sub>2</sub>O<sub>2</sub> induced inhibition of proliferation and migration of keratinocytes.

NAC significantly increases Non enzymatic antioxidants such as vitamin C and GSH. Vitamin C is essential for the proper functioning of procollagen hydroxylase a key enzyme in the synthesis of collagen. NAC, induced increase in vitamin C levels increases the formation of collagen a major component of wound healing.

Glutathione minimizes the effects of oxidative stress and accelerates wound healing by increasing the contraction capacity of fibroblasts and prevents keratinocytes from apoptosis.

NAC by strengthening enzymatic (SOD and CAT) and non-enzymatic antioxidants (vitamin C and GSH), decreases lipid peroxidation and limits the oxidative stress induced tissue damage, there by fastens the wound healing.

In conclusion, NAC decreases oxidative stress by increasing enzymatic (SOD and CAT) and non-enzymatic (vitamin C, GSH) antioxidants and thus fastens wound healing by increasing collagen synthesis and proliferation and migration of keratinocytes.

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