THE PROTECTIVE EFFECT OF N-ACETYLCYSTEINE (NAC) AGAINST OXIDATIVE STRESS ENZYME AND LIPID PROFILE FOR TOXICITY MALE RATS BY DIMETHYLNITROSAMINE

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ABSTRACT

The present study is conduct to evaluated the deleterious effects of administration of dimethylnitrosamine (DMN) on the some oxidative stress and lipid profile parameters of laboratory male rats (*Rattus rattus*), we used eighteen adult male rats randomly divided into three equal groups (six in each). Group 1 (control) the animals treated by given oral normal saline (0.2 ml), group 2 was treated by oral dimethylnitrosamine 30 mg/kg/day, group 3 the animals were treated by oral N-acetylcysteine 300 mg/kg/day followed by dimethylnitrosamine 30 mg/kg/day. At the end of experimental period, rats were sacrificed. Blood was collected by cardiac puncture to investigate lipid profile and oxidative parameters including serum MDA, glutathione, catalase, and SOD. Results showed a significant reduction in SOD, Catalase, glutathione, and HDL, and a significant increase in MDA, LDL, total cholesterol, triglyceride, after DMN treatment, while these changes were returned to nearly normal level when they combined NAC with DMN treatment for the 30 days treatment when compared with the control group.

INTRODUCTION

N-Acetylcysteine (NAC) is precursor of amino acid L-cysteine ,it is very important in cellular antioxidant that caused decrease in inflammation in many diseases, and inhibits lipid peroxidation[(1)], therefore NAC has a wide actions in different applications ,it can pass through the blood brain barrier which leads to

dysfunctions . Also, it applied for the treatment of neurological disorders and modulates neurotrophic and inflammatory pathways [2].

N-nitroso compounds can be divided into two categories: nitrosamines and nitrosoamide compounds, which includ N-nitrosoureas, N-nitrosocarbamates and N-nitrosoguanidines ,the chemical and biological effect of both groups differ considerably . Nitrosamines have biological properties especially in research cancer, it has more than 100 nitroso compounds studied in laboratory animals they were carcinogenic and some are induce cancer after a single dose , the most important nitrosamines are dimethylnitrosamine and diethylnitrosamine [3].

Dimethylnitrosamine (DMN) is a member of a family of carcinogens, it is also known as nitrosodimethylamine (NDMA), dimethyl- nitrosoamine, N, N-dimethylnitrosamine, N-methyl-N-nitrosomethamine, N-nitroso- N, N-dimethylamine. It caused generation of reactive oxygen species(ROS) which resulting in oxidative stress in cell and tissue . DMN has no used in commercial and industrial production and is generated from the reaction of DMN with monochloroamine or nitrosation of DMN by the nitrate [4].Nitrosamines are small molecules product from combination of nitrate and nitrite with amine compounds [5]. Nitrosamines are present in water soil, and air [6]. DMN is a predominate member of nitrosamine and is found in water ,food, beer, cured meats and rubber [7].

Non-dietary nitrosamines source include tobacco, cosmetics and occupational exposure in rubber or rocket fuel factories, and it leather tanneries [8]. The nitrate and nitrosamine are most commonly associated with consumption of meats, processed meat and fish with bear having the largest amount of nitrosamine [9].

MATERIALS AND METHODS

The experiment was conducted at the animal house of Veterinary Medicine College / university of Basra. Where 18 adult male rats (Rattus norvegicus), age 8 weeks and average body weight between (180-200 gr.) were selected randomly, they were maintained at standard experimental condition. All rats were housed in plastic cages in a room with controlled temperature and humidity. They were kept under good hygienic conditions. Food and water were provided daily (*ad libitum*). Rats were maintained on a natural 12 h light- 12 h dark cycle. The general condition and

behavior of rats were noticed. After the accommodation period, eighteen young male rats were randomly divided into three groups (6 in each group) as following:

Group one (control): the animals were given normal saline solution oral by gavage (0.2ml) for 30 days.

Group two: the animals were treated with di methyl nitrosamine 30 mg/kg BW/ day by oral gavage for 30 days.

Group three: the animals were treated with NAC 300 mg/kg BW after one hour treated di methyl nitrosamine at a dose of 30 mg/kg/day for 30 days.

At the end of experiment period all rats were anesthetized with chloroform, after that abdominal cavity was opened by midline incision. Blood samples were collected via cardiac puncture by using 5 ml disposable syringe, blood was collected into test tube free from anticoagulant to separate serum for estimation the lipid profile parameters (HDL, LDL, total cholesterol, triglyceride, and VLDL) by using chemistry autoanalyzer (Serial No.20628, Human Star, Germany).

Antioxidant Enzymes Measurement

Measurements of Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPX) and MDA had been used by ELISA kit Elabascience Biotechnology Inc. China .While, Serum Malondialdehyde estimation(MDA) which is the chief finale product of lipid peroxidation will be done in serum according to Yagi method [10] .

RESULTS

-Oxidative stress

As illustrated in table (1) there is a significant increase ($p< 0.05$) in MDA in group 2 when compared with the animals of group 3 and control groups ,while SOD ,catalase and Glutathione showed a significantly decreased ($p< 0.05$) in group 2 compared with other groups .

Table 1: Effect of DMN and NAC on oxidative stress enzyme . (m± SE) n=6

Parameter / group	SOD U/ml	MDA mmol/L	Catalase U/ml	Glutathione mg/dl
G1 Control	773.81±14.91 a	0.28 ±0.13 b	37.69 ±1.50 a	41.5 ±7.93 a
G2 DMN	230.80 ±13.11 b	2.55 ±0.35 a	6.63 ±1.462 b	12.5 ±1.80 b
G 3 NAC+ DMN	707.51 ±17.20 a	0.29± 0.20 b	37.10 ±2.21 a	48.3 ±5.20 a
LSD	66.3	0.71	5.3	14.6

The different small letters refer to significant differences at ($p< 0.05$).

2-Lipid profile

Lipid profile showed a significant ($p< 0.05$) increase in the TC,TG,LDL and VLDL in group of animals treated di methyl nitrosamine in group 2 as compared with group 3 the animals treated di methyl nitrosamine after one hour treated N-Acetylcysteine and control group. The same table showed that the HDL appeared decrease significantly ($p< 0.05$) in G2 as compared with control and other treated groups

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Table 2: Effect of DMN and NAC on lipid profile. (mean± SE),n=6

Parameter / group	TC mg/dl	TG mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
G1 Control	33.50±4.60 **b**	12.62 ± 1.710 **b**	13.01 ± 3.22 **a**	17.91 ± 4.81 **b**	2.53±0.35 **a**
G2 DMN	58.30 ±4.8 **a**	24.65 ± 3.57 **a**	7.40 ± 1.32 **b**	44.94 ± 4.30 **a**	4.93 ± 0.70 **a**
G 3 NAC+ DMN	32.32 ± 4.01 b	11.60 ± 3.41 b	12.01 ± 5.19 a	11.50 ± 5.50 b	4.50 ± 0.83 a
LSD	9.1	8.8	5.6	10.2	2.0

The different small letters refer to significant differences at ($p< 0.05$).

DISCUSSION

The finding of this work postulated the change in antioxidant enzymes and lipid peroxidation as shown on table (1and 2).There was a significant decrease in SOD, catalase, and GSH, while a significant increase in MDA levels during

administration of DMN for 30 days while these effects declined to non-significant levels when compared with the control group during combination of NAC with DMN.

The primary enzymatic defense in the liver against the damage effects of O2 in rats is SOD , if SOD activity is low, then O2 can interact with NO to form peroxynitrate ($ONOO^-$), which can react to form the potent OH and nitrogen dioxide (NO2) radicals ,therefor the oxidative stress in rats was prevented by SOD enzyme [11]. These radicals are highly damaging to cell proteins, lipids, and DNA, Glutathione is catalyzed by glutathione peroxidase (GPx), to reduced hydroperoxides and can remove free radicle in the body to protect cells from oxidative damage [12].

The present study showed that the exposed rats to DMN leads to accumulation of MDA in cell membrane which can be modified by lipid peroxidation products, such as trans-4-hydroxy-2-nonenal,4-hydroperoxy-2-nonenal, and MDA, Lipid peroxidation products can also modulate signaling molecules and alter functions of enzymes and proteins involved in inflammation [13]. N-Acetylcysteine as a precursor of cysteine, exerts antioxidant effect by reacting directly with electrophiles or by facilitating generation of GSH, after incorporation into cells, NAC directly scavenges H2O2, hydroxyl free radicals and hypochloric acid in vitro, therefore, NAC has been widely used as a research tool in as an antioxidant research [14],also it is suggested that NAC may also exert its antioxidant effect indirectly by facilitating GSH biosynthesis and supplying GSH for glutathione peroxidase-catalyzed reactions, depletion of GSH is one of the primary factors that permit lipid and protein oxidation [15].

The results of our study revealed a significant increase in total cholesterol, TG, LDL, and VLDL in male rats when compared with control group during

treatment with DMN for 30 days, while there HDL concentration were significantly decreased, but these changes become non-significantly when DMN combined with NAC in 30 days treatment when compared with control group.

These effect are in accordance with result obtained by [16], due to the effect of dimethyl nitrosamine as a potent oxidation material and free radicle promoter they induce a significant increase in LDL, also a different mechanism mediate conversion of HDL into LDL, and free radicles involved in such conversion , in agreement with these findings, levels of both generated free radicals and LDL were markedly increased, whereas HDL levels decreased after treatment of rats with different types of N-nitrosamines [17]. Due to antioxidant properties of NAC and as a free radicle scavenger they can ameliorate the toxic effect of DMN on lipid profile parameters. Administration of NAC decreased serum levels of triacylglycerols, VLDL-cholesterol and LDL-cholesterol in diabetic rats (DM+NAC), while increasing the concentration of HDL-cholesterol. Considering that cell recognition between apolipoproteins and membrane receptors depends on their structure being intact, the antioxidant action of NAC can prevent oxidation of proteins and ensure endocytosis of lipoproteins, decreasing in serum concentration [18].

دراسة تاثير الاسيتايل سيستايين-ن على بعض معايير الجهد التاكسدي وصور الدهون في ذكور الجرذان المختبرية المتسممه بمادة النايتروزامين

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الخلاصة

اجريت هذه الدراسة لمعرفه التاثير الوقائي اسيتايل سيستايين ضد التاثير التاكسدي وصور الدهون الناتج من التعرض للنايتروزامين لذكور الجرذان المختبرية (Rattus rattus).استخدمت ١٨ جرذ ذكري مختبري قسمت الى ثلاثة مجاميع (٦ لكل مجموعة). المجموعة الاولى: وتعتبر مجموعة سيطرة تم تجريعها عن طريق الفم بمحلول الملح الاعتيادي لمدة ٣٠ يوم ،المجموعة الثانية :تم تجريعها فمويا بمادة النايتروزامين بجرعة ٣٠ملغم/ كيلوغرام لمدة ٣٠ يوم ،المجموعة الثالثة : تم تجريعها عن طريق الفم بمادة الاسيتايل سيستايين ٣٠٠ملغم/كيلوغرام ثم بعد ساعه جرعت بمادة النايتروزامين بجرعة ٣٠ ملغم/كيلوغرام لمدة ٣٠ يوم ،وتم

اعطاء الحيوانات عليقة متوازنة من الغذاء وكذلك الماء عند الرغبة خلال فترة التجربة . في نهاية التجربة جمعت عينات الدم وفصل مصل الدم لحساب القراءات المطلوبة وتشمل انزيمات (SOD, Catalse) وكذلك MDA, glutathione وحساب صور الدهون الثلاثية(TG) والكوليستيرول TC والدهون عالية الكثافة HDL وقليلة الكثافة LDL. اظهرت الدراسة انخفاض نسبة HDL, catalase, SOD, glutathione وارتفاع نسبة LDL, TG, TC،MDA بعد التعرض للنايتروزامين ولكن هذه التغيرات عادت الى مايقارب مستواها الطبيعي بعد ان تم تجريع الحيوانات الاسيتايل سيستايين خلال نفس الفترة مما يدل على التاثير الوقائي للحامض الاميني الاسيتايل سيستايين ضد الجهد التاكسدي للمواد المؤكسدة وما ينتج عنه من ارتفاع في مستوى الدهون الضارة في ذكور الجرذان المختبرية.

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