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The Administration of N-Acetyl Cysteine has Dramatically Suppressed the Ox-LDL Level in High Fat Diet Mice: A Novel Approaches to Prevent Atherosclerosis using Antioxidant

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Introduction & Objective: Inflammation and oxidative stress were involved in atherogenesis. Under oxidative stress state, oxidized LDL (Ox-LDL) was formed. It needs neutralized by antioxidant. N-Acetyl Cysteine (NAC) contains glutathione, the antioxidant substances. We investigated the beneficial effect of NAC in suppressing the progressivity of early atherosclerosis phases. Methods: An experimental study was conducted in Laboratory of Prof. Noerjanto Hospital Teaching Veterinary Universitas Syiah Kuala. Thirty Rattus-Novergicus Wistar strain mice were separated to 6 groups which were negative control, positive control, NAC 600mg treatment and 1200mg treatment groups for 2 weeks and 6 weeks (N, DL, DLN6-2, DLN6-6, DLN12-2, and DLN12-2 subsequently). Before giving NAC, we induced mice to be atherogenic state for 8 weeks duration using high fat diet (HFD). After completing the intervention, we collected serum Ox-LDL from samples. We use Factorial ANOVA and LSD post hoc test to analyse the data. Results: Ox-LDL level between atherogenic mice and control negative group was significantly difference (p value < 0,05). The highest and lowest Ox-LDL was found in positive control group (348,96 ng/mL) and in the 2 groups given NAC for 6 weeks (DLN6-6 and DLN12-6 with Ox-LDL level were 148,37 ng/mL and 154,18 ng/mL, respectively). Duration of treatment was significantly difference (DLN6-6 vs DLN6-2, DLN12-6 vs DLN 12-2, p value <0.05). Conclusion: NAC can suppress Ox-LDL level significantly. Six weeks NAC treatment with 600mg/day are the best in lowering Ox-LDL. No difference of Ox-LDL level between DLN6-6 and DLN12-6.

Keywords: N-Acetyl Cysteine, Atherosclerosis, Antioxidant, Oxidized LDL, Dyslipidaemia

1. Introduction

Atherosclerosis is a chronic disease involving the arteries and has become a global problem despite reductions in prevalence and mortality in recent years through control of various risk factors such as smoking, blood pressure, and diabetes. Hyperlipidemia plays an important role in the process of atherosclerosis. In addition to resulting in heart disease, hyperlipidemia also leads to diabetes mellitus, non-alcoholic liver disease, and cerebrovascular disease.(1)(2)

Prevalence of atherosclerotic heart disease reaches 98.25-101.11 per 1000 people, and there was an increase in the prevalence rate in 2015 compared to 2014. Recently, several theories have been developed regarding the involvement of inflammation and oxidative stress in the process of atherogenesis. Long-term inflammatory conditions produce excessive levels of Reactive Oxygen Species (ROS) and trigger oxidative stress.(3)(4)(5)

The oxidation process mediated by ROS and Reactive Nitrogen Species (RNS) is proatherogenic. Under oxidative stress conditions, oxidized LDL is formed, known as Oxidized LDL (Ox-LDL). Oxidative stress plays a role in atherosclerosis through the ROS pathway. Ox-LDL levels are usually low but can increase if there is any mismatch between oxidative and decomposer agents as antioxidants. Oxidative stress that occurs in atherosclerosis can be suppressed by antioxidant agents.(6)(7) Antioxidants can be broadly categorized into enzymatic and non-enzymatic types. Endogenous enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and thioredoxin reductase (TrxR), which play critical roles in the detoxification of reactive oxygen species (ROS) within cellular environments. Conversely, endogenous non-enzymatic antioxidants encompass compounds such as glutathione (GSH), uric acid, bilirubin, coenzyme Q ($CoQ/CoQH_2$), and lipoic acid, which contribute to the overall redox balance and cellular protection against oxidative stress. Additionally, exogenous non-enzymatic antioxidants include dietary antioxidants such as α-tocopherol (vitamin E), ascorbic acid (vitamin C), B vitamins, carotenoids, and polyphenols. These compounds are obtained from external sources and are essential for neutralizing oxidative damage in biological systems.(8)(9)

N-Acetyl Cysteine (NAC), which has been widely used as a mucolytic and anti-inflammatory agent in various diseases, was found to inhibit endothelial cell senescence in atherosclerosis. NAC has been stated to be a precursor of the non-enzymatic antioxidant GSH. As a precursor of GSH, NAC may be beneficial in some chronic inflammatory diseases. By acting as antioxidant, it has possibility in preventing the process of foam cell formation induced by Oxidized Low Density Lipoprotein (Ox-LDL) by its anti-inflammatory effect.(10)(11)(12)

In this study, we want to compare the Ox-LDL levels in serum between of Wistar strains Rattus Novergicus rats, identify their patterns in 2 weeks and 6 weeks of NAC medication with different doses after high fat diet administration for 8 weeks of each group. This study wants to investigates the best NAC dose and duration that can suppress the Ox-LDL levels in atherosclerosis induced mice.

2. Methodology

This research was conducted at the Laboratory of the Veterinary Teaching Hospital of Syiah Kuala University from September 2023 to February 2024. All experimental procedures involving animals were rigorously carried out in accordance with the ethical guidelines established by the Faculty of Veterinary Medicine at Syiah Kuala University. The study protocol received approval from the Veterinary Ethics Committee of the Faculty (Approval Number: 267/KEPH/X/2023), ensuring adherence to ethical standards for the treatment and care of research animals. This study is a component of a broader investigation into oxidized low-density lipoprotein (Ox-LDL), which aims to elucidate the role of oxidative stress in various pathological conditions. The findings from this research are expected to contribute to a deeper understanding of the mechanisms underlying Ox-LDL's impact on animal health and disease progression, potentially informing future therapeutic strategies.

2.1 NAC

It was administered to the animal models daily as much as 5,4 mg/kg and 10,8mg/kg of rat body weight according to the conversion formula. The negative control groups were administered standard diet daily. High fat diet was given as much as 100 g/day with 92% standard diet, 5% goat fat, 2% egg yolk, and 0.2% cholic acid

2.2. Study design

We conducted the experimental study. Thirty Rattus-Novergicus Wistar strain male rats (5 weeks old age and 75–100 grams body weight) were obtained. After acclimatization period, rats were divided into 6 groups with randomization method, which were negative control, positive control and NAC 600mg doses and 1200mg doses groups for 2 weeks and 6 weeks (N, DL, DLN6-2, DLN6-6, DLN12-2, and DLN12-2 respectively). The negative control group was given a standard diet while positive control group and NAC group were given high fat diet (HFD). Blood samples were collected at 10^{th} week and 14^{th} week from vena for measuring the Ox-LDL serum level.

2.3. Measurement of Ox-LDL level

Ox-LDL Kit was purchased from Bioenzy. The levels of Ox-LDL in serum were measured by sandwich ELISA method using Rat Ox-LDL ELISA kit (Cat. No. BZ-22188197-EB) through standard protocol, and it was measured at 450 nm wavelength.

2.4 Statistical analysis

Data analysis was conducted using SPSS software, version 25 (IBM Corporation, New York). To assess the effects of N-acetylcysteine (NAC) administration on oxidized low-density lipoprotein (Ox-LDL) levels, factorial ANOVA was performed to evaluate the interactions between dosage and duration of NAC treatment. Subsequently, one-way ANOVA was utilized to determine the specific effects of NAC on Ox-LDL levels across different treatment groups. Post hoc analysis was conducted using the Least Significant Difference (LSD) method to identify significant differences in Ox-LDL levels among the various treatment groups. This comprehensive statistical approach enabled a robust evaluation of the effects of NAC on Ox-LDL levels, accounting for both the dose-response relationship and the duration of administration.

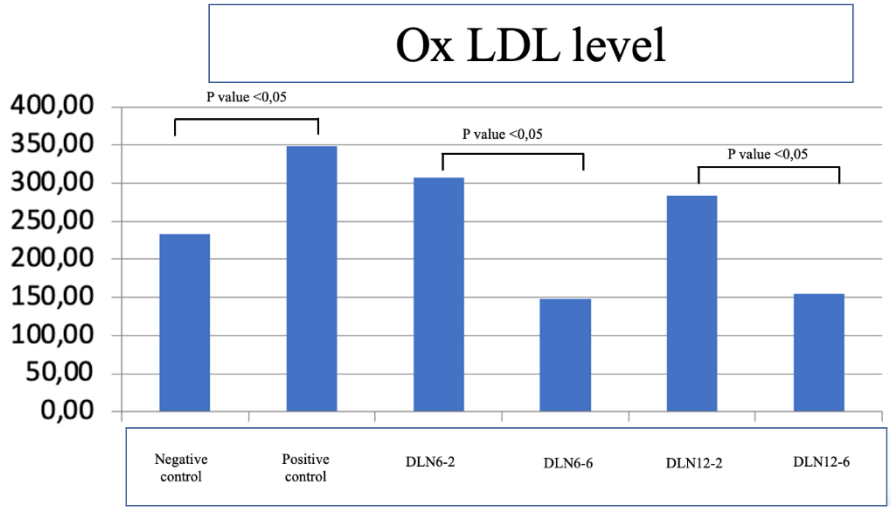
3. Results

The highest average Ox-LDL was in the positive control treatment which amounted to 348.96 ng/mL. Meanwhile, the lowest average OxLDL was in the DLN6-6 treatment which amounted to 148.37 ng/mL.

Intervention Group	Averages (Mean) OxLDL (ng/mL)
Negative Control (normal group)	233,53
Positive Control	348,96
DLN6-2	306,81
DLN6-6	148,37
DLN12-2	283,15
DLN12-6	154,18

Table 1. Level of Ox-LDL in each group

In all groups, NAC administration were a statistically significant (P value < 0.005, by LSD post hoc test) in suppressing the Ox-LDL levels but no significant differences between DLN 6-6 and DLN 12-6 arm groups. As graphic showed that the lowest level of Ox-LDL was found in 2 groups DLN6-6 and DLN12-6 with rate 148,37 ng/mL and 154,18 ng/mL respectively. At this findings, we conclude that the best level Ox-LDL can be reached in the two groups which are DLN6-6 and DLN12-6 but we could not determine which group were better. The NAC administration with doses 600 mg/day are effective in suppressing Ox-LDL with only 6 weeks of duration rather than the 12 weeks NAC treatment with same dose.



(Figure 1)

Figure 1. Level of Ox-LDL in each group showed the duration of NAC medication gave significant different between NAC 600mg group (DLN6-6 vs DLN6-2, p value <0.05) and significant different between NAC 1200mg group (DLN12-6 vs DLN 12-2, p value <0.05)

4. Discussion

The duration of atherogenic feeding for 8 weeks can increase markers of dyslipidaemia and

trigger the process of foam cell formation in male Wistar strain white rats significantly.(13) We gave the atherogenic diet to mice for 8 weeks. In this study, there was the highest level of Ox-LDL found in only dyslipidaemia group without NAC intervention (positive control) with rate achieved 348,96 ng/mL. After NAC administration, all intervention groups showed the lower Ox-LDL levels compared with control positive groups (p value < 0,005). We conclude that NAC can reduce the progressivity of dyslipidaemia by suppressing the Ox-LDL levels between high fat diet mice.

NAC, in middle 1970s, was used as an antidote for acetaminophen poisoning.(14) The protective effect of NAC in the context of acetaminophen poisoning is primarily attributed to its ability to replenish hepatic reduced glutathione (GSH) levels, which are crucial for cellular detoxification processes. GSH functions as a key non-enzymatic antioxidant, playing a significant role in neutralizing reactive oxygen species (ROS) and protecting cellular structures from oxidative damage. NAC is recognized as a vital precursor in the biosynthesis of GSH, thereby enhancing the body's antioxidant defences'.(15) Numerous studies have examined the in vivo efficacy of NAC in elevating GSH content across various tissues, both under physiological and pathological conditions. For example, in a study involving suckling piglets with acetaminophen-induced liver damage, NAC treatment significantly increased GSH levels as well as the ratio of reduced GSH to oxidized glutathione in the liver. This shift in GSH dynamics suggests a restoration of the antioxidant capacity, which is often compromised in toxicological scenarios.(10) In a model of oxidative stress induced by paraquat in rats, administration of NAC effectively mitigated the reduction of GSH levels in both the liver and brain, indicating a protective effect against oxidative damage. The ability of NAC to maintain GSH levels in critical organs underscores its potential utility in conditions characterized by oxidative stress and GSH depletion. Moreover, extensive animal studies have confirmed that both acute and chronic oral administration of NAC can significantly elevate GSH content in a variety of tissues, including the liver, kidney, skin, lungs, and brain. This broad tissue distribution and effect highlight NAC's therapeutic potential beyond acetaminophen poisoning, suggesting its applicability in a range of oxidative stress-related disorders. By enhancing GSH levels, NAC may play a crucial role in preserving cellular integrity and function, ultimately contributing to improved health outcomes in various pathological states.(14)

The atherosclerosis triad includes therapies targeting oxidative stress, aging, and inflammatory pathways and is an innovative approach to atherosclerosis management. This makes the researchers did some antioxidant approaches in managing atherosclerosis. Several studies on inhibiting the progress of atherosclerosis through inflammatory pathways, oxidative stress and aging were still ongoing.(16)

NAC has been shown to block the in vitro biotransformation of natural LDL into oxidized LDL, and attenuate the in vivo oxidation of natural LDL and ROS formation from oxidized LDL.(10)(15) In this study, between NAC intervention groups, the lowest Ox-LDL level can be achieved after 6 weeks administration of NAC. The two weeks therapy of NAC still not yet giving promising Ox-LDL levels. Between the 6 weeks NAC therapy group, no significant differences between the 600mg and 1200mg doses but the 600mg NAC therapy group had lower Ox-LDL levels than 1200 mg dose therapy group (148,37ng/mL vs 154,18 ng/mL, p value 0.752). No significant difference in level of Ox-LDL between 6 weeks NAC medication

group with 600mg/day and 1200mg/day may suggest the antioxidant effect had already worked in the plasma circulation as stated by Heriansyah et al.(17) There is a statistically significant difference between DLN6-6 and DLN6-2 (p value < 0.005) which means the 6 weeks treatment with NAC 600mg/day preserved the Ox-LDL levels in HFD mice.

NAC therapy has been shown to delay endothelial cell senescence in atherosclerosis patients and improve endothelium-dependent vasodilation in both atherosclerotic and non-atherosclerotic individuals. Additionally, NAC prevents foam cell formation induced by Ox-LDL and suppresses the matrix-degrading capacity of foam cells, thus preserving the structural integrity of arterial walls. In preclinical studies involving young apolipoprotein E-deficient (apoE-/-) and low-density lipoprotein receptor-deficient (LDLR-/-) mice on an atherogenic HFD, NAC significantly inhibited the progression of atherosclerosis. These findings highlighted NAC's therapeutic potential in enhancing vascular health and mitigating cardiovascular risk through its antioxidant and anti-inflammatory effects.(15)

Xin Fang et al. investigated the protective effects of N-acetylcysteine (NAC) on endothelial dysfunction, proposing that its efficacy may be associated with the modulation of methylglyoxal (MG) and reduced glutathione (GSH) levels, along with the attenuation of oxidative stress. Their study demonstrated that NAC significantly inhibited atherosclerotic progression in streptozocin (STZ)-induced and high-lipid diet (HLD)-fed apolipoprotein E-deficient (ApoE-/-) diabetic mice. At 12 weeks of age, atherosclerotic plaque formation at the aortic root in diabetic mice was markedly greater than that observed in the normal diet control group. Furthermore, the lesion area in the HLD group was nearly three times larger than that in the Diabetes Mellitus (DM) only group. Importantly, 12 weeks of NAC treatment resulted in a significant reduction of atheroma development, with a nearly 50% decrease in lesion area in HLD-treated diabetic mice compared to the DM+HLD group that did not receive NAC. These findings suggest that NAC may serve as a valuable therapeutic agent in the management of atherosclerosis, particularly in diabetic contexts characterized by oxidative stress and endothelial dysfunction.(18)

A recent animal study demonstrated that N-acetylcysteine (NAC) effectively inhibits atherosclerosis progression in low-density lipoprotein receptor knockout ($LDLr^{-/-}$) mice on a high-fat diet by mitigating reactive oxygen species (ROS) overproduction and reducing serum levels of Ox-LDL and inflammatory cytokines. In hypercholesterolemic rabbits, NAC treatment decreased the expression of gelatinases and matrix metalloproteinase-9 (MMP-9) in foam cells, suggesting a protective effect against plaque destabilization. Additionally, atherosclerotic rabbits receiving NAC for 8 weeks showed significant reductions in ox-LDL, MMP-9, and MMP-2 levels, along with inhibited atherosclerotic formation. Apolipoprotein E knockout (ApoE KO) mice treated orally with NAC for the same duration exhibited reduced plaque collagen content and nitrotyrosine expression. These findings suggest that NAC exerts protective effects against atherosclerosis, likely via the inhibition of oxidative stress and modulation of inflammatory pathways.(19)(20)

Several studies about NAC in mice used various doses and duration. Until now, still no standard dose recommendation about NAC therapy according to animal studies. For example, Redwan et al also gave NAC therapy for 6 weeks duration and found that NAC can significantly reduce body weight of mice.(21) Another study enrolled human with

dyslipidaemia, Cui et al found that NAC more effective lowering Ox-LDL compared to placebo. The study found that the suppression effect on Ox-LDL still remains at 1 week after NAC withdrawal.(12) This findings strengthen our result in this study that the DLN12-6 were not superior than DLN6-6 treatment group. We speculated that can be happened due to the treatment had reached enough the therapeutic threshold just only with 600mg/day NAC and elevating doses to be 1200 mg/day didn't give any beneficial effect anymore.

Our study only conducted in short period study for 14 weeks with limited numbers of mice , it may be necessary to observe the effect of NAC in more samples of atherogenic mice for longer duration in the future to learn the pattern of lipid parameters reduction after antioxidant medication. We conducted this study to enrich the articles about antioxidant role in preventing atherosclerotic process. Nowadays, a lot of study published about atherosclerosis prevention using antioxidant approaches. Ashiq et al (2023) stated that oxidative stress worsen the condition of cardiovascular disease (CVD). They highlighted the oxidative stress induced by xanthin oxidase activity that can generate uric acid. Moreover, several studies proved that there were association between urate levels to CVD. (22)

5. Conclusions

Our study has showed that N-Acetyl Cysteine dramatically can inhibit the progressivity of atherosclerosis by suppressing Ox-LDL serum marker. Duration of NAC medication was significantly difference resulting Ox-LDL level in this study. Six weeks NAC medication with 600mg/day are the best choice of therapy in HFD mice in this study. The Ox-LDL had important role in early development of atherosclerosis. By its antioxidant substance, NAC provides the protective effect in preventing the atherosclerosis progressivity. We hope this study can be one of the pioneer in using antioxidant as medication for preventing atherosclerosis in high risk population affecting metabolic disorders.

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