ORIGINAL ARTICLE

Hepatoprotective Effect of Acetylated Amino Acid on Methimazole Induced Hepatotoxicity in Mice

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ABSTRACT

Objective: To explore the hepatoprotective effect of N-acetylcysteine on methimazole induced hepatic damage in mice.

Study Design: Randomized control trial.

Place and Duration of Study: The study was carried out from Nov 2014 to Oct 2015 at the animal house of Army Medical College, Rawalpindi.

Materials and Methods: Thirty male BALB/c mice were randomly divided into three groups of ten animals each. Group I: Control group (C), Group II: Methimazole treated group (M), Group III: Pretreated N-acetylcysteine (NAC) group. Single dose of Methimazole (MMI) (1000mg/kg, i.p) was injected for induction of hepatotoxicity. N-acetylcysteine (NAC) (300mg/kg, i.p) was given pre Methimazole (MMI) administration. The extent of hepatic injury was determined by evaluation of serum alanine transaminase (ALT) and alkaline phosphatase (ALP) along with liver histopathology.

Results: Methimazole (MMI) produced liver damage as evident by markedly raised liver enzymes along with necrosis and inflammatory cell infiltration. N-acetylcysteine (NAC) treated group resulted in reduction in elevation of serum biomarkers and improvement of histological picture.

Conclusion: N-acetylcysteine (NAC) holds significant hepatoprotective effect against Methimazole (MMI) induced hepatotoxicity.

Key Words: *Methimazole, Hepatotoxicity, N-acetylcysteine.*

Introduction

Methimazole (MMI) is the most frequently prescribed antithyroid drug worldwide.[1] The preferred use of MMI for hyperthyroidism can be attributed to its better compliance and superior safety profile. However, its clinical use is associated with many extra thyroidal toxicities including hepatotoxicity which limit its usefulness. MMI induced hepatic injury has an incidence of 6.6% in adults presenting as hepatitis, cholestasis and acute liver failure.[2,3] Liver injury produced by MMI is dependent upon bio activation to its reactive metabolites i-e N- methylthiourea and glyoxal. These intermediates cause oxidative and carbonyl stress, mitochondrial impairment and immune reactions.[4] Previous studies have also identified cellular glutathione as an important susceptibility factor in MMI induced cytotoxicity.[5]

Despite reports of MMI induced liver damage, no specific protective has been developed against it. However, an indisputable area of medicinal benefit involves attenuation of oxidative hepatic damage. Researches have recognized the role of antioxidants in various types of drug induced liver injuries.[6] N-acetylcysteine; the acetylated non enzymatic antioxidant has been the antidote for acetaminophen poisoning since 1974. However, its safety and efficacy has been proven outside acetaminophen overdose in liver transplant related damage, fibrosis, hepatitis, alcoholism, heavy metal toxicity and wide range of glutathione deficient genetic and metabolic disorders.[7] NAC exerts its beneficial effects in these clinical settings through glutathione dependent and independent mechanisms.[8]

The aim of the conducted study was to explore the protective effect of NAC against MMI induced hepatotoxicity in mice.

Materials and Methods

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An experimental study was carried out at the Animal house of Army Medical College, Rawalpindi from Nov 2014 to Oct 2015. The conducted trial was a joint venture of Department of Pharmacology and Therapeutics and Department of Pathology, Army Medical College. Thirty male 8-10 weeks old BALB/c mice, weighing 30-40 gram were provided by National Institute of Health, Islamabad. Mice were housed under standard husbandry conditions (temperature 20 ± 2^0 C, humidity 40-60% and 12 hour light/dark cycle) with diet, water and libitum. Animal care and research was carried out in accordance with protocols of ethical committee of "Centre of Research in Experimental and applied Medicine (CREAM)".

MMI of analytical grade was purchased from Sigma Chemicals USA through a licensed dealer. NAC injections of Aumur Pharmaceuticals were purchased from Karachi based pharmacy.

Only adult healthy male mice weighing > 30 grams and < 40 grams with normal LFTs and no obvious abnormality were included in the study. After one week of acclimatization mice were randomly divided into three groups with ten animals each. Group I (n=10) served as the control group (C) and was given normal saline intraperitoneally (i.p). Group II was MMI treated group (M) and given MMI dissolved in normal saline (1000mg/kg i.p). 1000 mg/kg i.p of MMI was taken as the appropriate toxic dose after conduction of pilot study with various previously mentioned experimental doses.[9,10] Group III; NAC pretreatment (NAC -Pre) group was intervened with NAC (300 mg/kg i.p) one hour before MMI.[11]

Baseline sampling of all animals was done through tail vein obtaining at the start of research.[12, 13] After recommended recovery period[14], intervention was carried out and blood samples were collected by performing cardiac puncture five hours after MMI administration.[10] Serum obtained from clotted blood by centrifugation at 3000 revolution/min was used for analysis of ALT and ALP by commercially available kits of Cormay and Linear. ALT was estimated by IFCC method while calorimetric method (DGKC method) was applied for ALP. At the end of experimental period, liver was removed and fixed in 10% formalin. Hematoxylin and eosin (H&E) stained slides were prepared and studied under microscope for histopathological findings.

Data was analyzed using SPSS 21. All data was expressed as Mean ± S.E.M. Statistical difference between serum markers at initial and final hours was calculated using students t- test. One way ANOVA followed by Post hoc Turkey was applied for multiple comparisons between groups. p <0.05 was considered significant.

Results

Effect of N-Acetylcysteine on Serum ALT Levels

Baseline and final values of ALT of G-I (control group) and G-III (NAC pretreated group) remained within normal range during the experimental period (Fig 1). An insignificant p value was obtained when G-I was compared with G-III (Table II). MMI significantly raised ALT levels (261.33±20.30) as compared to initial values (45.33± 9.84) (Fig 1) and control Group I. (Table I). NAC affords protection against MMI induced hepatic insult by mitigating the rise in ALT as evident by significant difference between G-II and G-III (Table III).

Effect of N-Acetylcysteine on Serum ALP Levels

Results revealed an insignificant difference between initial and final levels of ALP of G-I and G-III (Fig 2) which was also reflected in their intergroup comparison (Table II). A significant increment was observed in final serum ALP levels (251 ±17.61) of drug treated Group II (Fig 2). Comparison of G-II (M) with control group G-I revealed statistically significant difference (Table I). A trend of lowering of ALP levels was displayed by NAC pretreatment as

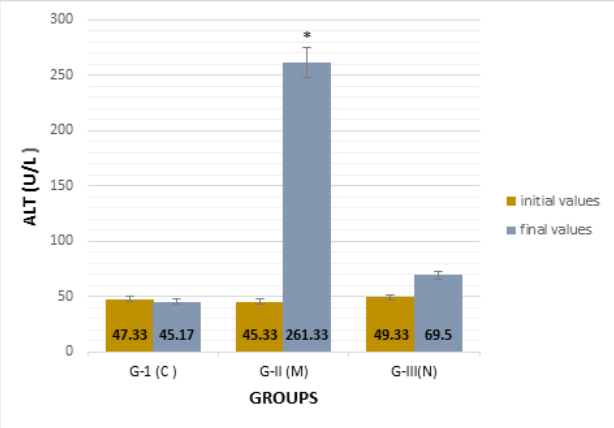

Fig 1: Initial and final values of ALT (U/L) of G-I, G-II and G-III *p < 0.05 =significant

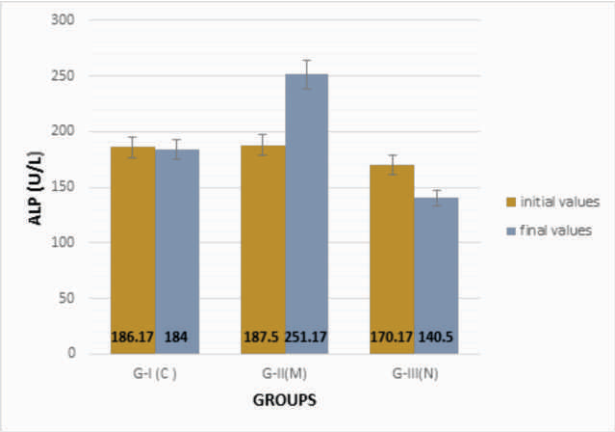

Fig 2: Initial and final values of ALP (U/L) of G-I, G-II and G-III *p < 0.05= significant

Table I: Comparison of ALT and ALP between G-I and G-III

Parameter	Initial values (0 hour)		Final values (5 hours)	
	G-I	G-II	G-I	G-II
ALT (U/L)	47.33	45.33	45.17	261.33
SEM	9.87	9.84	10.03	20.30
p-value	0.98		0.00*	
ALP (U/L)	186.17	187.50	184.00	251.17
SEM	16.81	14.46	15.69	17.61
p-value	0.99		0.03*	

**p* value < 0.05 = significant

Table II: Comparison of ALT and ALP between G-I and G-III

Parameter	Initial values (0 hour)		Final values (5 hours)	
	G-I	G-III	G-I	G-III
ALT(U/L)	47.33	49.33	45.17	69.50
SEM	9.87	7.24	10.03	6.13
p-value	0.98		0.43	
ALP(U/L)	186.17	170.17	184.00	140.50
SEM	16.81	14.75	15.69	13.50
p-value	0.74		0.21	

**p* value < 0.05 = significant

Table III: Comparison of ALT and ALP between G-II and G-III

Parameter	Initial values (0 hour)		Final values (5 hours)	
	G-II	G-III	G-II	G-III
ALT(U/L)	45.33	49.33	261.33	69.50
SEM	9.84	7.24	20.30	6.13
p-value	0.94		0.00*	
ALP(U/L)	187.50	170.17	251.17	140.5
SEM	14.46	14.75	17.61	13.50
p-value	0.71		0.00*	

**p* value < 0.05 = significant

evident by p value < 0.05 when compared with G-II (M) (Table III).

Histopathological Findings

Light microscopy of H & E stained slides of control group showed normal lobular appearance with central vein, portal triad and radiating hepatocyte cords (Fig 3). MMI administration caused cellular discontinuity, vascular congestion, loss of hepatocyte radial distribution and inflammatory cell infiltration (Fig 4 and 5). NAC pretreatment resulted

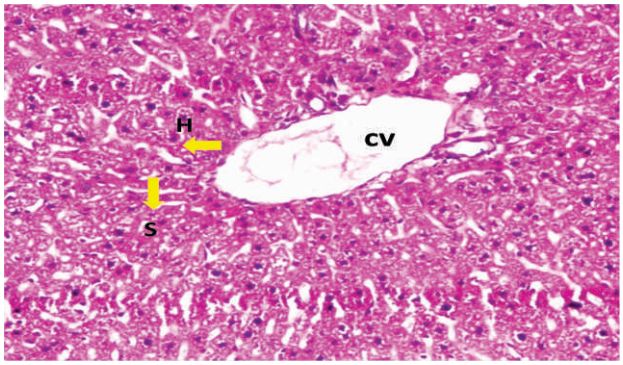

Fig 3: Pictograph of H & E stained liver specimen of control group at 40X showing central vein (CV), hepatocyte (H) and sinusoid space (S)

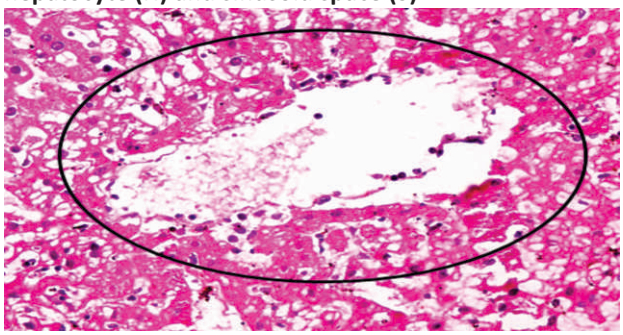

Fig 4: Pictograph of MMI treated group (M) AT 40X revealing cellular discontinuity and loss of radial distribution of hepatocytes.

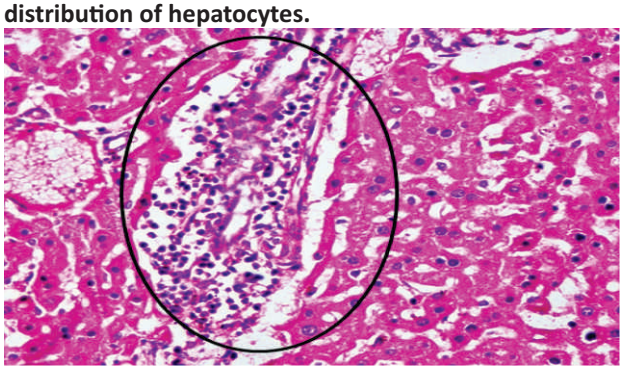

Fig 5: Pictograph of MMI treated group (M) AT 40X revealing portal inflammation

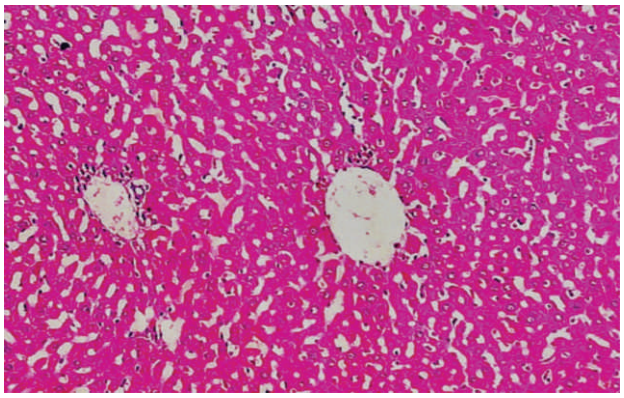

Fig 6: Pictograph of NAC treated group showing preserved architecture of hepatic parenchyma and portal triad with minimal inflammation

in preservation of liver architecture with minimal portal and lobular inflammation (Fig 6).

Discussion

Drug induced liver injury (DILI) is an unresolved health problem with an impact well beyond the number of cases occurring annually. It has become the most common reason for termination of drug development and withdrawal of approved products from market. Antithyroid medicines are included among the thousand (1000) drugs known to cause hepatic damage.[15]Although the precise mechanism of MMI induced hepatotoxicity requires further exploration, however the role of reactive metabolite, oxidative and carbonyl stress, intracellular target dysfunction and immunological reaction has been implicated. The hepatotoxic metabolites produced as a result of biotransformation are capable of reacting with enzymatic and non-enzymatic antioxidants (GSH) rendering them ineffective and ensuing toxicity.[16]

Liver is central to all detoxification processes and adversely targeted by MMI during its administration. Our results showed that MMI produced an abnormal increase in ALT and ALP ($p < 0.05$) which was reflected as cellular discontinuity, necrosis and portal inflammation on microscopy. The significant rise in biochemical markers and histopathological findings were in accordance with the research of Tashkandi and his fellows in 2014.[17] They demonstrated that administration of MMI produced a percentage increase of 202 and 191 in ALT and ALP along with 61% decrease in antioxidant enzymes. Coherent with our findings, Heidari and mates revealed elevation of liver enzymes with concomitant decrease in GSH levels as a consequence of MMI induced hepatic insult.[18] This highlights the role of oxidative stress in pathophysiology of MMI induced hepatic insult and the function of GSH in detoxification of its toxic intermediates. Thus GSH depleted hepatocytes are more susceptible to MMI induced injury producing remarkable damage at one thirtieth the normal toxic dose.[9]

Antioxidants have been documented to play their protective role in conditions set in the background of oxidative stress and impede progression of these diseases. Studies have revealed the hepatoprotective potential of many amino acids against drug induced liver injuries due to their antioxidant effects.[6] Present study evaluated the protective capacity of N-acetylcysteine (NAC) in MMI induced hepatotoxicity. Pretreatment with acetylated cysteine in G-III (N-group) prevented the elevation of ALT and ALP observed in MMI treated group –II ($p < 0.00$). Injection of NAC one hour before MMI also managed to preserve the hepatic architecture with minimal inflammatory changes on histopathology. Insignificant difference ($p > 0.05$) between ALT and ALP of control group (C) and NAC pretreated group-III also adds weight to the hepatoprotective effect of NAC. Heidari and colleagues demonstrated that administration of organosulfur compounds attenuated cell death and prevented ROS formation, mitochondrial damage and lipid peroxidation caused by MMI.[19]

Since many studies has recognized oxidative stress as a major causative factor of MMI induced cellular damage[4,16], NAC's beneficial effects can be attributed to its direct and indirect antioxidant properties. Acting as membrane permeable source of L-cysteine for endogenous GSH, it can facilitate neutralization of cytotoxic metabolites before they can initiate damage.[20,21] This acetylated amino acid can also defend against liver damage by scavenging free radicals and toxic aldehydes.[22] This attenuation of oxidative stress through prevention of GSH depletion and glyoxal trapping by NAC was also reported by Heidari and mates.[23]

The results affirmed the notion that NAC extends its protective effects against MMI induced liver damage evident by prevention of rise of serum ALT and ALP

along with preservation of hepatic lobular organization.

Conclusion

N-acetylcysteine holds hepatoprotective potential against MMI induced liver injury due to broad spectrum of physiological activities, however further investigation is required to endorse the prophylactic role of NAC in thionamide induced hepatotoxicity.

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