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Original Article

Formulating a New Pharmaceutical Drug; Acetaminophen Tablet Containing N-acetyl Cysteine, To Alleviate the Severity of Liver Damage in Rats: Phase I, Animal Study

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Abstract

Background and Aim: Acetaminophen (APAP) is a commonly used analgesic and also the leading cause of medication-induced liver damage. On the other hand, N-acetylcysteine (NAC) is a medication widely used to treat APAP overdose. Despite this interest, a few studies have investigated the co-administration effects of these medications. Therefore, this study aimed to evaluate the effects of NAC and APAP on renal and liver functions in rats when they use concurrently.

Methods: Male Wistar rats were orally treated with a single dose of APAP (700 mg/kg) alone or in combination of NAC at the three different doses (200, 500, and 700 mg/kg). After 24 hours, the blood and liver samples were collected for biochemical and histopathological evaluations.

Results: Liver damage was well established in the 700 mg/kg APAP-treated rats, as evidenced by elevated the plasma levels of aspartate transaminase (AST) and alanine transaminase (ALT). In addition, the plasma level of blood urea nitrogen (BUN) was significantly increased in the APPA group compared to the control group. Moreover, histological examinations revealed that liver degeneration was evident in APAP-treated animals. NAC only at the highest dose (700 mg/kg) could inhibit ALT elevation, but had no effect on AST and BUN levels. Interestingly, co-administration of NAC (700 mg/kg) with APAP (700 mg/kg) could slightly shift liver histological alterations from the irreversible stage (fibrosis) toward reversible lesions such as necrosis and hemorrhage.

Conclusion: The study findings indicate that co-administration of NAC and APAP can reduce the severity of APAP-induced liver damage in rats.

Keywords: Acetaminophen; Acetylcysteine; Drug Interaction; Drug-Induced Acute Liver Injury.

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Introduction

Paracetamol, also known as acetaminophen or APAP, is widely used to treat pain and fever. Even though APAP is generally safe at recommended doses, its overdose can cause liver toxicity (1). APAP liver toxicity is among the most commonly occurring poisonings worldwide (2). N-acetylcysteine (NAC) was first proposed in 1970 as an effective antidote for APAP poisoning, inhibiting the binding of N-acetyl-P-benzoquinone (NAPQI) to

liver cells (3). NAC is a well-known and readily available antidote for the treatment of APAP-induced toxicity. It also has antioxidant and anti-inflammatory properties (4, 5). NAC is not only prescribed as an antidote for APAP toxicity, but also it is effective in eliminating the toxicity of heavy metals such as lead (6). Moreover, it is administered in internal medicine to treat idiopathic lung fibrosis and as an agent with antimicrobial and vasodilator effects (7-9). It is used to prevent contrast-induced nephropathy and prevent possible hearing loss following gentamicin use, especially in children and infants (10). Despite the widespread use of high doses of NAC, no toxicity has yet been reported. Evidence demonstrated that the golden time for NAC consumption as an antidote after APAP overdose is 8-hour (11).

In 2011, Mehrpour et al. suggested that a drug can be produced by mixing of APAP and NAC in order to prevent the toxic effects of APAP(12). Subsequently, some studies have examined the concomitant effect of the two drugs (13). We recently found that co-administration of NAC with APAP can improve the antinociceptive effect of APAP(14). Therefore, we are aiming to formulate a new pharmaceutical drug, acetaminophen tablet containing NAC. So, in the first step, we designed this animal study to evaluate the efficacy of this combination on the severity of liver and kidney damage in rats.

Methods

Animals and study design

The protocol of this study was based on the international rules of handling laboratory animals approved by the Ethics Committee of Birjand University of Medical Sciences (Permit code: IR.bums.REC.1395.296).

48 male Wistar rats weighing 200 ± 20 g were obtained from the animal facility of Research Centre of Experimental Medicine, Birjand University of Medical Sciences. The animals were kept in propylene cages, temperature-controlled room (22±2º C) with a 12-h light/dark cycle. The animals had free access to the standard laboratory animal diet (Behparvar, Iran) and tap water during the study period. The rats were randomly assigned into six equal groups (n= 8, each). Before starting investigations, the rats were kept on fast for 12-h but had free access to water.

Experimental protocol

The rats were randomly divided into six equal groups (n=8, each) and treated as follows:

- Group I (control): This group only received saline;
- Group II (NAC): This group was treated with 700 mg/kg NAC dissolved in saline;
- Group III (APAP): Treated with 700 mg/kg APAP dissolved in saline as a model group;
- Group IV (APAP+NAC200): Concurrently treated with 700 mg/kg APAP and 200 mg/kg NAC;
- Group V (APAP+NAC500): Concurrently treated with 700 mg/kg APAP and 500 mg/kg NAC;
- Group VI (APAP+NAC700): Concurrently treated with 700 mg/kg APAP and 700 mg/kg NAC.

All the treatments were done orally in equal volume (1mL) using a gastric tube. After 24 hours of the investigations, the animals were anesthetized using intraperitoneal injection of ketamine and xylazine (75:10 mg/kg) (15). Afterward, the blood samples were collected from the heart, and serum was separated by centrifugation at 2500 g for 10 minutes. The biochemical parameters, including blood urea nitrogen (BUN), creatinine (Cr), aspartate transaminase (AST), and alanine transaminase (ALT), were evaluated using standard diagnostic kits (Bionick, Iran).

Following the blood collection, the liver of each animal was immediately dissected out, weighed, and placed in 10% formalin. After 48 hours, the liver samples were processed for paraffin embedding. Sections of 5 thick were cut, deparaffinized in xylene, rehydrated in serial graded ethanol solutions, and then used for Masson's Trichrome Staining to evaluate liver damage.

Statistical analysis

Data analysis was performed using SPSS19 software. Shapiro-Wilk test was used to evaluate the data homogeneity. ANOVA test was employed in case of the normal distribution; otherwise, the Kruskal-Wallis test was applied. Tukey post hoc test

was used to compare each pair of groups. P values ≤0.5 were considered significant.

Results

Biochemical parameters

The results of serum concentrations of liver enzymes (AST, ALT) and kidney function markers (BUN, Cr) were presented in Table 1. Accordingly, Both liver enzymes and BUN levels were significantly elevated in APAP-treated group in comparison with the control ($p < 0.01$, each). Co-administration of APAP with NAC in all doses could not ameliorate AST elevation in rats. However, NAC-treated rats exhibited a significant decrease only in ALT level compared to the APAP-poisoned group. In other words, ALT level of the APAP+NAC700 group was significantly lower than APAP-poisoned group ($p < 0.05$).

Co-administration of NAC with APAP at all doses could not mitigate BUN increasing in APAP-treated rats. Despite the fact that Cr levels in the APAP-poisoned animals tended to increase, but the change was not statistically significant in comparison with control group ($p = 0.051$).

Macroscopic change of livers

Figure 1 shows the results of the liver-to-body weight ratio in the studied groups. Compared to the control group, the mean liver-to-body ratios of the APAP, APAP+200 mg/kg NAC, APAP+500 mg/kg NAC, and APAP+700 mg/kg NAC were significantly decreased ($p <0.05$). None of the treatments could prevent this reduction in the animals.

Histological evaluation

Histological examination of the liver sections of normal and experimentally treated animals was presented in Figures 2 and 3. The liver tissues belong to the control group exhibited normal appearance without any significant inflammation, sinusoidal hemorrhage or dilatation, and hepatocytes degeneration. Similarly, NAC-treated normal rats showed normal liver architecture without any evident degeneration, inflammation, or hemorrhage (Figure 2).

On the other hand, the liver sections of APAP-treated animals exhibited severe liver damage, which represents diffuse fibrotic lesions between hepatocytes, portal space, lobular central vein, and even sinusoids (Figure 3). Moreover, other histological changes such as infiltration and sinusoidal hemorrhage were evident in this group. Histological examination of APAP-treated rats which were concurrently treated with NAC at the doses of 200 and 500 mg, revealed pathological appearance as same as APAP group. However, at the dose of 700 mg/kg, NAC could mitigate APAP histological damages from irreversible changes such as fibrosis to reversible changes like necrosis (Figure 3).

In the present study, concurrent administration of NAC with a toxic dose of APAP (700 mg/kg) was investigated to determine whether NAC could prevent APAP poisoning. Accordingly, biochemical parameters regarding liver function, including AST and ALT and kidney function markers such as BUN and Cr, were evaluated. Furthermore, liver histology was performed to assess the severity of APAP-induced hepatic injury in rats. This study clearly demonstrated that APAP (700 mg/kg) induced liver toxicity and renal damage by increasing liver enzymes activity and elevating BUN levels, respectively. Histological examination of liver tissues showed that APAP induced severe and irreversible pathological alterations, mainly collagen diffusion and fibrosis.

NAC at the highest dose could significantly prevent ALT elevation in rats. None of the NAC doses could ameliorate the elevations of AST and BUN levels and also improve liver-to-body weight ratio in APAP-poisoned rats. It worth be noted that 24 hours after APAP poisoning, all of the rats in the model group (APAP 700 mg/kg) were lethargic and sedentary, while those receiving NAC demonstrated normal behavior, suggesting an effect of NAC against APAP toxicity.

Based on the current protocols, NAC is recommended as a known antidote to control the toxic effects of APAP. The FDA-approved dosage regimen for oral NAC starts with a loading dose of 140 mg/kg followed by 17 doses, each at 70 mg/kg, given every 4 hours. An important factor in evaluating the effectiveness of NAC is the timing, and it is practically ineffective if it is taken after 10 hours from APAP overdose (16). Several studies

indicate that NAC consumed within 8 hours from APAP overdose can inhibit its toxic effects (11).

As stated earlier in this paper, in 2011, Mehrpour et al. hypothesized that a drug composed of APAP and NAC be produced to prevent the toxic effects of APAP (12). In 2015, Owumi et al. tested this hypothesis on an animal model. The results of their study indicated that oral co-administration of these two drugs positively prevented APAP poisoning (13). In 2017, Mast et al. treated rats with foods containing 1% APAP and 0.5% NAC for two weeks, which effectively reduced ALT levels and inhibited weight loss (17). Another pharmacological issue of formulating a combination of APAP and NAC is its efficacy. Therefore, our team carried out an experimental study in which the antinociceptive activity of APAP+NAC formulation has been investigated. We found that co-administration of NAC with APAP can improve the antinociceptive effect of APAP (14).

The liver is the main body organ in the metabolism and detoxification of drugs and is prone to be harmed by a large number of medicinal and environmental chemicals. Regardless of its cause, liver damage occurs with five general types of responses (inflammation, degeneration, necrosis and apoptosis, fibrosis, and cirrhosis) (18). Our results revealed that NAC in a dose dependent manner inhibited pathological alterations in the liver tissue of APAP-poisoned rats. NAC could prevent liver fibrosis, as the sign of the last stage of liver damage, although there were still some complications such as necrosis, hemorrhage, and dilation of the sinusoid space.

Liver cell necrosis is also one of the most common responses of the liver to toxins so that in the case of severe coagulation necrosis, liver cells get weakly stained and become mummified, and their nuclei are often destructed. In our study, the poisoned group received a dose of 700 mg/kg of NAC, and more mildly, the group APAP+NAC 500 showed necrosis of liver cells, especially in the space around the lobular central vein, indicating ischemic injury due to toxic reactions to drugs. Although liver cell necrosis can precede inflammation, the reverse can also occur (19). Inflammation after the onset of mononuclear cells' exposure to liver cells is one of the common causes of liver degeneration, which was not observed in this study. The fibrous tissue is generally formed in response to swelling or direct damage to the liver. Unlike all previous responses that are reversible, fibrosis (similar to the case observed in the poisoned group, NAC doses of 200 and 500 mg/kg) is generally considered to be an irreversible complication of liver damage. Collagen deposition causes a change in liver parenchymal hematuria. At the initial stages, fibrosis can occur around the portal tracts and the lobular central vein (similar to our findings) or directly in the space of Disse (as in the APAP group). As the fibrosis continues, nodules from regenerating liver cells are formed, a state that is known as cirrhosis (long-term effects not studied in our study) (19). According to the findings of this study, NAC co-administration with APAP could effectively modify the liver tissue damage from the irreversible phase (fibrosis) to reversible stages, such as necrosis and apoptosis.

Table 1. Comparison of liver enzymes and renal function biochemical markers between the studied groups

Groups	**AST** (IU/L) Mean ± SD	**ALT** (IU/L) Mean ± SD	**BUN** (mg/dl) Mean ± SD	**Cr** (mg/dl) Mean ± SD
Control	125.57±23.39	63.75±16.52	38.71 ± 4.95	0.90±0.05
NAC 700 mg/kg	86.86±25.61	52.86±9.08	32.42 ± 6.47	0.83±0.05
APAP (poisoned)	506.25±30.21***	315.57±46.70***	73.83 ± 15.95**	1.23±0.34
APAP+200mg/kg NAC	523.83±171.10***	318.17±112.72***	79.0 ± 35.69**	0.95±0.33
APAP+500mg/kg NAC	437.00±126.76***	327.20±86.15***	70.8 ± 6.45**	1.02±0.18
APAP+700mg/kg NAC	564.14±79.95***	187.57±77.19*,#	70.7 ± 19.29**	0.94±0.15

APAP: acetyl-para-aminophenol, NAC: N-acetylcysteine, AST: aspartate transaminase, ALT: alanine transaminase, BUN: blood urea nitrogen, Cr: creatinine. Values are expressed as mean ± S.D., n = 8 animals per group. * $P < 0.05$, ** $P < 0.01$, and ***$P < 0.001$ versus control group; # $P < 0.05$ versus APAP group..

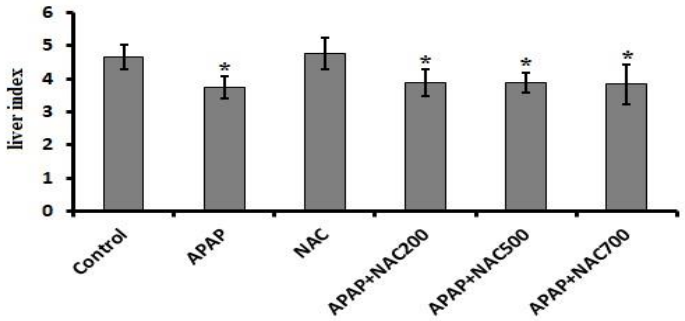

Figure 1. Liver index [(liver weight/body weight) × 100] in the studied groups. Values are presented as mean ± S.D, n = 8 in each group. APAP: acetyl-para-aminophenol, NAC: N-acetylcysteine. One way ANOVA followed Tukey's post-hoc test*p < 0.05: compared to control group.

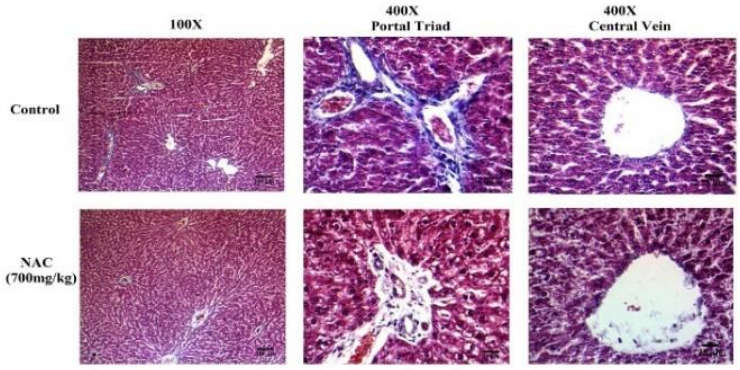

Figure 2. Histological evaluation of liver sections of normal rats treated with saline (control) or 700mg/kg of N-acetylcysteine (NAC), stained with Masson's Trichrome technique. 100x magnification of the liver sections showing the lobular central veins, the portal spaces, the normal sinusoidal radial branches, and the normal structure of the liver in both control and NAC groups. The portal space histology in control group indicated the presence of a small connective tissue and a very small number of mononuclear cells. Minimum connective tissue without any infiltration observed in the portal triad of NAC group. The lobular central vein of control and NAC groups indicating normal structure with normal sinusoidal space, without any signs of fibrosis.

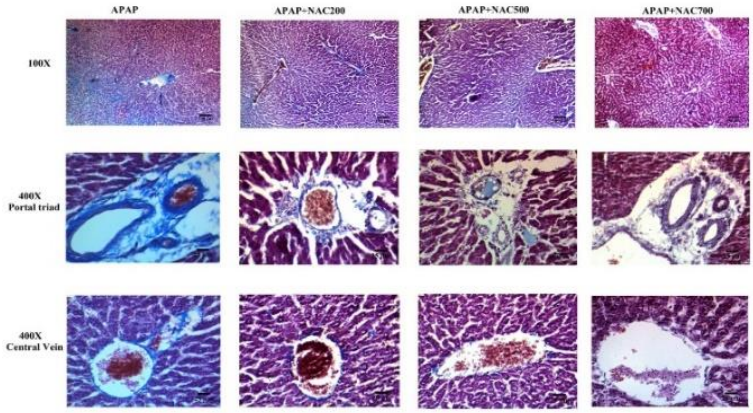

Figure 3. The liver sections of rats received 700 mg/kg acetyl-para-aminophenol concurrently with saline (APAP), or 200-700 mg/kg of N-acetylcysteine (APAP+NAC200, APAP+NAC500, and APAP+NAC700). The sections were stained with Masson's Trichrome technique.100x magnification indicating the wide diffusion of collagen fibers between hepatocytes and lobular central veins of all groups except APAP+NAC700. The portal triad space representing extensive collagen deposition in APAP group; whereas, in NAC treated rats the diffusion dose dependently has been moderated as far as normal in APAP+NAC700. In APAP, APAP+NAC200 and APAP+NAC500 groups, central vein fibrosis is evident, but normal structure with the least collagen deposition observes in APAP+NAC700 group.

Discussion

In the present study, concurrent administration of NAC with a toxic dose of APAP (700 mg/kg) was investigated to determine whether NAC could prevent APAP poisoning. Accordingly, biochemical parameters regarding liver function, including AST and ALT and kidney function markers such as BUN and Cr, were evaluated. Furthermore, liver histology was performed to assess the severity of APAP-induced hepatic injury in rats. This study clearly demonstrated that APAP (700 mg/kg) induced liver toxicity and renal damage by increasing liver enzymes activity and elevating BUN levels, respectively. Histological examination of liver tissues showed that APAP induced severe and irreversible pathological alterations, mainly collagen diffusion and fibrosis.

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Conclusion

Based on the results of this study, NAC concomitant use with APAP can reduce the severity of APAP-induced liver toxicity in rats. Further clinical studies are needed to determine the effectiveness of NAC in conjunction with APAP to create a combined drug in individuals at risk of overdose and suicide, such as those with a history of psychiatric disorders (especially those with depression and personality disorders).

Conflict of Interest

The authors declare no conflict of interest.

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Ethics

This study was approved by the Ethics Committee of Birjand University of Medical Sciences (Permit code: IR.bums.REC.1395.296).

References

1. Saljoughian M. Acetaminophen intoxication: a critical-care emergency. US Pharm. 2016;41(12):38-41.

2. Yoon E, Babar A, Choudhary M, Kutner M, Pyrsopoulos N. Acetaminophen-Induced Hepatotoxicity: a Comprehensive Update. J Clin Transl Hepatol. 2016;4(2):131-42.

3. Chun BJ, Moon JM, Kim SH. Antidote for acetaminophen poisoning: N-acetylcysteine. J Korean Med Assoc. 2013;56(12):1067-75.

4. Mokhtari V, Afsharian P, Shahhoseini M, Kalantar SM, Moini A. A Review on Various Uses of N-Acetyl Cysteine. Cell J. 2017;19(1):11-7.

5. Pei YP, Liu H, Yang Y, Yang YW, Jiao Y, Tay FR, et al. Biological Activities and Potential Oral Applications of N-Acetylcysteine: Progress and Prospects. Oxidative Medicine and Cellular Longevity. 2018;2018:2835787.

6. Wang L, Wang ZY, Liu JZ. Protective effect of N-acetylcysteine on experimental chronic lead nephrotoxicity in immature female rats. Human & Experimental Toxicology. 2010;29(7):581-91.

7. Demedts M, Behr J, Buhl R, Costabel U, Dekhuijzen R, Jansen HM, et al. High-dose acetylcysteine in idiopathic pulmonary fibrosis. New England Journal of Medicine. 2005;353(21):2229-42.

8. Dodd S, Dean O, Copolov DL, Malhi GS, Berk M. N-acetylcysteine for antioxidant therapy: pharmacology and clinical utility. Expert Opin Biol Ther. 2008;8(12):1955-62.

9. Girouard H, Chulak C, Wu L, Lejossec M, De Champlain J. N-acetylcysteine improves nitric oxide and α-adrenergic pathways in mesenteric beds of spontaneously hypertensive rats. American journal of hypertension. 2003;16(7):577-84.

10. Millea PJ. N-Acetylcysteine: Multiple Clinical Applications. American Family Physician. 2009;80(3):265-9.

11. Kerr F, Dawson A, Whyte IM, Buckley N, Murray L, Graudins A, et al. The Australasian Clinical Toxicology Investigators Collaboration randomized trial of different loading infusion rates of

N-acetylcysteine. Ann Emerg Med. 2005;45(4):402-8.

12. Mehrpour O, Ballali-Mood M. Why not formulate an acetaminophen tablet containing N-acetylcysteine to prevent poisoning? Journal of Medical Toxicology. 2011;7(1): 95–6.

13. Owumi SE, Andrus JP, Herzenberg LA, Herzenberg LA. Co-administration of N-Acetylcysteine and Acetaminophen Efficiently Blocks Acetaminophen Toxicity. Drug Development Research. 2015;76(5):251-8.

14. Nakhaee S, Dastjerdi M, Roumi H, Mehrpour O, Farrokhfall K. N-acetylcysteine dose-dependently improves the analgesic effect of acetaminophen on the rat hot plate test. BMC Pharmacology and Toxicology. 2021;22(1):4.

15. Hassanzadeh-Taheri M, Hosseini M, Salimi M, Moodi H, Dorranpour D. Acute and sub-acute oral toxicity evaluation of Astragalus hamosus seedpod ethanolic extract in Wistar rats. Pharmaceutical Sciences. 2018;24(1):23-30.

16. Greene SC, Noonan PK, Sanabria C, Peacock WF. Effervescent N-Acetylcysteine Tablets versus Oral Solution N-Acetylcysteine in Fasting Healthy Adults: An Open-Label, Randomized, Single-Dose, Crossover, Relative Bioavailability Study. Current therapeutic research, clinical and experimental. 2016;83:1-7.

17. Mast C, Pourpe C, Voyard G, Remond D, Migne C, Centeno D, et al. Dietary supplementation with cysteine prevents adverse metabolic outcomes of repeated cures with paracetamol in old rats. The British journal of nutrition. 2017;118(11):889-96.

18. Hassanzadeh-Taheri M, Hassanpour-Fard M, Doostabadi M, Moodi H, Vazifeshenas-Darmiyan K, Hosseini M. Co-administration effects of aqueous extract of turnip leaf and metformin in diabetic rats. Journal of traditional and complementary medicine. 2018;8(1):178-83.

19. Kumar V, Abbas AK, Fausto N, Aster JC. Robbins and Cotran pathologic basis of disease. 9th ed. Elsevier. 2014. p.610-621.