INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND DEVELOPMENT (IJPRD)
Platform for Pharmaceutical Researches & Innovative Ideas
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CARDIO PROTECTIVE NATURE OF N-ACETYL CYSTEINE AGAINST B ADRENERGIC AGONIST INDUCED MYOCARDIAL INDUCED MYOCARDIAL INFARCTION IN RATS

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ABSTRACT
The Biochemical effects of NAC pretreatment against isoproterenol induced Myocardial infarction was studied in male albino rats. The activities of mitochondrial enzymes and levels of antioxidants were estimated in heart mitochondria. The levels of cholesterol, triglycerides and FFA were also estimated in the serum of control and experimental rats.Isoprotrenol levels of antioxidants and mitochondrial enzymes, and increased the levels of triglycerides, cholesterol, FFA. Treatment with NAC confirms the protective and inhibitory effect against isoproterenol induced lipid per oxidation.

KEY WORDS: NAC, Isoproterenol, Antioxidants, Myocardial infarction

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INTRODUCTION

Myocardial infarction a Killer disease is the cause of death and disability in all industrialized nations. It is the major origin of Chest pain in most Western Societies. The term Myocardial infarction is thought to reflect death of Cardiac myocytes due to prolonged ischemia. Myocardial infarction is an acute coronary syndrome that can occur during the natural course of coronary atherosclerosis.

Induction of Myocardial infarction by isoproterenol is well established in animal model to study the protective role of various cardio protective agents.

N-Acetyl Cysteine is one of the drugs taken as antipyretic agent. It has been observed that frequent intake of NAC reduces the risk of heart dysfunction and cardiac arrest. NAC, a precursor generated by isoproterenol.

Induction of myocardial infarction is accompanied by the generation of free radicals. The role of antioxidants and antioxidant enzymes in heart mitochondria has been studied.

Rearrangement of lipid metabolism is of considerable importance in the development of ischemic heart disease. Hence, the individual lipids in serum were estimated.

NAC has been chosen for the present study because of its antioxidant nature directly or indirectly. Therefore an attempt has been made in the present study, to evaluate the protective

efficacy of NAC on experimental myocardial infarction.

It is hoped that the results would open new avenues enlightening on the protective role of NAC in the treatment of myocardial infraction.

MATERIALS AND METHODS

Wistar strains of male Albino rats weighing 150 – 250 g were used as the experimental model. The animals were kept in well ventilated cages and were fed with commercial pelleted rat chow and water *ad libitum*.

The rats were divided into four groups.

Group 1: Control

Group 2: Administered isoproterenol (20 mg /100 g body wt, subcutaneously6 twice at an interval of 24 hours)

Group 3: NAC treated (75mg/kg body wt administered orally for 21 days)

Group 4: NAC treated alone (75 mg / kg body wt administered orally for 21 days)

Group 2 and 3 were also given isoproterenol at the above mentioned dose after pretreatment with NAC, twice at an interval of 24 hours (**Wexler BC** *et al.*, **1978**).

The animals surviving after the second dose of isoproterenol administration were sacrificed by cervical decapitation and blood was collected and serum separated. The heart was dissected out and washed in ice-cold saline and homogenized in 0.1 M Tris – Hcl, pH 7.4 and used for various experiments.

The Following Biochemical parameters have been estimated in Serum and in Heart Mitochondria namely,

Proteins

Antioxidant Enzymes

Lipid Profile

Mitochondrial Enzymes

Statistics: The values are expressed as mean ± SD.Statistical difference was analyzed by students – t-test and 'P'values were determined.

RESULT AND DISCUSSION

Mitochondria is the major oxygen consuming organelle of the myocardial cell, and they are known to reduce oxygen univalently.Mitochondria serve as a locus in the cell where free radical reactions may originate. Mitochondrial respiratory chain generates a large continuous flux of oxygen radicals. These oxygen radicals including super oxide anion, hydrogen peroxide, and hydroxyl radical and single oxygen, attack cellular macromolecules oxidizing membranous phospholipids and damaging protein and DNA. Lipid per oxidation is a major mechanism of oxygen free radical toxicity. The reactive radical species attack the membrane and converts polyunsaturated fatty acids into lipid peroxides.

TABLE 1: Table 1 shows the levels of LPO, SOD, and Catalase in the tissue of control and experimental animals. Values are expressed as mean ± SD for 6 rats in each group.

Parameters	Group 1	Group2	Group3	Group4
LPO(n moles of TBARS/100 mg ptn)	3.82 ± 0.29	5.37 ± 0.5 ^{a***}	4.21± 0.38 ^{a***}	4.07 ±0.35 ^{a***} b^{NS}
SOD(units/min/100 mg ptn)	12.32 ±1.21	6.57 ± 0.59 ^{a***}	11.02± 1.07 ^{a***}	10.72± 1.04 ^{a***} b^{*}
Catalase(n moles of H_2O_2decomposed/min/100 mg ptn)	1.37 ± 0.11	0.78 ± 0.07 ^{a***}	1.12±0.10 ^{a***}	1.23±0.11 ^{a***} b^{NS}

Statistically significant variations

'a' as compared to group 1

'b' as compared to group 2

*** P < 0.001

In table 1, the level of LPO was found to be increased in the heart tissue during ISO administration in Group 2 rats. Lipid per oxidation has been shown to increase the level of LPO during myocardial ischemia. **(Klonner RA *etal.*, 1980).** Group 3 and 4 rats Pretreated with NAC maintained the levels of LPO to near normal.

SOD and Catalase was found to be decreased in ISO induced rats. The antioxidants regained the levels in treated rats. SOD and Catalase play a major role in eliminating Reactive oxygen Species.

Table 2: Table 2 depicts the levels of Glutathione, Glutathione reductase and Glutathione-S- Transferase in the mitochondrial tissue of control and experimental animals.

Values are expressed as mean ± SD for 6 rats in each group.

Parameters	Group 1	Group2	Group3	Group4
GSH(n moles of GSH oxidized/mg ptn)	7.21 ± 0.69	4.24 ± 0.39 ^{a***}	5.64± 0.52 ^{a**}	^{b***} 5.73 ±0.53 ^{a***}
GSH Reductase(n moles NADPH oxidized/min/mg ptn)	1.98 ±0.17	1.04 ± 0.97 ^{aNS}	2.17± 0.97 ^{a*}	^{b*} 2.26± 0.18 ^{a*}
GPX(µg GSH utilized/min/mg/ptn)	1.27 ± 0.98	0.93± 0.07 ^{aNS}	1.13±0.12 ^{a***}	^{bNS} 1.17±0.09 ^{a***}
GST(n moles CDNB conjugated min/mg ptn)	63.12 ± 5.78	42.7± 4.1 ^{a***}	54.72±5.22 ^{a***}	^{b*} 53.12±5.6 ^{a***}

Statistically significant variations

'a' as compared to group 1

'b' as compared to group 2

***** P < 0.001, **P<0.01,*P<0.1 and NS-Non significant**

The level of Glutathione was found to be decreased in ISO treated rats, similar to the observation made by **(Ebenezer KK *etal*. 2001).** The diminished Glutathione concentration may be due to either increased degradation or decreased synthesis of the total GSH (Jozwiak, *et al.*, 1985. The level of glutathione was significantly elevated in group 3 and 4 rats following NAC administration. NAC acts as a direct antioxidant and scavenger of free radicals generated from other sou8rces (Villa P *et al.,* 1995). The levels of glutathione peroxides and Glutathione reductase were found to be diminished in isoproterenol induced rats. The decreased activity may be due to lipid per oxidation and generation of free radicals by isoproterenol. Pretreatment with NAC in group 3 and 4 rats maintained the normal levels of glutathione reductase and glutathione peroxides.

Table 2 shows a significant decrease in the activity of glutathione- S – transferase in group 2 rats when compared to group 1 rats. Due to the decreased glutathione- S – transferase activity, the free radicals are not neutralized and hence myocardium shows enhanced susceptibility to the per oxidation in the presence of promotion of lipid per oxidation . NAC pretreatment brought back the glutathione- S – transferase activity in group 3 and 4 rats to near normal level.

TABLE 3: The Levels of MDH, ICDH, and SDH in heart mitochondria of the Experimental animals. Values are expressed as mean ± SD for 6 rats in each group**.**

Parameters	Group 1	Group2	Group3	Group4
MDH(nano moles of NADH oxidized/min/mg ptn)	305.15 ± 22.3	249.3±23.2 ^{a***}	293.25±21.7 ^{a**}	^{b***} 287.21±21.7 ^{a***}
ICDH(nano moles of α – KG produced/hr/mg ptn)	712.2 ±57.3	607.2 ± 45.3 ^{a**}	657.6±45.15 ^{aNS}	^{bNS} 671.7± 42.8 ^{a*}
SDH(µ moles of Succinateoxidized/min/mg ptn)	218.6 ± 18.5	162.3± 14.7 ^{a***}	192.7±13.3 ^{a***}	^{bNS} 201.3±15.4 ^{a***}

Statistically significant variations
'a' as compared to group 1
'b' as compared to group 2
***** P < 0.001, **P<0.01,*P<0.1 and NS-Non significant**

Mitochondria are seen as a compartmentalized system, with specific enzymes located within the inner and outer membrane. Mitochondria consumes more than 90% of the oxygen used by cells, and mitochondrial respiratory chain leaks large amount of super oxide anion radicals, which react with membrane phospholipids to develop lipid per oxidation **(Toshiho *et al*., 1995).**

The activities of TCA cycle enzymes in heart mitochondria are presented in table 3. The enzyme activities MDH, ICDH and SDH decreased significantly in group 2 rats received only isoproterenol, when compared to control and treated rats. This is also evidenced by the observed reduction in MDH, SDH and ICDH **(Manjula TS *et al*., 1993)**. Reductions in the activities of these enzymes prove the defect in aerobic oxidation of pyruvate which might lead to low production of ATP molecules. TCA cycle enzymes which are located in the outer membrane of mitochondria could have been affected by the free radicals produced by isoproterenol (L H. Opie J., 1985).

The activities of MDH, SDH and ICDH were increased in group 3 and 4 rats received both NAC and isoproterenol as compared to grou8p 2 rats. NAC pretreatment has been observed to increase the activity of TCA cycle enzymes. The mechanism of action has been due to the inactivation enzymes cyclooxygenase and lipooxygenase, preventing the formation of lipid peroxides to protect against the injury mediated by free radical **(Arstall MA *et al*; 1995)**

TABLE 4: The activities of NADH dehydrogenase, Cytochrome - C – oxidase and a- KGDH were found on table 4. Values are expressed as mean ± SD for 6 rats in each group.

Parameters	Group 1	Group2	Group3	Group4
NADH dehydrogenase(n moles of NADH oxidized/min/mg ptn)	125.1 ± 9.7	95.3±8.3 ^{a***}	108.6±8.3 ^{a**}	^{b***} 98.87±8.7 ^{aNS}
Cyt-C-Oxidase(n moles min/mg ptn)	0.27 ±0.017	0.20 ±0.013 ^{a***}	0.23±0.018 ^{a**}	^{b*} 0.21± 0.013 ^{aNS}
α-KGDH (n moles of pot.ferrocyanide liberated/mg ptn)	69.7 ± 6.2	45.3± 3.7 ^{a***}	58.3±5.1 ^{a***}	^{b*} 60.3±3.8 ^{a***}

Statistically significant variations
'a' as compared to group 1
'b' as compared to group 2
***** P < 0.001, **P<0.01,*P<0.1 and NS-Non significant**

Table 4 depicts decreased activity of NADH dehydrogenase, Cytochrome – C – oxidase and a-KGDH in isoproterenol treated rats. The decreased activity in mitochondria was due to the unavailability of lipid for its functional activity **(Varghese A *et al*., 1990).**

NAC pretreatment was observed to increase the activity of Cyt-C-oxidase and NADH dehydrogenase due to reduced degradation of phospholipids. It also preserves oxidative phosphorylation in mitochondria and facilitates the recovery of tissue ATP.

N-acetylcysteine also increases mitochondrial complex I and IV specific activities both invitro and in vivo in synaptic mitochondrial preparations from aged mice (Beck. 2001).

Fig (1) the Levels of Cholesterol in Serum of Experimental Animals Values are expressed in mean ± SD for 6 rats in each Group

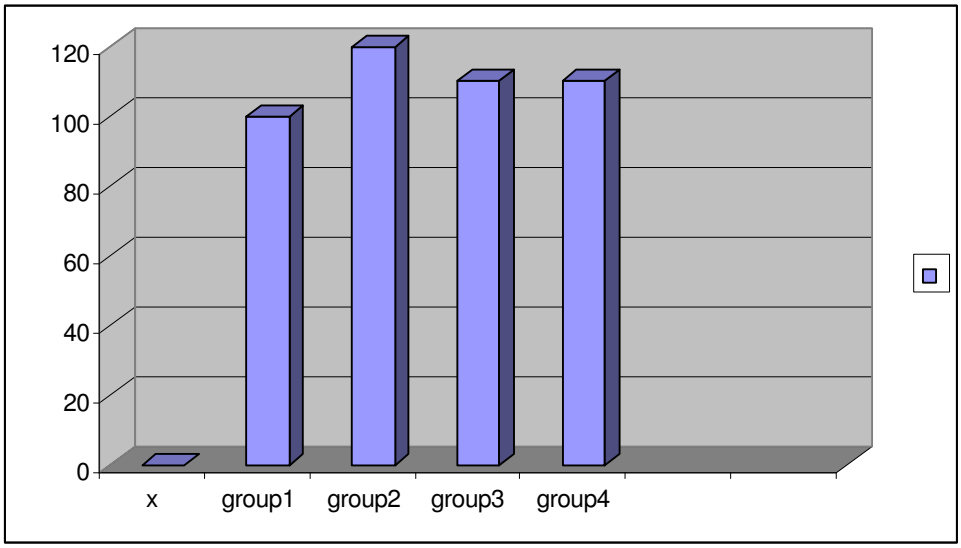


Fig (2) the Levels of Triglycerides in Serum of Experimental Animals Values are expressed in mean ± SD for 6 rats in each Group

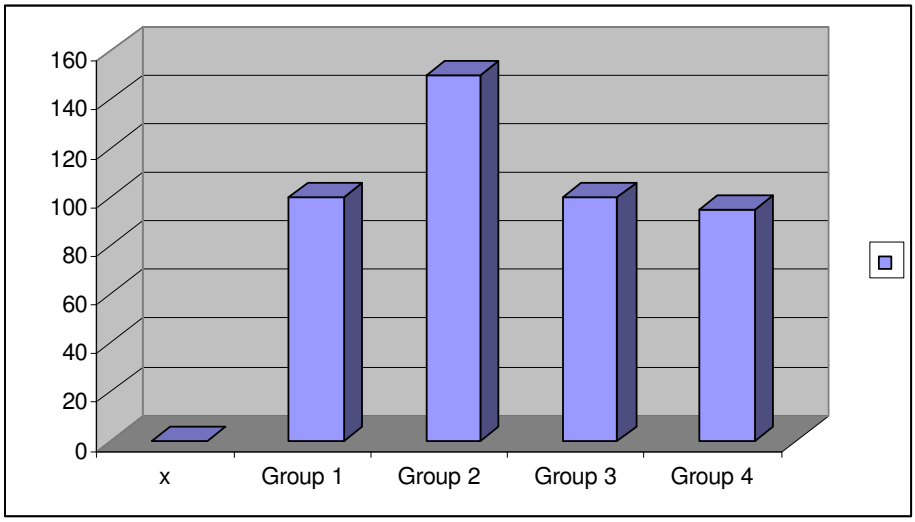
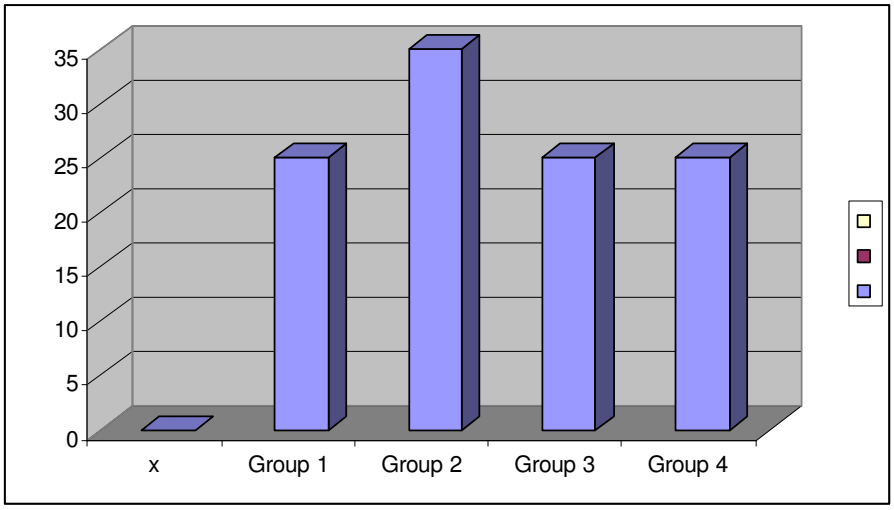


Fig (3) the Levels of Free Fatty Acids in Serum of Experimental Animals Values are expressed in mean ± SD for 6 rats in each Group



The increase in the level of serum lipids due to isoproterenol administration is an evidence for its known hyperlipidemic effect.

In isoproterenol treated rats, there was a significant increase in the levels of the lipids. The elevation is significantly attenuated in group 4 rats. Similar results were also observed by **(Sree Priya *et al*., 1998, Manjula TS *et al*., 1992).** NAC reversed the levels of cholesterol probably by regulating cholesterogenesis and by its inhibitory effect lipid per oxidation, thereby reducing the levels of lipid components.

Hypertriglyceridemia was observed in isoproterenol treated rats, and such a state was also reported in association with cardiovascular disturbances **(Freedman DS *et al*., 1998).** The levels of triglycerides were reduced in group 4 rats when compared with group 2 rats. The increased per oxidation of polyunsaturated fatty acids is recognized as one of the possible biochemical mechanisms for the genesis of membrane injury in the myocardium **(Narasimhan L *et al*., 1990).**

A significant increase in free fatty acid in isoproterenol induced rats might have been due to the breakdown of membrane phospholipids **(Narasimhan L *etal*., 1990).** The increased per oxidation of the membrane phospholipids releases free fatty acids the action of phospholipids A2 **(Chein KR *et al.,* 1980).**

SUMMARY & CONCLUSION

The biochemical effects of N-acety1 cysteine pretreatment against isoproterenol induced myocardial infarction was studied in male Albino rats. The activities of mitochondrial enzymes and levels of antioxidants were estimated in heart mitochondrial. The levels of cholesterol, triglycerides and free fatty acids were also estimated in the serum of control and experimental rats.

Isoproterenol decreased the levels of antioxidants and activities of mitochondrial enzymes. The levels of triglycerides, cholesterol and free fatty acids were increased in isoproterenol induced rats.

Free radical generation may be the major pathogenic factor responsible for initiation of myocardial cell damage. The lipid per oxidation and thiol depletion is responsible for the inactivation of mitochondrial enzymes in isoproterenol induction.

Treatment with NAC confirms the protective and inhibitory effect against isoproterenol induced lipid peroxidation.

NAC will shortly become the most talked nutrient supplement in the world

because it directly addresses each of these concerns. It may be said that NAC is the universal antioxidant because it activates and increase the potential of all the other antioxidants.

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