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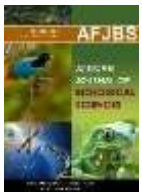

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TITLE:

"ROLE OF OXIDATIVE STRESS AND DIMINISHED GLUTATHIONE IN COPD: BASIS OF N-ACETYLCYSTEINE AS AN ANTIOXIDANT AND ANTI-INFLAMMATORY AGENT."

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ABSTRACT

Chronic obstructive pulmonary disease (COPD) is characterized by airway obstruction with episodes of symptom exacerbation that contributes to the progression of the disease and progressive loss of lung function. The role of increase in oxidative stress, inflammation and marked reduction in the levels of endogenous antioxidants such as reduced glutathione (GSH) and lung GSH biosynthesizing enzymes is involved. GSH is one of the most important antioxidant defence systems in lung cells and is accountable for maintaining cellular redox status in endothelial cells, in maintaining intracellular GSH/Glutathione oxidized (GSSG) homeostasis. Level of Reactive Oxygen Species (ROS) in the lung is progressively increased in COPD patients. Reactive Oxygen Species released as a result of COPD may enhance the likelihood of a patient developing cardiovascular disease, diabetes and osteoporosis. As per the study of the Burden of Obstructive Lung Disease (BOLD) its global prevalence is of 10.1% and COPD is estimated to be the third leading cause of death.
N-acetylcysteine possesses the sulfhydryl group responsible for antioxidant effects, directly scavenges ROS. Thus, it may prevent tissue damage to various organs by scavenging oxygen free radicals (e.g., superoxides). Researchers have shown higher NAC daily produces control on the activation of inflammatory factor like Nuclear Factor - NF-kb. Further, it was shown to produce positive effect on nuclear erythroid 2–related factor–2 transcription factor that has a crucial role as regulator of cellular redox status. Thus, NAC shows potential beneficial antioxidant and anti-inflammatory effect in COPD.

Keywords: Chronic Obstructive Pulmonary Disease, Glutathione, oxidative stress, N-Acetylcysteine, antioxidant, anti-inflammatory

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INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is a progressive disease characterized by long-term breathing problems, "air flow resistance that is not reversible"; a major cause of disability [1,2]. As stated by World Health Organization (WHO), COPD is occasionally called by terminology such as emphysema or chronic bronchitis. Chronic bronchitis and emphysema are older terms used for different types of COPD [1,2]; 'emphysema' an anatomically distinct entity associated with enlarged and distorted lung alveoli; whereas it differentiates from Chronic bronchitis, a clinical entity associated with disease of small bronchioles with chronic airflow obstruction, chronic cough and marked expectoration. COPD is the fourth leading cause of death in the United States [1]. Common signs and symptoms are cough with mucus production (Phlegm); shortness of breath, especially with physical activity leading to limited daily activity; wheezing sound on breathing; chest tightness; and more likely patients of COPD may often suffer colds or other respiratory infections such as the flu, or influenza. As stated by World Health Organization (WHO) [1], the main difference between asthma is the airway obstruction which is reversible with inhaled medicines, whereas in contrast to COPD; it is mostly fixed. COPD only affects adults and usually becomes worse with time.

Risk factors for chronic respiratory diseases include **tobacco smoking** (including second-hand smoke), indoor air pollution (such as biomass fuel used for cooking and heating), outdoor air pollution, occupational dusts and chemicals (vapours, irritants, and fumes); or allergens. Hence, COPD can be prevented by reducing or avoiding exposure to these risk factors [4,5]. As stated by Vogelmeier (2017), the WHO Global Alliance against CRDs (GARD) vision is "a world in which all people breathe freely" [4,5]. COPD claims the lives of over 3 million people every year, as per WHO, and moreover, tobacco smoking accounts for over 70% of COPD cases in high-income countries, whereas, in low-and-middle-income-countries tobacco smoking accounts for 30–40% of COPD cases with household air pollution being the other major risk factor [4,5]. As per WHO, there is an estimated 392 million people living with COPD and three quarters of them live in low- and middle-income countries. As predicted by World Health Organization (WHO) COPD will become the third leading cause of death worldwide by 2030 [1]. People living with COPD face a greater risk of developing lung cancer, cardiovascular diseases, and type 2 diabetes. The COVID-19 pandemic has further underscored the challenges in accessing healthcare for these individuals. Quitting smoking will not only reduce the risk of COPD but also significantly reduces the risk of these

severe coexisting conditions [4,5]. Rarely, a genetic condition called alpha-1 antitrypsin (AAT) deficiency may play a role in causing COPD. People who have this condition have low blood levels of alpha-1 antitrypsin (AAT), a protein made in the liver [4]. However, therapy and lifestyle modifications can help the patient feel better, stay more active, and slow the progress of the disease. Smoking cessation treatment should be integrated into the management of COPD [2]. Chronic obstructive pulmonary disease is confirmed by spirometry test, whereas the symptoms can be treated by medications and physical treatments. In patients with COPD those who smoke tobacco, the most effective treatment available is cessation of smoking, which can slow down the progression of the disease and decrease COPD-related deaths. In specific cases, people may benefit from using inhaled corticosteroid medicines [4,5,6]. Several researchers [1-3] have demonstrated that oxidative stress is increased further, and depletion of levels of Glutathione (GSH) during severe COPD exacerbations. N-Acetyl-L-Cysteine (NAC) is a precursor of GSH and acts as free oxygen radical scavengers.

As stated by numerous research studies [6-9] signifies the incidence of increased oxidative stress and decreased antioxidants such as glutathione in patients with COPD. People with COPD and smokers without COPD tend to have lower glutathione levels, which correlate with worse lung function. Hence, the role of oxidative stress and diminished glutathione in COPD is under current research area. Therefore, the present review study aims to provide a comprehensive overview of the role of oxidative stress, particularly COPD, and to focus on the potential role of N-Acetyl-L-Cysteine (NAC), a precursor of GSH, as an adjunct to standard therapy for the treatment of COPD; as Acetylcysteine is an antioxidant and anti-inflammatory agent.

CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD): AN OVERVIEW

As stated by report [2] from Global Strategy for the Diagnosis, Management, And Prevention Of Chronic Obstructive Pulmonary Disease (2018) [2], "Chronic Obstructive Pulmonary Disease (COPD) is a common, preventable and treatable disease that is characterized by persistent respiratory symptoms and airflow limitation that is due to airway and/or alveolar abnormalities usually caused by significant exposure to noxious particles or gases". Globally, the COPD burden is projected to increase in coming decades because of continued exposure to COPD risk factors and aging of the population.

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality in patients older than 40 years of age [7], and it is projected to become the fourth leading

cause of premature death by 2040 [5-7]. It has a morbidity of approximately 10% in adults aged 40 and above, increasing with age and approximately 86–98% of COPD patients suffer from at least one comorbid condition [7]. Researchers [7] also stated that there is interaction between Cardiovascular Diseases (CVD) and COPD involves several additional biological processes which includes hypoxia, systemic inflammation, endothelial dysfunction, increased platelet reactivity and/or arterial stiffness [7]. Therefore, as the study indicated [7] the pathophysiology and inflammation are interlinked between COPD and CVDs, the patients of COPD should be assessed for cardiac comorbidities.

Patients with COPD cover a higher risk for other health problems (Murray & Lopez, 1997) [1,2,7] as mentioned below:

Risk Factors for COPD and Co-Morbidities:

1) Endogenous and Exogenous pro-oxidants - Tobacco smoking (including second-hand smoke)
2) Indoor air pollution such as biomass fuel used for cooking and heating
3) Outdoor air pollution
4) Occupational dusts and chemicals such as vapours, irritants, and fumes; or by breathing in second-hand smoke or dusts.
5) Allergens
6) Occupational risks
7) Respiratory system problems such as Lung cancer; Respiratory infections such as flu or pneumonia
8) Osteoporosis – weak muscles and brittle bones
9) Central nervous system conditions such as Anxiety, Depression
10) Metabolic syndrome such as Diabetes
11) Aging Populations
12) Nutrition deficiency
13) Socio-economic status of individuals

Cardiovascular Comorbidities [7]

1) Heart Failure (HF)
2) Ischemic Heart Disease (IHD)
3) Coronary Artery Disease (CAD)
4) Cardiac Dysrhythmia
5) Systemic Hypertension
6) Stroke
7) Pulmonary and Peripheral Vascular Disease (PVD)

History and Prevalence of COPD:

The evolution of knowledge concerning COPD and its components – emphysema, chronic bronchitis, and asthmatic bronchitis – covers 200 years. The foundation of modern Pulmonary Function Test (PFT) is since 1846 A.D., when John Hutchinson invented the spirometry. He coined the term Expiratory ‘Vital Capacity’ [10]. The stethoscope and

spirometer became important early tools in diagnosis and assessment. Spirometry remains the most effective means of identification and assessment of the course of COPD and responses to therapy. The spirometry is a key to the diagnosis and management of COPD, yet its use is still poorly applied to the diagnosis and management of COPD in most locations in the world today. Though spirometry is an old procedure, simple to use and inexpensive but highly significant in health care [10]. The Burden of Obstructive Lung Disease (BOLD) study found a global prevalence of 10.1%. In Europe, COPD is ranked as the third most common cause of death (Pauwels et al 2001) [2].

ROLE OF REACTIVE OXYGEN SPECIES: SIGNIFICANCE OF GLUTATHIONE

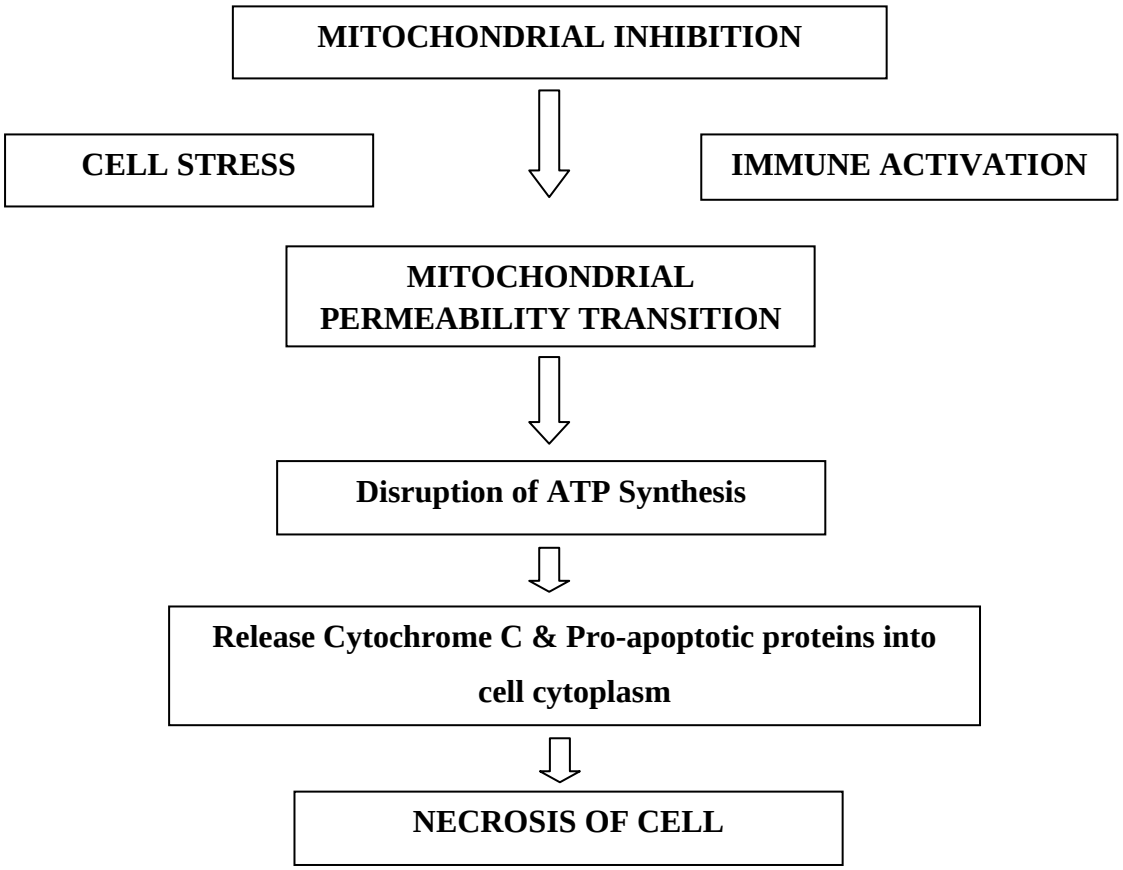
Glutathione (GSH), the most abundant mammalian antioxidant, is mainly responsible for maintaining cellular redox status in endothelial cells, in maintaining intracellular GSH/Glutathione oxidized (GSSG) homeostasis, hence represents one of the most important antioxidant defence systems in lung cells [3]. The role of increase in oxidative stress, inflammation & marked reduction in the levels of endogenous antioxidants such as Reduced Glutathione (GSH) & Lung Glutathione biosynthesizing enzymes are significant in patients with COPD [14,15]. Research on Oxidative Stress, indicated that oxidative stress occurred in response to the production of cellular pro-oxidant Reactive Oxygen Species (ROS) & Reactive Nitrogen Species (RNS), during endogenous metabolic reactions [1, 2, 16].

As stated by researchers [3], based on a pulmonary and extrapulmonary inflammation with increase also in reactive oxygen species (ROS). Oxidants have long been recognized to have an important role in the pathogenesis of COPD [3, 4, 10–12], because, besides a direct lung damage, they also enable the activation of transcription factors like nuclear factor NF-kB with consequent production of inflammatory proteins and damage of antiproteases [13].

To counter the oxidative stress and cellular damage, several antioxidant enzymes have been developed including Superoxide Dismutase (SOD), Glutathione Peroxidase and Catalase, that detoxify the Reactive Oxygen Species & the cells contained endogenous antioxidants that scavenge the free radicals and reduces the cellular damage of which Glutathione plays a major role, as depicted in figure no. 1. With the discovery of Superoxide Dismutase strengthened the theory of free radical mechanism of cell injury [16]. GSH is mainly responsible for maintaining cellular redox status in endothelial cells, in maintaining intracellular GSH/Glutathione Oxidized (GSSG) homeostasis, hence represents one of the most important antioxidant defence systems in lung cells [16].

The regulation of production of GSH is at the substrate level from cysteine as depicted in figure no. 2. L-cysteine is synthesized from homocysteine via the trans-sulfuration pathway [19]. Glutathione is key cofactor for glutathione S-transferase conjugation reaction in mammalians. The synthesis of glutathione is further catalyzed by essential enzyme Glutamate Cysteine Ligase (GCL) [17]. Orally administered NAC i.e. N-acetyl derivative of the naturally occurring amino acid L-cysteine; undergoes deacetylation reaction, where NAC is converted into L-cysteine. L-cysteine a good source of sulfhydryl containing antioxidant is a direct precursor in the synthesis of intracellular glutathione, also; acts as free radical scavenger. Its antioxidant action is believed to originate from its ability to stimulate GSH synthesizing level and scavenging Reactive Oxygen Species (ROS) and nitrogen species [19-22].

Figure 1: Role of Mitochondria in Cell Necrosis



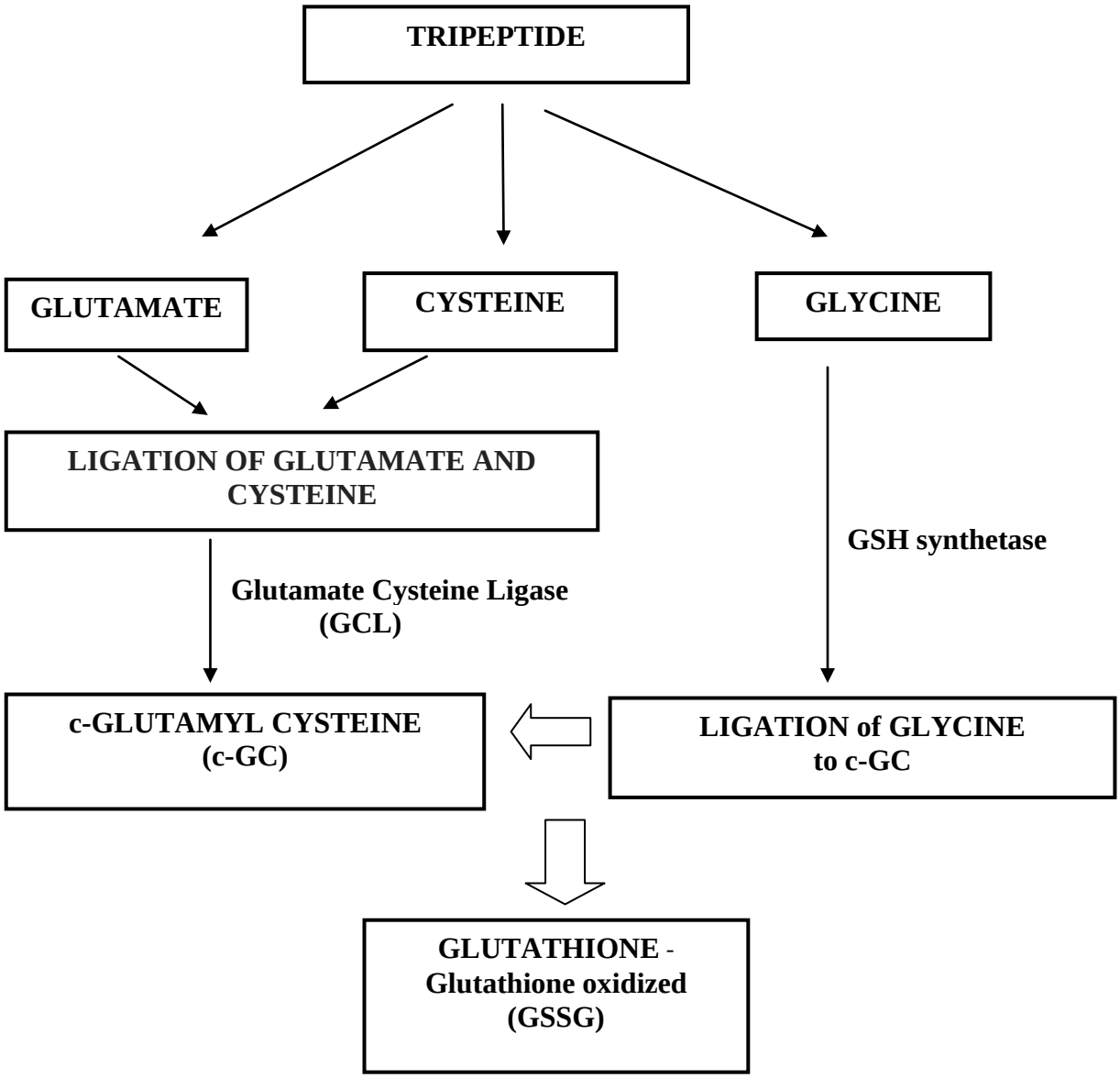
N-ACETYL-L-CYSTEINE:

ANTIOXIDANT & ANTI-INFLAMMATORY PROPERTIES

N-Acetyl-L-Cysteine (NAC) is a mucolytic agent with both antioxidant and anti-inflammatory properties, is documented to be able to reduce acute exacerbations of COPD through an antioxidant effect [7-12, 17]. It has been classically used in paracetamol overdose [18] and as well as to combat the toxicity of various substances [19, 20]. Cysteine is present in most high-protein food. N-acetylcysteine is a nutritional supplement with antioxidant and anti-inflammatory properties. After ingestion, N-acetylcysteine is metabolized into glutathione, a key antioxidant with effects throughout the body. In the lungs, glutathione deficiency (common in alcoholics) is linked to a higher risk for air way diseases. Acetylcysteine, precursor in the formation of the antioxidant glutathione (GSH) in the body; is the N-acetyl derivative of the amino-acid L-cysteine. GSH is a cofactor for glutathione S-transferase–mediated xenobiotic conjugation and is an important determinant of cellular redox status in endothelial cells, in maintaining intracellular GSH/Glutathione oxidized (GSSG) homeostasis, hence represents one of the most important antioxidant defence systems in lung cells. Formation of GSH is depicted in Figure 1 [16, 20, 22].

Antioxidant protection in the tissues and also in the respiratory tract is mainly provided by the glutathione (GSH), a redox-cycler thiol present in epithelial lining fluid, that, by transforming from the reduced to oxidized form, exerts a powerful antioxidant effect and then is converted again to the reduced form by the enzyme glutathione reductase. Hence as indicated by researchers [8, 9] a high dosage of NAC is required to exert an antioxidant action in patients with COPD [8, 9].

Figure 2: Formation of antioxidant glutathione from cysteine [8,9,22]



Acetylcysteine enhances the effects of nitric oxide (NO) by combining with NO to form S-nitrosothiol, a potent and biologically stable vasodilator. Acetylcysteine may thus compete with the superoxide radical for NO and thus prevent the formation of a damaging perioxinitrite free-radical. Thus, acetylcysteine exhibits multiple potential mechanisms of action that may limit ischemia and promote cellular repair and survival. Studies using acetylcysteine as an antioxidant for organ protection in settings of clinically-relevant heart, lung, liver, and renal ischemia are intriguing and continue to be pursued [8,9,22].

GSH is mainly responsible for maintaining cellular redox status in endothelial cells, in maintaining intracellular GSH/Glutathione oxidized (GSSG) homeostasis, hence represents one of the most important antioxidant defence systems in lung cells [9, 3]. High dose oral N-Acetyl-L-Cysteine (NAC) is deacetylated into L-cysteine which is a direct precursor in the synthesis of intracellular GSH, also a good source of sulfhydryl containing antioxidant; acts directly as free radical scavenger. Its antioxidant action is believed to originate from its ability to stimulate GSH synthesizing level and scavenging reactive oxygen species (ROS) and nitrogen species [16].

Owing to its antioxidant properties, NAC is able to maintain the redox-dependent cell-signaling and transcription, in particular the Nuclear Factor–kB (NF-kB) [24]. It has been suggested by meta-analysis studies [8] that NAC, which is a mucolytic agent with both antioxidant and anti-inflammatory properties, would be able to reduce acute exacerbations of COPD through an antioxidant effect [8]. The results of this meta-analysis study [8] clearly presented good evidence that NAC can be considered an important, safe and well-tolerated therapeutic agent to be integrated into the regular treatment regimens of patients with COPD, but also that NAC should be administered at high dose [8]. They suggested that a low dose is sufficient in providing benefit to patients with chronic bronchitis, but not to patients with COPD [8]. As stated by Mario Cazzola, et al in 2015, their metanalysis study [8] concluded with their statement that, "the strong signal that comes from our meta-analysis leads us to state that if a patient suffers from COPD with an objective confirmation of airways obstruction, NAC should be administered at a dose of ≥1200 mg per day to prevent exacerbations, while if a patient suffers from chronic bronchitis but is without airways obstruction, a regular treatment with 600 mg per day seems to be sufficient" [8]. As stated clearly by Rushworth GF, et al [25]; NAC does have some antioxidant potential due to its ability to increase intracellular glutathione, which plays an important role in the control of oxidative–carbonyl stress in COPD [25].

XENOBIOTIC ENZYME: MICROSOMAL EPOXIDE HYDROLASE (EPHX1) [26]

As indicated by researchers Zhang JQ, Zhang JQ, et al (2015) [26], that, "Microsomal Epoxide Hydrolase is a xenobiotic enzyme that detoxifies highly reactive epoxides, which are created by cigarette smoking. Study [26] have shown that NAC treatment is useful in COPD

patients with extremely slow/slow microsomal Epoxide Hydrolase (EPHX1) enzyme activity. Polymorphism in the EPHX1 gene may have a significant role in differential responses to treatment with NAC in patients with COPD". Further researchers stated [26], that the main known oxidation inhibition enzymes in the body including glutathione-S-transferase, microsomal epoxide hydrolase (EPHX1), & heme oxygenase; hydrolyze & inactivate oxygen metabolites, thus fighting against or neutralizing the oxidative damage caused by oxidative stress, & eventually maintaining the dynamic balance of oxidation/anti-oxidation in the body [26, 5-9].

CONCLUSIONS AND PROSPECTS

Hence, N-Acetyl-L-Cysteine (NAC) acts as an antioxidant by restoring the pool of intracellular reduced GSH; with consequent increase in the concentration of reduced glutathione in plasma and airways, and by the active uptake of glutathione from plasma by the lungs, which are the main place of GSH synthesis together with the liver. Further, NAC maintains the redox-dependent cell-signaling and transcription, in particular the Nuclear Factor–kB (NF-kB) critical for proinflammatory genes regulation. Thus NAC shows potential beneficial effect as a valid add-on therapy in COPD. Thus, the antioxidant and anti-inflammatory properties of high doses oral NAC can be useful adjunct in chronic bronchitis and COPD therapy, and can be further studied in our country for labeled use.

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