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NLM : WG 505

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The Effect of N-Acetyl-L-Cysteine (Nac) on Plasma Adma Level and The Expression of Vcam-1 Protein on Endotelial Dysfunction in Diabetic Rats

Bulletin of Health Research
Vol. 44 No. 3, September, Pages. 147–152

Endothelial dysfunction is an early state of symptoms in a cardiovascular disease. An elevated oxidative stress plays a key role in the pathogenesis of macrovascular diabetic complication. The present study was design to evaluate the effect of NAC on plasma ADMA level and the expression of VCAM-1 protein on endothelial dysfunction in diabetic rats. Thirty male Sprague–Dawley *rats were divided into 5 groups i.e. normal rats, diabetic rats, treatment with NAC 30 mg/kgBW, NAC 56 mg/kgBW and NAC 100 mg/kgBW. Diabetic rats model was induced by intraperitonial administration of alloxan monohydrate at dose of 150 mg/kgBW, diabetes occurred on 3nd day after alloxan injection and then started treatment of* N-acetyl-L-cystein *for 28 days. ADMA plasma level was analyzed with Elisa Reader and the expression of VCAM-1 protein was evaluated by immunohistochemistry. Conclusion of this research is that treatment with NAC 30 mg/kgBW, NAC 56 mg/kgBW and NAC 100 mg/kgBW for 28 days may prevent oxidative stress indicated by the decreasing of plasma ADMA level by 45.8%: 55.75% and 65.92%, respectively and the decreasing of the expression of VCAM-1 protein on endothelial dysfunction in diabetic rat by 24%: 31.75% and 58.92%, respectively.*

Key words : (NAC), Endothelial dysfunction, ADMA, VCAM-1

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Dominant Risk Factors of Coronary Heart Disease in Indonesia

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Coronary heart disease is still a major cause of mortality and morbidity with great socio-economic impact like stroke. Coronary heart disease is preventable by early detection and risk factors control. This study aimed to identify dominant risk factors of coronary heart disease in Indonesia using National Basic Health Survey 2013 data. Data were analysed using SPSS 16 with complex sample. *A total of 722.329 respondent age ≥15 years old, consisting of 347.823 male and 374.506 female were analyzed. Prevalence of coronary heart disease was 1.5 % (95% CI 1.4–1.5). Several factors were identified as risk factors of coronary heart disease such as hypertension, mental disorder, diabetes mellitus, stroke, age ≥ 40 years old, smoking, female, low level of education, central obesity, and low level of social economic status with adjusted odds ratio range from 1.30 to 10.09. The dominant risk factors were hypertension, mental disorder, and diabetes mellitus. Promotion of healthy lifestyle and early detection since early age need to be enhanced to minimize the risk factors as well as coronary heart disease.*

Key words : coronary heart disease, risk factor, basic health survey 2013.

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Analysis of the Influence of Environmental Factors and Motivation on Job Satisfaction In Healthcare of Banjarbaru Hospital

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Efek N-Asetil-L- Sistein (NAC) terhadap Kadar Adma Plasma dan Ekspresi Protein Vcam-l pada Disfungsi Endotel Tikus Diabetes

THE EFFECT OF N-ACETYL-L-CYSTEINE (NAC) ON PLASMA ADMA LEVEL AND THE EXPRESSION OF VCAM-1 PROTEIN ON ENDOTELIAL DYSFUNCTION IN DIABETIC RATS

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Abstract

Endothelial dysfunction is an early state of symptoms in a cardiovascular disease. An elevated oxidative stress plays a key role in the pathogenesis of macrovascular diabetic complication. The present study was design to evaluate the effect of NAC on plasma ADMA level and the expression of VCAM-1 protein on endothelial dysfunction in diabetic rats. Thirty male Sprague–Dawley *rats were divided into 5 groups i.e. normal rats, diabetic rats, treatment with NAC 30 mg/kgBW, NAC 56 mg/kgBW and NAC 100 mg/kgBW. Diabetic rats model was induced by intraperitonial administration of alloxan monohydrate at dose of 150 mg/kgBW, diabetes occurred on 3nd day after alloxan injection and then started treatment of* N-acetyl-L-cystein *for 28 days. ADMA plasma level was analyzed with Elisa Reader and the expression of VCAM-1 protein was evaluated by immunohistochemistry. Conclusion of this research is that treatment with NAC 30 mg/kgBW, NAC 56 mg/kgBW and NAC 100 mg/kgBW for 28 days may prevent oxidative stress indicated by the decreasing of plasma ADMA level by 45.8%: 55.75% and 65.92%, respectively and the decreasing of the expression of VCAM-1 protein on endothelial dysfunction in diabetic rat by 24%: 31.75% and 58.92%, respectively.*

Key words : (NAC), Endothelial dysfunction, ADMA, VCAM-1

Abstrak

Disfungsi endotel merupakan tahap awal pada penyakit kardiovaskular. Peningkatan stres oksidatif berperan penting dalam patogenesis komplikasi makrovaskular pada penyakit diabetes. Penelitian ini bertujuan untuk mengevaluasi efek *N-asetil sistein* (NAC) dalam menurunkan kadar ADMA plasma dan ekspresi protein VCAM-1 pada disfungsi endotel tikus diabetes. 30 tikus jantan *strain sprague- Dawley* dibagi menjadi 5 kelompok yaitu kelompok normal, kelompok diabetes, kelompok perlakuan NAC pada dosis 30, 56 dan 100 mg/kgBB. Model tikus diabetes diperoleh dengan diinduksi aloksan monohidrat 150 mg/kgBB secara intraperitonial, kondisi diabetes terjadi pada hari ke-3 setelah induksi aloksan selanjutnya dilakukan pemberian *N-asetil sistein* selama 28 hari. Kadar ADMA plasma dianalisis dengan metode ELISA dan ekspresi protein VCAM-1 dianalisis secara imunohistokimia. Kesimpulan dari penelitian ini bahwa pemberian NAC pada dosis 30, 56 dan 100 mg/kgBB selama 28 hari dapat mencegah peningkatan stres oksidatif yang ditandai dengan menurunnya kadar ADMA plasma berturut-turut 45,80%; 55,75% dan 65,92% serta menurunnya ekspresi dari protein VCAM-1 pada disfungsi endotel tikus diabetes berturut-turut 24,00% ;31,75% dan 58,92%.

Kata kunci : ADMA, Endothelial dysfunction, NAC, VCAM-1

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease characterized by elevated blood sugar levels resulting from either a lack of insulin production or resistence to insulin.[1] WHO estimated that in 2030 prevalence of DM in Indonesia will be increasing by 21,3 million people.[2] The global figure of people with diabetes is projected to increase to 333 million in 2025 and 430 million in 2030.[1]

Diabetic Mellitus sufferers have many chance getting complications including macrovascular complications (CVD), such as atheroclerosis, stroke and myocardial infarction. CVD accounts for more than half of the mortality seen in the diabetic population and diabetes equates to an approximately threefold increased risk of myocardial infarction compared with the general population.[3,4]

Endothelial dysfunction is an early state of symptoms in cardiovascular disease. Endothelial dysfunction are characterized by alteration of homeostasis endothelium of the antihemostatic properties, vascular tone, increase of leukocyte adhesion and production of cytokines and growth factors.[5] Endothelium-derived nitric oxide (NO) is formed in the endothelium by the endothelial isoform of nitric oxide synthase (eNOS), NO is a potent endogenous vasodilator mediators play a crucial role in vascular homeostasis. A reduction in NO can result in endothelial dysfunction and in an increased risk for cardiovascular disease.[6] An elevated oxidative stress plays a key role in the pathogenesis of macrovascular diabetic complication.[1]

The most important mechanism involved in the complex series of reaction associated with accelerated atherosclerosis indiabetes is the increase in the irreversible formation and deposition of reactive advanced glycation endproducts (AGEs). The intermolecular collagen cross-linking caused by AGEs leads to diminished arterial and myocardial compliance and increased vascular stiffness, phenomena that are considered to partly explain the increase in diastolic dysfunction and systolic hypertension seen in diabetic subjects.[7] AGE interaction with receptor of advanced glycation endproducts (RAGE) is the induction of oxidative stress, leading to NF-κB activation and the induction of the endothelial expression of various cell adhesion molecules, including vascular cell adhesion molecule-1 (VCAM-1). Endothelial dysfunction is indicated by the expression of VCAM-1 protein, increasing of VCAM-1 expression promotes transendothelial migration of monocytes, thereby contributing to the formation of foam cells and initiation of atherosclerosis formation.[5]

Futhermore, reactive oxygen species (ROS), which oxidize lipids, proteins and DNA causing cellular damage and subsequent cell death.[8] One of protein which oxidized is indicated by the increasing of plasma asymmetrical dimethylarginine (ADMA) level. Increasing of ADMA level caused by increasing of regulation protein arginine methyltransferase (PRMT) expression and decreasing of dimethylarginine dimethylaminohydrolase (DDAH) activity. ADMA is a naturally occurring endogenous inhibitor of nitric oxide (NO) synthase. ADMA inhibits eNOS by competitive displacement of the physilogical substrate, L-arginine, from the enzyme.[9] ADMA reduces NO production and consequently could thus lead to endothelial dysfunction and development to cardiovascular events.[6,9]

An elevated oxidative stress that induce to increase of plasma ADMA level and the expression of VCAM-1 protein caused by imbalance between the increasing of reactive oxygen spesies (ROS) and decreasing of endogen antioxidant capacity in cell, particularly decreasing of endogen glutation antioxidant (GSH).

Endogen gluthation antioxidant can be increased by using NAC. NAC is precursor cystein and GSH.[10] NAC deacetylation become cystein, then cystein involve glutation metabolism pathway and increase intraceluler glutation Formation.[11] NAC is a source of sulfhydryl (-SH) as act antioxidant group in cells and scavenger of free radicals in direct such as superoxide (O_2•-) hydrogen peroxide (H_2O_2) and hydroxyl (OH•).[12]

The aims of this research are to determine the effect of N-acetyl-L-cysteine to decrease ADMA level and the expression of VCAM-1 protein that is marker of endothelial dysfunction in diabetic rats.

The advantage of this research is to give the scientific information of the role of NAC as antioxidant on endothelial dysfunction in the prevention the development of macrovascular diabetic complication in diabetic state.

MATERIALS AND METHODS

The present study was conducted by using the postest-only control group design.[13] The treatment of animals in this experiment was approved by Airlangga University Animal Care and Use Committee (ACUC), Surabaya, Indonesia.

Materials

N-acetyl-L-cysteine(NAC)(Sigma-Aldrich, USA), alloxan monohydrate (Sigma-Aldrich, USA), Antibody VCAM-1, sc-1504 (Santa Cruz Biotechnology,USA) and kit for immunohistochemistry (Biocare Medical, USA). ADMA Elisa Kit (Elabscience) and other reagens, such as formaldehyde, PBS, and aquadest (PT. Bratachem Ltd,Surabaya, Indonesia).

Animals

Thirty male rats of Sprague–Dawley strain, weighing between 180 – 250 g, two month age, blood glucose < 200 mg/dl. Animals were maintai ned in the climatically controlled housing facility in Animal Laboratory, Faculty of Pharmacy, Airlangga University, Indonesia for a week before the initiation of the experiment.

Experimental Protocol

Rats were divided into five groups. Rats in naive group received buffer citrat 0.1 M pH 4.5 diabetic rats model was induced by intraperitonial administration of alloxan monohydrate at dose 150 mg/kgBW, diabetes occurred on 3rd day after alloxan injection. Both the naive and diabetic groups receive drinking water, whereas the treatment groups received NAC in different dose i.e 30 mg/kgBW, 56 mg/kgBW, and 100 mg/kgBW p.o. Both NAC or drinking water administered everyday for 28 days. During the treatment, blood glucose level of naive and diabetic groups were observed with glucometer on day 14 and 28. On 29th day, all of rats were sacrificed then the plasma were collected for plasma ADMA level data and the aortic tissue section are also collected and prepared by imunohistochemestry method for measuring the expression of VCAM-1 protein. Rats were sacrificed and were taken blood and its aortic tissue then was put into a plastic bag and wrapped with paper, covered and stored in the refrigerator or directly be burnt.

Determination of blood glucose levels

Blood samples were taken from the tail vein of rat for determination of blood glucose level using one call plus glucometer®

Plasma ADMA level assay

Plasma levels of ADMA were determined by an ELISA kit.[9] It uses Competitive-ELISA as the method. The mechanism of this method is based on the interaction between antibody and antigen in sample which using enzym as label reporter.[14]

Immunohistochemistry and VCAM-1 expression scoring

Aortic tissues were collected and fixated in 10% neutral buffered formalin for 24 h at room temperature. The tissues were then embedded in paraffin wax and sections were cut at 4 μm thickness and stained with rat antibody VCAM-1, for overnight incubation at 40°C. Then, VCAM-1 expression was observed on the endothelial cell surface and calculated using Allred scoring system by combining the percentage of positive cells and intensity of the reaction product in most of the examined field.[15]

Statistical analysis

The results are expressed as means ± standard deviation ($\bar{x}$± SD) for plasma ADMA level and VCAM-1 data. Comparison between the groups were performed by using one-way analysis of variance (ANOVA), continued with Tukey's procedure for multiple comparison tests in order to obtain plasma ADMA level data. However, scoring of VCAM-1 expression were analyzed with Mann Whitney test. A value of $p < 0.05$ was considered to be significant.

RESULT

1. The effect alloxan monohidrate of blood glucose level in diabetic rat model.

As illustrated in Table 1, diabetic model was induced by alloxan monohidrate 150 mg/kgBB, shown by significantly increased in blood glucose level ($p<0.001$).

Table 1. Increasing of blood glucose level in diabetic state after induction of alloxan monohidrate at dose 150 mg/kgBB

Group	Blood glucose level (mg/dl) x̄± SD			
	0	3	14	28
Naive	108.7 ± 11.8	107.7 ± 13.8	107.8 ± 16.1	109.2 ± 20.4
DM	109.7 ± 10.6	> 600.0 ± 0.0	> 600.0 ± 0.0	594.7 ± 10.8***

***) significant differences ($p< 0.001$) vs naive group

2. The effect NAC to decrease plasma ADMA level in diabetic rat model

As illustrated in Fig. 1, in diabetic condition, elevated oxidative stress as shown by significant increasing in plasma ADMA level from 1.05 ± 0.03 to 3.58 ± 0.12 . As illusrated in Fig 2, NAC administration at different doses (30, 56, and 100 mg/kg BW) decreased plasma ADMA level by 45.8%: 55.75%, and 65.92%, respectively ($p<0.001$).

3. The effect NAC to decrease the expression of VCAM-1 protein on endothelial dysfunction in diabetic rat model

As illustrated in Fig. 3 and Table 2, staining with rat antibody VCAM-1 sc-1504 (1:100), on the expression of VCAM-1 proteinon endothelial cell surface was conducted, as shown in Fig.3, negative staining was shown in aortic tissue from naive group, as shown in panel A, positive staining was shown in dark brown (Red arrow) on endothelial cell surface in all groups induced by alloxan. The strongest expression was observed in diabetic untreated group, as shown in panel B significantly increasing from 1.87 ± 0.20 to 6.33 ± 0.55. As illustrated in Fig. 4 and Table 3, the expression of VCAM-1 protein on EC surface decreased gradually after NAC administration for 28 days, as illustrated in panel C (NAC 30 mg/kg BW), D (NAC 56 mg/kg BW), and panel E (NAC 100 mg/kg BW). The Scoring for the expression of VCAM-1 protein was determined by Allred scoring system (data was not shown). And was analyzed with Mann Whitney test showed NAC administration in the treatment group (30, 56, and 100 mg/kgBW) significantly decreasing the expression of VCAM-1 protein by 24%: 31.75% and 58.92%, respectively ($p= 0.001$).

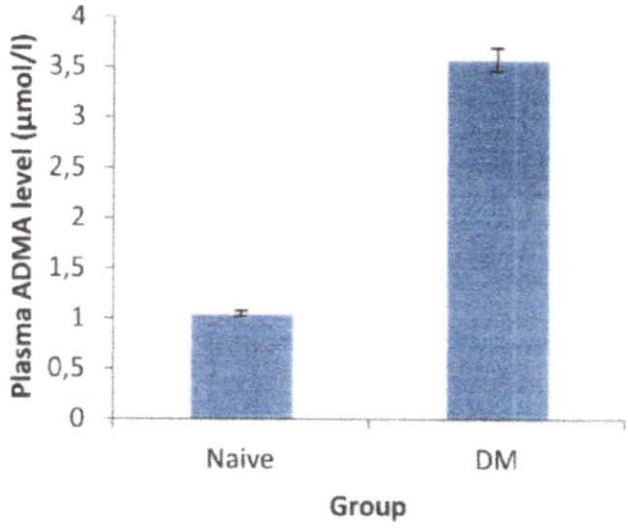

Figure 1. Plasma ADMA level after induction of all-oxan monohidrate at dose 150 mg/kgBB

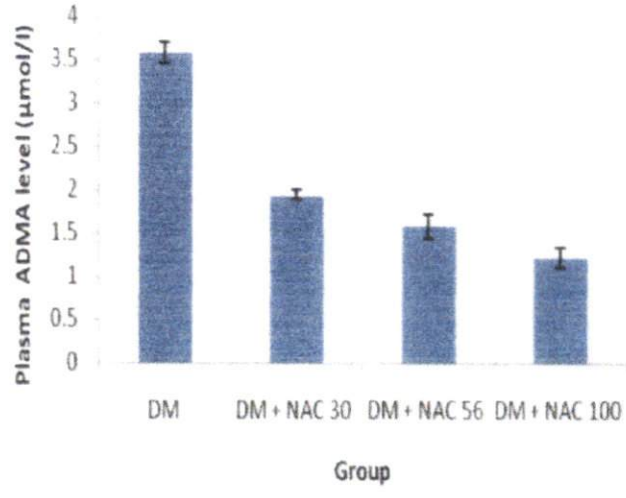

Figure 2. Plasma ADMA level after NAC administration for 28 days

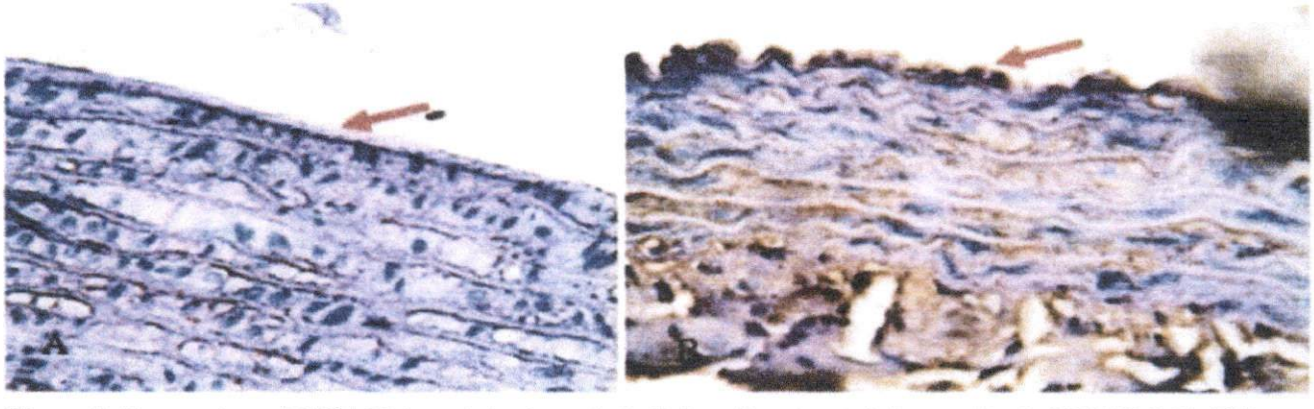

Figure 3. Expression of VCAM-1 protein in endothelial surface by staining antibody VCAM-1 (1:100), shown in dark brown (Red arrow). Naive group, shown in panel A (400x) and DM group in panel B (400x).

Table 2. Score expression of VCAM-1 Protein

Group	Score expression of VCAM-1 protein (x̄± SD)
Naïve	1.87 ± 0.20
DM	6.33 ± 0.55*

*) Significant differences (p=0.003) vs naive group

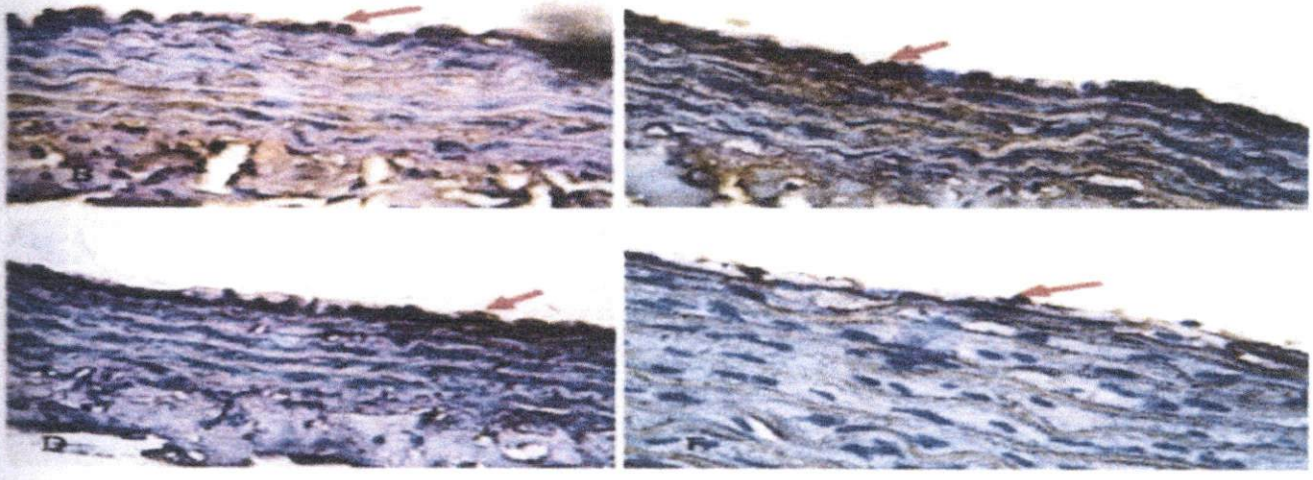

Figure 4. Expression of VCAM-1 protein in endothelial surface by staining antibody VCAM-1 (1:100), 400x. shown in dark brown (red arrow) (B) DM group(C) DM+NAC 30 mg/kg BW, (D) DM+NAC 56 mg/kg BW, and (E) DM +NAC 100 mg/kgBW

Table 3. Score expression of VCAM-1 protein after NAC administration for 28 days

Group	Score expression of VCAM-1 protein (x̄± SD)
DM	6.33 ± 0.55
DM + NAC 30	4.80 ± 0.99
DM + NAC 56	4.32 ± 0.41
DM + NAC 100	2.60 ± 0.24

DISCUSSION

Diabetes is an important and independent risk factor for the development of cardiovascular complications and obstructive vascular diseases involving several body systems. Being the distinguishing feature of diabetes, hyperglycemia has been suggested to play a key role, via oxidative stress, inmediation of these complications.[16]

Endothelial function is important for the homeostasis of the body and its dysfunction is associated with several pathophysiological conditions, including atherosclerosis, hypertension and diabetes. Understanding and treating endothelial dysfunction is a major issue in the prevention of vascular complications associated with all forms of diabetes mellitus.[17]

The key role played by reactive oxygen species in the mediation of the vascular complications of diabetes is also reinforced by studies showing that antioxidants such as vitamin E, superoxide dismutase, catalase, ascorbic acid and glutathione (GSH) are all decreased in blood and tissue of diabetic animals. This decrease in endogenously occurring antioxidants will also result in increased oxidative injury by failure of protective mechanisms.[16]

An elevated oxidative stress can be reduced by using NAC. NAC is precursor cystein and GSH.[10] NAC deacetylation become cystein, then cystein involve in glutation metabolism pathway and increase intraceluler glutation formation. NAC is a source of sulfhydryl (-SH) as act antioxidant group in cells and scavenger of free radicals in direct such as superoxide ($O_2^{\bullet-}$) hydrogen peroxide (H_2O_2) and hydroxyl ($OH^{\bullet}$).[12]

This study used male sprague – dawley (SD) rat induced by intraperitoneally alloxan monohidrate 150 mg/kgBB,an intraperitoneal dose below 150 mg/kg may be insufficient for inducing diabetes in this animal species.[18] Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals

undergo dismutation to hydrogen peroxide with a simultaneous massive increase in cytosolic calcium concentration, which causes rapid destruction of pancreatic β-cells.[19]

Increasing of plasma ADMA level and expression of VCAM-1 protein that marker of oxidative stress are consequently could thus lead to endothelial dysfunction and development to cardiovascular events.[6]

Increasing of endogen antioxidant capacity in cell, particularly increasing of endogen glutation antioxidant (GSH) by NAC causes a decrease in markers of oxidative stress that plasma ADMA level and expression of VCAM-1 protein. So that prevention of vascular complications associated with all forms of diabetes mellitus.

CONCLUSION

Our data show that the treatment with NAC as an antioxidant can reduce plasma ADMA level and the expression of protein VCAM-1 on endothelial cell dysfunction in diabetic rat model indicate in the prevention the development of macrovascular diabetic complication.

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