

Hepato-Protective Effect of Folic Acid and Vitamin B12 in Comparison to N-acetylcysteine in Experimentally Induced Acetaminophen Toxicity in Rats

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ABSTRACT

To compare the effectiveness of vitamin B12 and folic acid in comparison to N-acetylcysteine (NAC) as hepato-protective agent against liver damage in experimentally induced acute acetaminophen toxicity in Sprague dawley rats. 18 male Sprague dawley rats were randomly divided into three groups of six each. Group A was administered NAC, group B - vitamin B12 and group C - folic acid intraperitoneally one hour after an acute over-dosage of acetaminophen 3mg/kg. After 48 hours, 1.5 ml of blood was withdrawn and the animals were sacrificed for histopathological examination of liver. The mean AST and ALT of vitamin B12 group did not differ statistically from that of the NAC group. The folic acid group had higher mean AST though not statistically significant whilst the mean ALT was significantly higher compared to NAC group. The reduction in the mean ALP and GGT with vitamin B12 showed statistically significant reduction whereas the folic acid group did not. Histopathology of liver revealed that the tissue architect was totally disturbed with portal tract appearing hemorrhagic and congested in addition to centrilobular necrosis in the folic acid group. In vitamin B12 group, the architecture of tissue was preserved with only mild inflammation. Vitamin B12, unlike folic acid has proved to be an efficacious hepato-protective agent in animal model of acute acetaminophen toxicity.

Keywords: Acetaminophen toxicity, Folic acid, Vitamin B12, Liver enzymes, Histopathology.

INTRODUCTION

Acetaminophen or paracetamol is one of the commonly used non-steroidal anti-inflammatory drugs. Though considered as safe analgesic-antipyretic, an overdose can lead to hepatotoxicity.

Each year in the United States, approximately 6% of adults are prescribed acetaminophen doses of more than 4 g/day and 30,000 patients are hospitalized for acetaminophen toxicity.¹ Acetaminophen overdose is clinically significant as it is the most common cause of drug-induced liver failure.² Liver

injury occurs in 17% of adults with unintentional acetaminophen overdose.¹ Acute tubular necrosis is yet another manifestation of acute acetaminophen toxicity and it can occur either isolated or along with hepatotoxicity.³

N-acetylcysteine (NAC) is used as the treatment of choice for acetaminophen overdosage.⁴ Intravenous and oral formulations of NAC are available for the treatment of acetaminophen overdose. Though the current standard of care involves NAC, the use of this drug has many adverse effects like allergy, anaphylaxis, angioedema, bronchospasm. These reactions are dose-dependent and preceded by release of histamine. Hypotension can occur due to the vasodilatory effect and in severe cases, there can be consequent oliguria which may lead on to the issue of pre-renal pathology for an acute renal failure.⁶ Animal studies and previous case reports strongly suggest that massive NAC overdose can cause cerebral edema and seizures.⁷

Though it has been well established that NAC can reduce mortality in acute overdose toxicity of acetaminophen, it is important to understand that the risk of adverse reactions to NAC do exist as is the risk of its overdosage.^{6, 8} Noteworthy, the preparation of NAC for intravenous administration is quite complex.⁶ Cases of iatrogenic NAC overdose have been reported in literature and it is important as this could even lead to death of the patient due to progressive hemolysis, thrombocytopenia and acute renal failure.^{6, 7}

A high incidence of more than 60% patients treated with NAC has been reported to develop ADR.⁸ Vitamin B12 and folic acid have been demonstrated to possess hepato-protective effect in other models of liver injury. Though one animal study indicating the effectiveness of folic acid in liver injury induced by acetaminophen has been reported in literature,⁹ there are no studies evaluating the protective role of vitamin B12 in this animal model and in addition, this is the first study comparing the hepato-protective activity of vitamin B12 with folic acid in experimentally induced acute acetaminophen toxicity in rats in order to identify the potentially less toxic alternative to NAC by evaluating the effects of vitamin B12 and folic acid on liver enzymes and

histopathology of liver tissue in comparison to that of NAC.

MATERIALS AND METHODS

The institutional animal ethics committee approved the protocol of the study (Approval number: 289/2015/IAEC). 18 Adult, male Sprague dawley rats weighing 200-300g were housed at constant ambient temperature in 12hr light, 12hr dark cycle. They were group housed as 3 animals per cage in polypropylene cages. Pellet diet and tap water were provided *ad libitum*. The animals were randomly allocated into the following three groups of 6 rats per group.

Group A: 6 rats in which acetaminophen at a dose of 3g/kg was given and NAC at a dose of 150mg/kg once after 1 hour of acetaminophen toxic dose. Group B: 6 rats in which acetaminophen at a dose of 3g/kg was given and vitamin B12 at a dose 10mg/kg once after 1 hour of acetaminophen toxic dose. Group C: 6 rats in which acetaminophen at a dose 3g/kg was given and folic acid 8mg/kg administered once after 1 hour of acetaminophen toxic dose.

The animals are weighed and the drugs were orally gavaged based on the body weight. 48 hours later, 1.5ml of blood was collected from the tail vein of the rats and liver function test (LFT) was performed. Quantitative analyses of liver enzymes namely, aspartate-aminotransferase (AST) or serum glutamic-oxaloacetic transaminase (SGOT), alanine-aminotransferase (ALT) or serum glutamic-pyruvic transaminase (SGPT), alkaline phosphatase (ALP) and α -glutamyl transpeptidase (GGT) were performed. The values of these enzymes thus estimated were indicative of the severity of liver damage and were statistically analyzed.

All the rats were then sacrificed and liver specimens were taken, fixed in 10% formaldehyde and embedded in paraffin. Sections were cut from the tissue blocks, mounted on slides, and stained with Van Gieson's stain. They were examined under microscope for liver cell damage and disruption of normal architecture. [Figure1]

Statistical analysis: The data was entered in Excel and analyzed using SPSS software version

19. Comparison of the mean of liver enzymes was done using one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test. The difference between the groups was considered statistically significant if $p < 0.05$. Histopathological changes between groups were qualitatively analyzed.

RESULTS

The mean AST (260.2 ± 54.7) of vitamin B12 group was lower compared to that of the group which received NAC with mean AST (319.8 ± 57.6) and though the mean ALT for vitamin B12 group (114.3 ± 32.4) was higher than that of NAC

Table 1: Comparison of liver enzymes between groups using one way ANOVA

Parameter	Between groups comparison	Mean difference	p value
AST	Groups A and B	59.667	0.103
	Groups A and C	-30.167	0.393
ALT	Groups A and B	-27.167	0.348
	Groups A and C	-207.333*	0.000
ALP	Groups A and B	119.167*	0.001
	Groups A and C	-378.000*	0.000
GGT	Groups A and B	-0.400	0.372
	Groups A and C	-1.650*	0.002

*The mean difference is significant at the 0.05 level.

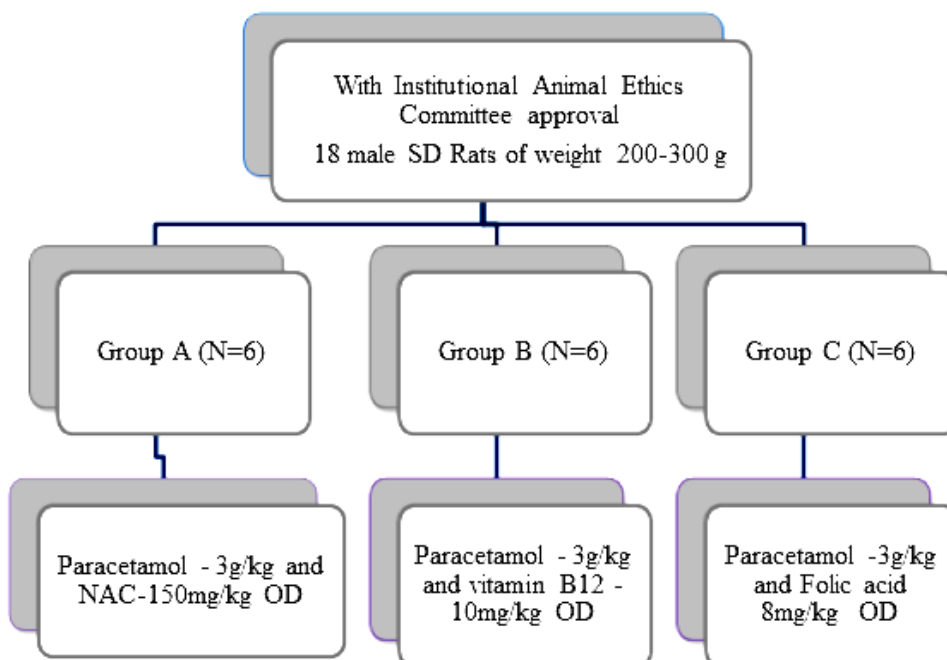


Fig. 1: Methodology

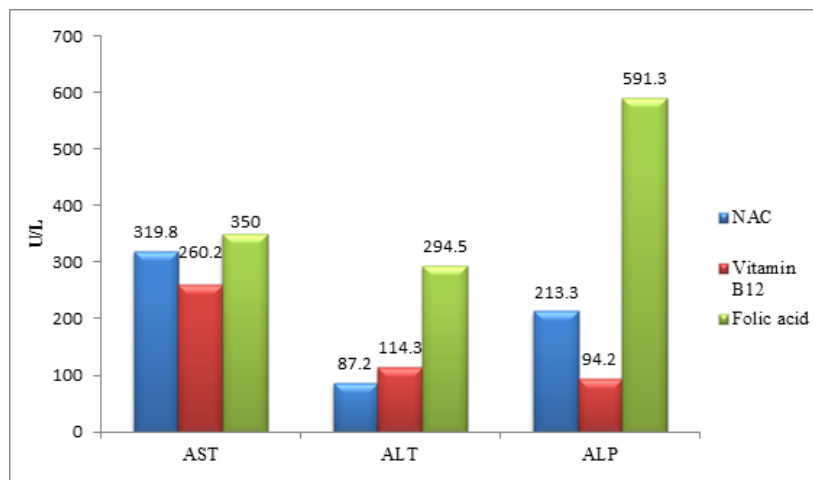


Fig. 2: Comparison of mean AST, ALT, ALP between groups

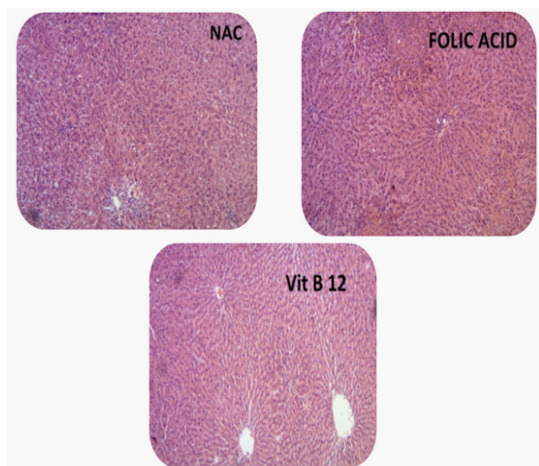


Fig. 3: Histopathology picture of the study groups

group (87.2 ± 41.5), [Figure2] the difference was not statistically significant by ANOVA with *post-hoc* LSD test either for AST ($p=0.103$) or ALT ($p=0.348$) between the two groups. [Table1]

On the contrary, folic acid group demonstrated a higher mean AST (350 ± 65.4) and mean ALT (294.5 ± 65.5) than NAC group. Comparison of the folic acid group with the group that received standard NAC treatment using ANOVA revealed that there was no statistical significance for the difference in the mean AST ($p= 0.393$) unlike that for mean ALT ($p< 0.001$). In addition, the vitamin B12 group had a significantly lower AST with mean difference of -89.8 ± 34.3 ($p= 0.019$) as well as

for ALT with mean difference of -180.2 ± 28.0 ($p< 0.001$) when compared to the group given folic acid. [Figure2 and table1]

Interestingly, there was a statistically greater reduction in mean ALP for vitamin B12 (94.2 ± 61.2) compared to NAC group (213.3 ± 48.2) whereas folic acid group (591.3 ± 44.8) had a statistically higher mean ALP ($p< 0.001$) indicating that folic acid did not demonstrate ALP reduction analogous to vitamin B12. [Table1]

Though mean GGT was slightly higher for the vitamin B12 group (1.18 ± 0.77) compared to the NAC group (0.78 ± 0.68), there was no statistical significance ($p=0.372$) for the difference in the mean values. However GGT for folic acid group (2.43 ± 0.79) mirrored the ALP wherein there was a statistically significant higher mean GGT ($p=0.002$) observed for folic acid group compared to the NAC group. [Table1]

Histopathological examination of the liver specimens revealed interface hepatitis, focal hepatic necrosis and thinning of sinusoidal plate in group A. In contrast, the group which received folic acid demonstrated tissue architect being totally disturbed with portal tract appearing hemorrhagic and congested in addition to centrilobular necrosis. Group B or vitamin B12 group had the architecture of tissue preserved with only mild inflammation. [Figure3]

DISCUSSION

This animal study has brought out the effective lowering of the liver enzymes by vitamin B12 in acute acetaminophen toxicity. The protective efficacy of vitamin B12 in reducing the liver damage after acetaminophen overdose is also evident from the histopathological changes observed in the study. It has been well established that acetaminophen induced liver damage causes centrilobular necrosis associated with a marked increase in AST, ALT activity.^{10, 11, 12} Also, the increase in serum ALP activities is supported by the findings of earlier studies that, acetaminophen overdose is also accompanied by an increase in ALP activity due to hepatocellular injury.^{10, 13} The reactive oxygen species-sensitive transcription factor namely nuclear factor-kappaB (NF-kappaB) is said to play a vital role in genesis of acetaminophen induced hepatotoxicity¹² and it has been reported that oxidative stress mediated by the metabolite N-acetyl-P-benzoquinone-imine (NAPQI) is considered to be the main cause of hepatic injury which involves depletion of cellular ATP, reduced Ca^{+2} ATPase activity with increased cytosolic Ca^{+2} levels, apoptosis and lipid peroxidation mediated by reactive oxygen species associated with a concomitant increase in AST, ALT, ALP.^{14,15} It has also been hypothesized that reactive oxygen species induced lipid peroxidation generates peroxynitrite, a powerful oxidant and nitrating agent. Peroxynitrite can consequentially modify cellular macromolecules, aggravating mitochondrial dysfunction and ATP depletion, thus leading to cellular necrosis in hepatocytes and sinusoidal endothelial cells.¹⁶

The effectiveness of vitamin B12 parallels that of NAC since there is no significant difference in the mean AST, ALT and GGT values between the two groups [Table1]. In addition, the reduction in mean ALP was statistically greater for the vitamin B12 group than the NAC group. In comparison, folic acid did not demonstrate a similar effectiveness either in reduction of raised hepatic enzymes or reversal of histopathological changes induced by acute administration of high dose acetaminophen. [Figure3] This is in contrast to an earlier study which indicated lowering of liver enzymes and improvement in hepatocellular damage histopathologically following administration of folic acid in acetaminophen induced toxicity in rats. Folic acid, though has

plausible hepato-protective effect attributed to its property of eliminating the changes that are related to the oxidative stress such as lipid peroxidation and oxidative DNA damage,^{9, 17} our study did not replicate a similar efficacy and this can be ascribed to a lower dose of acetaminophen for induction of toxicity in the previous studies and disparity in the time duration of blood withdrawn for estimation of liver enzymes. Such hepato-protective property of folate supplementation regimens after induction of liver toxicity using either chromium in rabbits or folate deprivation with copper exposure in rats have been reported in literature.^{18,19} The contradiction in findings between different studies probably stems from the fact that there is some inherent variation in the pathways and diversity in the intensity of oxidative stress induced by different models.

On the other hand, our study has delineated a significant hepato-protective effect of vitamin B12 demonstrating its efficacy in acute acetaminophen toxicity rat model.[Table1 and Figure3] The potential mechanisms contributory to this beneficial effect is the ability of vitamin B12 to maintain the sulphhydryl level under oxidative conditions despite lack of radical scavenging property.²⁰ Moreover, vitamin B12 has been shown to protect rat primary hepatocytes from hepatotoxin-induced cell death in *in-vitro* experiments.²⁰ Evidence suggests that the activity of glutathione reductase is significantly lower in B12 deficient liver²¹ and that the interaction between vitamin B12 and glutathione could protect against disease related to vitamin B12 deficiency.²² The presence of cobalt complex in vitamin B12 has been shown to effectively inhibit the hepatic inflammation and fibrosis.²⁰ Besides, protective effect of vitamin B12 in experimental liver injury by carbon tetrachloride^{23, 24} has been well documented in literature wherein the beneficial effect has been primarily hypothesized as owing to the improvement in mitochondrial integrity and this could be another contributory factor in the hepato-protection in acetaminophen toxicity amelioration also.

CONCLUSION

This study highlights that vitamin B12 exhibits significant hepato-protective effect and hence can be considered as a potential therapeutic

agent, alternative to N-acetyl cysteine in acute acetaminophen overdose after appropriate human trials.

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