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ORIGINAL ARTICLE

N-Acetyl Cysteine Ameliorates Hepatotoxicity Associated with the Use of Methotrexate in Mice

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ABSTRACT

Background: Liver is not only involved in maintaining homeostasis but also exhibits significant role in metabolism and detoxification of various drugs and toxins.
Aim: To explore the hepato-protective role of N-acetylcysteine against methotrexate induced hepato-toxicity.
Study design: Randomized controlled trial.
Methodology: This study having mice (n=18) was carried out after ethical review committee's (ERC) approval at Foundation university medical college in collaboration of National institute of health, Islamabad in 2017. Single intraperitoneal injection (20mg/kg) of methotrexate induced hepato-toxicity. Hepatoprotective effect was assessed by oral administration N-acetylcysteine (50mg/kg) alone with methotrexate. The extent of liver damage and effect of protective agents were determined by measuring serum ALT, AST, ALP after 24 hours of respective treatment. Liver samples were taken for histological analysis. One way ANOVA followed by Post Hoc Tukey test was applied for multiple comparisons of biochemical markers between the groups. Histopathological findings were analyzed by Chi Square test. $p < 0.05$ was considered significant.
Results: Significant ($p < 0.05$) hepatotoxicity was seen with substantial elevation in serum ALT, AST and ALP with methotrexate. N-acetylcysteine attenuated the methotrexate induced hepatotoxicity significantly ($p < 0.05$). The histopathological examination showed mild steatosis along with focal pleomorphism in the liver of mice that received methotrexate in comparison to group treated with N-acetylcysteine and methotrexate though minimal inflammation was seen. **Conclusion:** We concluded that N-acetylcysteine ameliorates the methotrexate induced hepatotoxicity on when used concomitantly**.**
Keywords: Hepatotoxicity, N-acetylcysteine and Methotrexate.

INTRODUCTION

Liver is one of the essential and functionally diverse organs of the body. It is not only involved in maintaining and regulating body homeostasis but also it exhibits significant role in metabolism and detoxification of various drugs, xenobiotics, environmental pollutants and toxins[1]. Drug induced liver injury is a serious health issue faced by physicians, pharmaceutical industries and drug regulating bodies. It is major devastating adverse effect that leads to the failure of drug development prior to approval and also the withdrawal of approved drugs after marketing[2,3].

Methotrexate (MTX) is an immunosuppressive and antineoplastic mediator which is used for the treatment for lymphoma, leukemia, psoriasis, rheumatoid arthritis and other autoimmune diseases[4]. However, despite its advantages, it has been seen that prolonged administration of MTX causes elevation in serum liver enzymes along with the development of chronic cirrhosis of liver and progressive fibrosis[5]. Though the exact action of MTX induced altered hepatic morphology is still a mystery, substantial experimental and clinical evidence suggested cellular damage due to oxidative stress as an important susceptibility factor[6].

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N-acetylcysteine (NAC) derived from L-cysteine which offers protection against oxidative stress by neutralizing free radicals. It is a precursor of antioxidant glutathione (GHS). It has proven efficacy against hepatic damage caused by acetaminophen overdose by replenishing intracellular GSH, and thereby helping to restore cells' capability to fight damage from oxidative stress. By initiating the electrophilic metabolic blockade, NAC imparts the anti-neoplastic as well as the anti-mutagenic activity. NAC also modulates the mitochondrial functions and the immune systems[7,8]. Beneficial effects of NAC has been shown in liver transplant related damage, fibrosis and various genetic and metabolic disorders involving GHS deficiency[9].

In the light of above description and increasing hepatotoxicity due to drugs, we planned the current project to the hepato-protective role of N-acetylcysteine against methotrexate induced hepato-toxicity.

The objective of the study was to explore the hepato-protective potential of NAC in MTX induced hepatotoxicity in mice**.**

METHODOLOGY

This study was carried out after ethical review committee's (ERC) approval at Foundation university medical college in collaboration of National institute of health, Islamabad in

2017. Eighteen mice were randomly divided into three groups. Group A (control group) received 0.2 ml normal saline intraperitoneally. Group B (hepatotoxic group) received single intraperitoneal injection of MTX at the dose of 20 mg/kg[10]. Group C (hepato-protective group) was given NAC 50 mg/kg orally[6] via oral gavage for 7 days with MTX at day 4. The extent of liver damage and effect of protective agents were determined by measuring serum ALT, AST, ALP after 24 hours of respective treatment. Liver samples were taken for histological analysis.

Statistical Analysis: Data was analyzed by using SPSS 23. Results were presented as mean ± S.E.M. One way ANOVA followed by Post Hoc Tukey test was applied for multiple comparisons of biochemical markers between the groups. Histopathological findings were analyzed by Chi Square test. $p < 0.05$ was considered significant

RESULTS

Significant ($p < 0.05$) hepatotoxicity was seen with substantial elevation in serum liver enzymes with MTX in group B. In group C, NAC attenuated the methotrexate induced hepatotoxicity significantly ($p < 0.05$) when compared with group B (Table-1). Figure-1 is showing normal liver architecture.

Table-1: Serum AST, ALT, ALP levels of control, MTX, MTX+NAC group

Parameters	Control	MTX	MTX+ NAC
ALT (U/L)	31.33±3.28	73.67±3.66[a]	39.83±1.64[b]
AST (U/L)	87.83±3.57	128.50±7.77[a]	90.33±2.41[b]
ALP (U/L)	94.67±4.9	315.33±12.44[a]	148.17±2.56[b]

*Data are expressed as mean±SEM; [a] p < 0.05 versus control group; [b] p <0.05 versus MTX group

Figure-1: Histology of liver parenchyma in Group A (Control) showing normal liver architecture (H&E 10X)

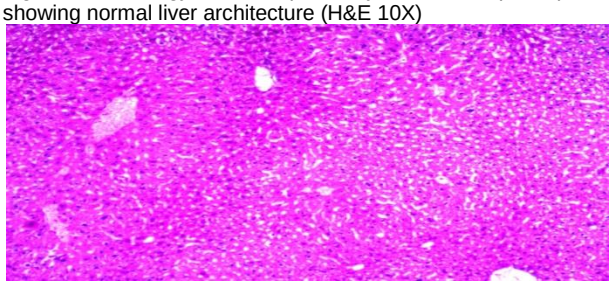
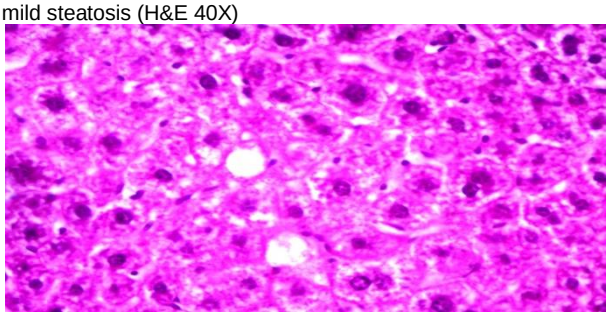
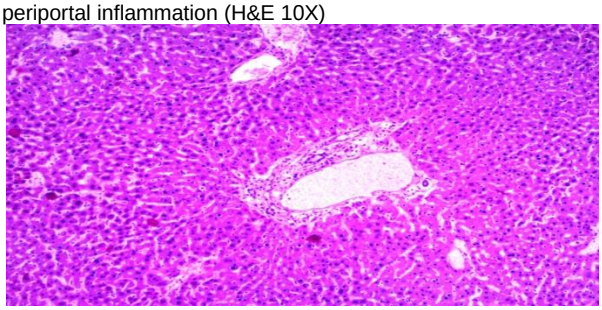


Figure-2: Histology of liver parenchyma in Group B (MTX) showing mild steatosis (H&E 40X)



The liver histopathological examination showed mild steatosis along with focal pleomorphism in group B (Figure-2), which were markedly reduced in mice of group C that were treated with N-acetylcysteine along with methotrexate though minimal inflammation was seen (Figure-3).

Figure-3: Histology of liver parenchyma in Group C showing mild periportal inflammation (H&E 10X)



DISCUSSION

Liver damage is one of the use-limiting adverse effect of MTX. Exploration of its underlying mechanism and establishment of its hepatoprotective agent has been the focus of researchers from many years. Approaches to protect hepatic damage by MTX are mandatory not only to improve the quality of life of patients but also to ensure a rewarding treatment.

Single intraperitoneal injection of MTX results in significant elevation of serum liver enzymes in mice, along with steatosis and mild pleomorphism on histopathological analysis[11]. Elevated serum hepatic enzymes with morphological distortion affirm the hepatotoxic potential of MTX reported in experimental exploration by Akbulut et al[6], Tag[10] and Demircet et al[12]. The exact molecular mechanism of MTX induced altered hepatic morphology is still unclear though different mechanisms are suggested like hepatic aggregation of MTX-polyglutamates with resultant increased homocysteine and decrease GSH production lead to increasing sensitization to ROS along with lipid peroxidation of biological membranes[5]. High homocysteine levels result in fatty infiltration of live by triggering oxidative and endoplasmic reticulum stress. Increased homocysteine levels also enhance pro-inflammatory cytokine like TNF, IL-6, IL-8 and IL-1β. The combination of all of these effects of homocyteine can activate HSC resulting in liver fibrosis[13,14].

In our study, administration of NAC along with MTX in mice showed protective effects on liver as depicted by significant decrease of serum liver markers. Our findings were in-line with the experimental studies of Akbulut et al[6] and Demircet et al[12] which showed significant reduction in the levels of serum hepatic enzymes on concomitant administration of these two drugs. NAC protects liver by increase the hepatocytes GSH levels[15,16]. GSH is important for the maintenance of cellular redox state, regulation of cell cycle, cell proliferation, apoptosis, immune function and fibrogenesis. GSH in its reduced state is mandatory for the detoxification of xenobiotics[17]. NAC reacts with ROS rapidly deactivates hydroxyl radicals and hydrogen peroxide. NAC is also involved in the regulation of the apoptosis as well as the cell cycle up regulation[15,16].

Limitations: Our study had limitations like financial constraints, lack of resources and small sample size. We did not perform acute toxicity.

CONCLUSION

We concluded that N-acetylcysteine can be the powerful hepatoprotective agent against methotrexate induced hepatic damage in mice most probably by increasing hepatocytes GSH levels. may provide protection by their anti-oxidant potential. However, further exploration into underlying mechanisms by which N-acetylcysteine prevents MTX hepatotoxicity is needed.

Authors' Contribution: AK & IKK: Conception and design of work, SL & AS: Collecting and analyzing the data, MA & AH: Drafting the manuscript, TL: Drafting and revising the manuscript for intellectual content.

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