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PROTECTIVE EFFECT OF N-ACETYL CYSTINE AGAINST METHOTREXATE INDUCED HEPATOTOXICITY IN MICE

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Abstract:

Background and objective: *The liver is the largest internal organ by percent weight in the human body and has crucial functions. N-acetylcysteine (NAC) is a well-established cytoprotective drug that has proven efficacy against drug (acetaminophen overdose)-induced hepatotoxicity. Therefore, the basic aim of this study is to analyses the protective effect of N-Acetyl Cystine against Methotrexate induced hepatotoxicity in mice.*

Material and methods*: This experimental study was conducted in Shahida Islam medical and dental college Lodhran during March 2019 to December 2019. In this study we used the 55 male albino mice. The 55 rats were randomly assigned to one of five groups, with an equal number of rats contained within each group. Forty-four rats were administered a single dose of 20 mg/kg methotrexate without or with daily treatments of 50 mg/kg oral NAC (MTX + NAC group), 50 mg/kg per day (single dose). The dose and duration of MTX and antioxidant agents were selected according to results from previous studies.*

Results: *This experimental study consist of 55 male albino mice. As compared to the control group, the experimental group showed significantly higher levels of serum ALT (*P *= 0.001) and significantly lower levels of AST (*P *= 0.001). None of the antioxidant treatments affected the MTX-induced ALT levels (all,* P *> 0.05), but the NAC treatment produced a significant decrease in the MTX-induced serum AST level (*P *< 0.05). MTX exposure appeared to have no effect on either ALP or TBil levels in serum (*P *> 0.05* vs *control group).*

Conclusion: *It is concluded that the mechanism of MTX-induced hepatic injury likely involves oxidative stress pathways; development or progression of this life-threatening condition may be prevented by prophylactic or therapeutic delivery of antioxidant agents, such as NAC.*

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INTRODUCTION:

The liver is the largest internal organ by percent weight in the human body and has crucial functions, including cholesterol production, intermediary metabolism, hormone synthesis, bile and urea production and drug detoxification. The liver has many functions. Some of the functions are: to produce substances that break down fats, convert glucose to glycogen, produce urea (the main substance of urine), make certain amino acids (the building blocks of proteins), filter harmful substances from the blood (such as alcohol), storage of vitamins and minerals (vitamins A, D, K and B12) and maintain a proper level or glucose in the blood[1]. The liver is also responsible for producing cholesterol. Several diseases can affect the liver. Some of the diseases are hepatitis , liver cancer, and cirrhosis (a chronic inflammation that progresses ultimately to organ failure) [2].

Alcohol alters the metabolism of the liver, which can have overall detrimental effects if alcohol is taken over long periods of time. Medications have side effects that may harm your liver. Some of the medications that can damage your liver are: serzone, anti-cancer drugs, and medications used to treat diabetes [3]. Unfortunately, the liver is often injured by environmental toxins, which damage and weaken the liver and eventually lead to different hepatic disorders. Additionally, it is also handling the metabolism and excretion of drugs and other xenobiotics from the body thereby providing protection against foreign substances by detoxifying and eliminating them [4].

The folic acid antagonist methotrexate (MTX) disrupts cellular metabolism, effectively inhibiting cellular growth. As such, MTX is an effective cytotoxic agent and has been widely applied in chemotherapeutic-based treatments for malignancies as well as inflammatory diseases [5]. While MTX is generally well-tolerated by patients, its cytotoxic nature also lends a substantial risk of life-threatening side effects - the most severe of which are hematopoietic suppression, hepatotoxicity and pulmonary toxicity. While the molecular mechanism of MTX-induced hepatotoxicity is not yet completely understood, considerable experimental and clinical evidence have implicated oxidative stress as a contributing factor. Increased reactive oxygen species (ROS) generation, along with decreased antioxidant defense activities, have been shown to promote development and progression of MTX-induced hepatotoxicity in a rabbit model [6].

N-acetylcysteine (NAC) is a well-established cytoprotective drug that has proven efficacy against drug (acetaminophen overdose)-induced hepatotoxicity. As the acetylated precursor of glutathione (GSH), NAC exerts effective antioxidant and anti-inflammatory functions. *In vitro* studies of the molecular mechanisms of NAC have demonstrated that the drug can protect a cell from oxidative stress by inhibiting H_2O_2 formation [7].

Therefore, the basic aim of this study is to analyses the protective effect of N-Acetyl Cystine against Methotrexate induced hepatotoxicity in mice.

MATERIAL AND METHODS:

This experimental study was conducted in Shahida Islam medical and dental college Lodhran during March 2019 to December 2019. In this study we used the 55 male albino mice. The animals were housed under regular laboratory environment conditions (21 ± 2 °C, 60% ± 5% humidity, and 12:12 h light-dark cycle) with free access to standard rat chow and water.

The 55 rats were randomly assigned to one of five groups, with an equal number of rats contained within each group. Forty-four rats were administered a single dose of 20 mg/kg methotrexate without or with daily treatments of 50 mg/kg oral NAC (MTX + NAC group), 50 mg/kg per day (single dose). The dose and duration of MTX and antioxidant agents were selected according to results from previous studies.

Groups	Treatment
A	Control
B	MTX (1ml/kg body weight)
C	MTX (1ml /kg body weight) + NAC (50mg/kg/body weight)
D	MTX (1ml /kg body weight) + NAC (100mg/kg/body weight)

Pharmacokinetic studies*:*

The pharmacokinetic parameters, peak serum concentrations (Cmax) and time to reach peak concentration (Tmax) were directly obtained from concentration-time data. Various pharmacokinetic parameters like area under the curve (AUC), elimination half life ($t_{1/2}$), volume of distribution (Vd), and total body clearance (CL), for each subject were calculated.

Blood and liver separation:

At the end of experiment the mice were sacrificed and blood samples was withdrawn from retro orbital of the eye and collected in glass tubes. Serum was separated by centrifugation for 10 minutes at 3000 rpm and stored at -80 ºC until biochemical analysis. Some liver lobes was kept in 10 % formalin for histopathological examination.

Tissue homogenate:
Some liver tissue was separated and homogenized in phosphate buffer to yield 25% homogenates. The homogenate was centrifuged for 15 minutes at 3000 rpm. The supernatant was stored at -20ºC then used for biochemical analysis.

Biochemical analyses:
The thiobarbituric acid substrate assay was used to measure malondialdehyde (MDA; nmol/g wet tissue) with a spectrophotometer (at 535 and 520 nm). Ellman's method was used to measure reduced GSH (nmol/g wet tissue) with a spectrophotometer. The xanthine/xanthine oxidase (XO) assay was used to estimate superoxide dismutase (SOD) activity (U/mg protein) by measuring the amount of reduced nitroblue tetrazolium (NBT), with one unit of SOD defined as the amount of protein that inhibits the rate of NBT reduction by 50%. Aebi's method was used to estimate catalase (CAT) activity (U/g protein) with a spectrophotometer (240 nm) to determine the rate constant *k* (dimension: s-1, *k*) for scavenging H_2O_2 (initial concentration 10 mmol/L). Plasma was separated from blood samples and directly applied to the Architect 16000c Autoanalyzer (Abbott Diagnostics, Abbott Park, IL, United States) to measure the concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin (TBil).

Statistical analysis:
The data was collected and analyzed using SPSS version 19. All the values were expressed in mean and standard deviation.

RESULTS:

This experimental study consist of 55 male albino mice. As compared to the control group, the experimental group showed significantly higher levels of serum ALT ($P = 0.001$) and significantly lower levels of AST ($P = 0.001$). None of the antioxidant treatments affected the MTX-induced ALT levels (all, $P > 0.05$), but the NAC treatment produced a significant decrease in the MTX-induced serum AST level ($P < 0.05$). MTX exposure appeared to have no effect on either ALP or TBil levels in serum ($P > 0.05$ *vs* control group).

Table 01: Effects of methotrexate on biochemical parameters of liver damage in serum

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	T. bilirubin (mg/dL)
Control	38 (34-48)	96.5 (74-135)	187 (168-271)	0.1 (0.1-0.2)
MTX treated group	121.5	39	112	0.1
MTX + NAC (50mg/kg/BW)	107.5	50	84.5	0.1
MTX + NAC (100mg/kg/BW)	70.5	32	57.9	0.1

As compared to the control group, the MTX treated group showed a significantly higher level of MDA activity and significantly lower levels of GSH, SOD, and XO activities (all, $P < 0.05$); however, MTX exposure appeared to have no effect on CAT activity ($P > 0.05$). None of the antioxidant treatments produced a significant change in the MTX-stimulated MDA activity or in the MTX-reduced XO activity (all, $P > 0.05$). The NAC treatment produced a significant increase in both the MTX-reduced GSH activity ($P < 0.05$) and SOD activity ($P < 0.01$).

Table 02: Effects of methotrexate and antioxidant treatments on biochemical parameters of liver damage in liver tissues

Groups	MDA (nmol/g)	GSH (μmol/g)	SOD (U/g)	CAT (K/g)
Control	409 (352-466)	3.02 (2.85-3.43)	71.78 (61.88-97.81)	25.12 (21.08-29.28)
MTX treated group	445.5	2.42	61.46	23.89
MTX + NAC (50mg/kg/BW)	432	3.12	70.22	22.16
MTX + NAC (100mg/kg/BW)	432.5	3.01	64.78	20.88

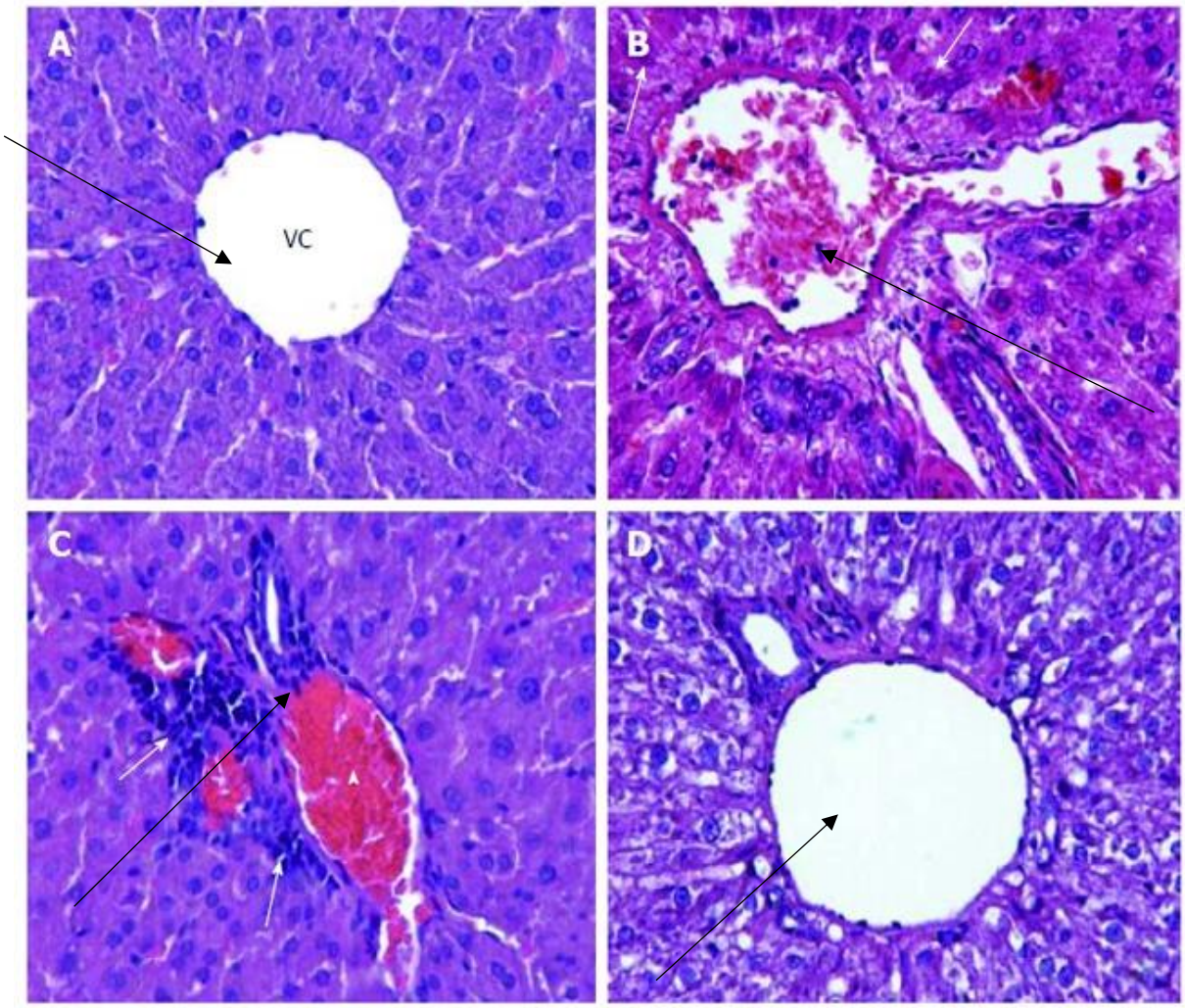

Figure 01: Methotrexate-induced effects on hepatic structure in rats. **A:** Normal histological appearence of H-E stained control liver tissues, **B:** Abnormal histological appearance of H-E stained model liver tissues, showing hepatocytes with eosinophilic cytoplasm; **C:** Inflammatory cells in the portal area and **D:** Hydropic degeneration (vacuolization/cellular swelling) in hepatocytes.

DISCUSSION:

Methotrexate-like chemotherapeutic agents have been used broadly in the treatment of various cancer diseases and some inflammatory diseases. One of the serious adverse effects caused by use of MTX is hepatotoxicity. Approaches to reduce this complication are valuable in order to improve the quality of life of patients and to ensure that treatment is more successful [8-10].

In this study, we also found that serum AST, ALT and GGT activities were significantly increased ($p<0.05$) in the methotrexate group compared to the control group. In the NAC + MTX group, we detected that NAC given for protection purpose decreased the levels of these enzymes significantly compared to the MTX group. Antioxidant functions and mechanisms of NAC have been identified in human studies. NAC realizes this feature by inhibiting H_2O_2 formation and thus protecting the cells from oxidative stress. Because of antioxidant feature of NAC, it serves in reducing liver injury. It has been detected that applied NAC in hepatotoxicity significantly attenuates serum AST and ALT levels and decreases proportion of damaged hepatocytes [11].

Previous studies in rats have also shown that prophylactic delivery of multiple antioxidant agents can prevent MTX-induced hepatotoxicity. For example, Tunali-Akbay et al and Dalaklioglu et al demonstrated that resveratrol, a potent antioxidant in rats but with unknown and questionable efficacy in

humans, protects against MTX-induced hepatotoxicity by decreasing hepatic MDA tissue level and increasing hepatic GSH and CAT activities [12]. Ali et al demonstrated the protective effect of chrysin against MTX-induced hepatotoxicity and showed that the drug was capable of enhancing the therapeutic index of MTX when the two were administered simultaneously [13]. Demiryilmaz et al demonstrated that administration of thiamine phosphate to rats led to significant reductions in oxidant parameters (*i.e.*, MDA and MPO) and to increases in antioxidant parameters (*i.e.*, GSH and SOD) in the liver tissue. Uraz et al showed that ursodeoxycholic acid provided protection against MTX-induced hepatocyte, again by using the rat model system [14].

CONCLUSION:

It is concluded that the mechanism of MTX-induced hepatic injury likely involves oxidative stress pathways; development or progression of this life-threatening condition may be prevented by prophylactic or therapeutic delivery of antioxidant agents, such as NAC. The hepatoprotective effects of NAC depend on its ability of the enhancement of antioxidant defense system, and its ability to reduce pro-inflammatory and apoptosis signal pathway.

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