ORIGINAL ARTICLE

HEPATOPROTECTIVE EFFECTS OF CHOLECALCIFEROL AND N-ACETYLCYSTEINE IN ACETAMINOPHEN INDUCED HEPATOTOXICITY IN MICE

Farzana Munir, Uzma Naeem, Salma Salim*, Aeman Choudhary**

Department of Pharmacology, Islamic International Medical College Rawalpindi, *Mohi-ud-din Islamic medical college Mirpur Azad Kashmir, **Postgraduate trainee, Pharmacology, Islamic International Medical College Rawalpindi, Pakistan

Background: The liver is a critical factor that has a vital role in metabolism and excretion of various chemical substances and drugs. Liver harm is a major problem to health that challenges healthcare vendors and the pharmaceutical industry. Acute liver injuries caused by various toxic chemicals, drugs, high alcohol intake, and microbes are studied well. This study aimed to measure the out-turn of cholecalciferol in comparison to N-acetylcysteine in acetaminophen-induced hepatotoxicity in mice. **Methods:** It was an experimental randomized control trial carried out in the Department of Pharmacology, Islamic International Medical College in collaboration with National Institute of Health Islamabad. Forty adult Balb-C mice were divided into four groups with 10 mice in every group. Group 1 was the Negative control group, and the experimental groups 2, 3, and 4 were administered acetaminophen to set off hepatotoxicity which was confirmed after one week through measuring alanine transaminase (ALT) levels. Group 3 was then administered cholecalciferol, and group 4 was given N-acetylcysteine. Terminal sampling was done on day 28 to evaluate the results. Statistical analysis was accomplished on SPSS-22. Comparison among groups was done using one-way ANOVA and student's *t*-test, and $p<0.05$ was taken statistically significant. **Results:** Mice of groups 3 and 4 significantly reduced ALT levels as compared to group 2. Cholecalciferol significantly decreased ALT levels in hepatotoxic mice with efficacy greater than N-acetylcysteine. **Conclusion:** Cholecalciferol is more efficacious in the reversal of acute liver injury as compared to N-acetylcysteine.

Keywords: Acetaminophen, Hepatotoxicity, Alanine Transaminase, Cholecalciferol, N-acetylcysteine

Pak J Physiol 2023;19(1):14–6

INTRODUCTION

Liver is one of the most crucial organs that features as a centre for the nutrients metabolism and waste metabolites excretion.[1] The liver is not only the most important organ for detoxification of foreign substances but also a great object of their toxicity because of its ability to detoxify toxic substances and functionally intervene between the site of resorption and the systemic circulation.[2] The constant liver injury involves oxidative stress, which is an imbalance between pro- *vs* anti-oxidants and the generation of reactive oxygen species which attack the hepatocytes.[3] This causes liver distress and presents with distortion of the metabolic functions, a reduction in glutathione (GSH) levels, a rise in tissue lipid peroxidation, and cellular necrosis.[4] Furthermore, serum levels of some biomarkers like transaminases, alkaline phosphatase, and bilirubin are generally increased in liver diseases.[4] Acute liver injury can be experimentally induced by various chemicals and medications which cause liver inflammation. One of those agents is acetaminophen which causes dose-dependent liver toxicity.[5]

Acetaminophen (APAP) commonly called paracetamol, is extensively in use anywhere in the international as a well-place analgesic and antipyretic drug. It is the main suggested reason for acute failure of liver in the world.[6] The most important concern with the paracetamol usage is to keep away from overdose; the consumption of an increase dose of acetaminophen often reasons intense acute harm to the liver. The acute acetaminophen toxicity might also supply high to probably fatal hepatic and renal damage in humans and laboratory animals.[7]

Cholecalciferol, called as vitamin D3, is generated through pores and skin and is found in some foods too. Its basic purpose is to preserve ordinary levels of serum calcium and phosphate to maintain bone strength. Cholecalciferol requires two hydroxylation tactics for its activation. The first hydroxylation occurs in the liver through the 25-hydroxylase enzyme and yields 25-hydroxycholecalciferol. It has an extended half-life compared to 1,25-dihydroxycholecalciferol. The other hydroxylation takes place in the kidneys through the 1-α-hydroxylase enzyme.[8]

N-acetylcysteine (NAC) is a spinoff of the aminoacid L-cysteine. It is a reduced glutathione precursor with direct antioxidant activities.[9] Detoxifying action of NAC was discovered during the 1970s and then it was used as an acetaminophen antidote as the usual remedy for the APAP toxicity. N-acetylcysteine acts as an antioxidant showing direct and indirect activities.[10] This study aimed at evaluation of comparative hepatoprotective effects of Cholecalciferol and N-acetylcysteine in acute liver injury.

METHODOLOGY

This experimental randomized controlled study was performed at Multidisciplinary Research Laboratory and Pharmacology Laboratory, Islamic International Medical College, Rawalpindi, and National Institute of Health, Islamabad after approval of Ethical Review Committee (Appl. #Riphah/IRC/21/60) of the institute, from Sept 2021 to Aug 2022. A sum of 40 male[10] mice weighing 30–50 grams without any bodily anomaly and with normal baseline criterion were included in the study and fed on commercial feeding diet with water *ad libitum*. The cages of mice were located in a nicely-aerated room maintained at 24±3 °C, humidity 60±11%, and a light/dark cycle of 12-hour.[11] After acclimatization for 1 week, the mice were divided into 4 groups of 10 each. Group 1 was normal control group fed on a normal diet with no intervention. The experimental groups 2, 3, and 4 were administered a single intraperitoneal injection of acetaminophen at a dose of 300 mg/Kg[12] on the 8th day. Hepatotoxicity was confirmed after one week (on day 15) in these groups by taking LFTs and comparing them with the control group. After confirmation, the disease control group mice (Group 2) continued normal diet, the group 3 mice received cholecalciferol at a dose of 1,000 IU/Kg[13] and Group 4 mice received N-acetylcysteine 300 mg/Kg dissolved in 0.9% physiological saline solution[10] for 2 weeks. Final sampling was performed via cardiac puncture on day 28. The blood was centrifuged at 5,000 rpm, and serum was collected and stored at -9 ºC in Eppendorf tubes for ALT estimation.[14] Student's *t*-test was used to analyze individual parameters in the groups and $p<0.05$ was considered statistically significant.

RESULTS

Alanine transaminase levels were increased significantly ($p<0.05$) in groups 2, 3, and 4 compared to group 1 on day 15 (Table-1).

On day 28 when different groups were compared, serum ALT levels decreased significantly in groups 3 and 4 mice compared to group 2. The values of groups 3 and 4 mice were almost equal to control mice (Figure-1).

Mice in group 3 and 4 mice were compared using paired *t*-test and it was observed that group 3 mice that were treated with cholecalciferol showed a significantly more decrease in ALT levels as compared to N-acetylcysteine treated group 4 mice (Table-2)

Table-1: ALT values of groups on days 0 and 15 of the experiment (Mean±SD)

Groups	ALT at day 0	ALT at day 15	*p*
Group-1	44.6±2.1	44.3±1.91	<0.001
Group-2	45.1±1.98	162.3±1.67	
Group-3	43.5±1.58	164.0±0.48	<0.001
Group-4	44.8±1.67	160.8±2.11	<0.001

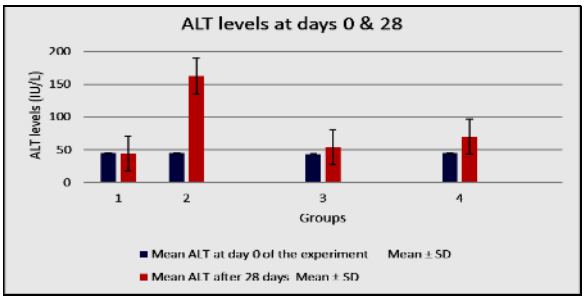

Figure-1: ALT levels on days 0 and 28

Table-2: ALT levels in groups 3 and 4 on day 28

Group 3	Group 4	*p*
54.0±0.48	69.8±2.11	0.001

DISCUSSION

Drug-induced liver injury is an unsolved health issue. Acetaminophen is a drug that most commonly induces hepatic damage at high doses due to the accumulation of its metabolites.[14] High levels of ALT make important confirmation for evaluating the extent of liver damage experimentally.[15]

In this study acetaminophen 300 mg/Kg was given intraperitoneally as a single injection which resulted in markedly high serum ALT levels in comparison to the control group. Munawar R *et al*[14] also used acetaminophen in this way to induce hepatotoxicity. Kazemifar *et al*[16] used one 800 mg/Kg oral dose of acetaminophen for induction of hepatotoxicity in rats, and they notified a marked increase in ALT and AST levels. This study is in harmony to another study[17] that showed marked changes in LFTs due to the toxic liver effect of acetaminophen.

Both Cholecalciferol and N-acetylcysteine have antioxidant potential and show hepatoprotective activity.[18] Emphasis on cholecalciferol has been given in this study as it is a commonly used supplement. The comparison with N-acetylcysteine has been done because its hepatoprotective effects are well-established.[19,20] According to our findings, reversal of the hepatic damage in the mice was seen with the usage of both cholecalciferol and N-acetylcysteine. Targher G, *et al*[21] determined the potential benefit of vitamin D3 supplementation in reducing the development and progression of non-alcoholic fatty liver disease. Recently Zhai *et al*[22] have observed the supplementation with cholecalciferol and calcitriol in patients with insulin resistance and with nonalcoholic fatty liver disease. Beneficial effects of vitamin D3 (1,25-dihydroxycholecalciferol) on hepatotoxicity induced by thioacetamide (TAA) were studied in rats by Özdemir-Kumral *et al*[23] who concluded that vitamin D3 provided defence against liver harm in a rat model of TAA-induced hepatotoxicity through inhibition of oxidative stress, inflammatory reaction and apoptosis. There was a

marked difference (p<0.05) in the mean ALT levels of groups 3 and 4, although both the groups showed a decrease in ALT levels compared to group 2 (p≤0.05).

CONCLUSION

Cholecalciferol is more efficacious than N-acetylcysteine in reversing acetaminophen-induced acute liver injury in mice model.

RECOMMENDATIONS

The molecular mechanism responsible for reducing/ reversing acetaminophen-induced hepatotoxicity can be explored by further studies. Histopathology aspects of this reversal of hepatotoxicity should also be explored.

REFERENCES

1. Balaban YH, Aka C, Koca-Caliskan U. Liver immunology and herbal treatment. World J Hepatol 2017;9(17):757–70.
2. Lu J, Tang M, Zhang T. Review of toxicological effect of quantum dots on the liver. J Appl Toxicol 2019;39(1):72–86.
3. Chen Z, Tian R, She Z, Cai J, Li H. Role of oxidative stress in the pathogenesis of nonalcoholic fatty liver disease. Free Radic Biol Med 2020;152:116–41.
4. Fracanzani AL, Petta S, Lombardi R, Pisano G, Russello M, Consonni D, *et al*. Liver and cardiovascular damage in patients with lean nonalcoholic fatty liver disease, and association with visceral obesity. Clin Gastroenterol Hepatol 2017;15(10):1604–11.e1.
5. Sarin SK, Kumar M, Eslam M, George J, Al Mahtab M, Akbar SMF, *et al*. Liver diseases in the Asia-Pacific region: a Lancet Gastroenterology & Hepatology Commission. Lancet Gastroenterol Hepatol 2020;5(2):167–228.
6. Chowdhury A, Nabila J, Adelusi Temitope I, Wang S. Current etiological comprehension and therapeutic targets of acetaminophen-induced hepatotoxicity. Pharmacol Res 2020;161:105102.
7. Yan M, Huo Y, Yin S, Hu H. Mechanisms of acetaminophen-induced liver injury and its implications for therapeutic interventions. Redox Biol 2018;17:274–83.
8. Al-Hashimi N, Abraham S. Cholecalciferol. StatPearls [Internet]. 2021 Jan 22. Available from: https://www.ncbi.nlm.nih.gov/books/NBK549768/ [cited 2021 Sep 16]
9. Elswefy SE, Abdallah FR, Wahba AS, Hasan RA, Atteia HH. Antifibrotic effect of curcumin, N-acetyl cysteine and propolis extract against bisphenol A-induced hepatotoxicity in rats: Prophylaxis versus co-treatment. Life Sci 2020;260:118245.
10. Sulaiman S, Hussain M, Nauman Shad M, Chiragh S. Hepatoprotective effect of Prazosin is comparable to N-Acetylcysteine in acetaminophen induced hepatotoxicity in mice. J Ayub Med Coll Abbottabad 2020;32(1):28–32.
11. Shendge AK, Panja S, Basu T, Ghate NB, Mandal N. Ameliorating effects of white mulberry on iron-overload-induced oxidative stress and liver fibrosis in Swiss albino mice. Food Chem Toxicol 2021;156:112520.
12. Dunn KW, Martinez MM, Wang Z, Mang HE, Clendenon SG, Sluka JP, *et al*. Mitochondrial depolarization and repolarization in the early stages of acetaminophen hepatotoxicity in mice. Toxicology. 2020;439:152464.
13. Anisiewicz A, Kowalski K, Banach J, Łabędź N, Stachowicz-Suhs M, Piotrowska A, *et al*. Vitamin D metabolite profile in cholecalciferol- or calcitriol-supplemented healthy and mammary gland tumor-bearing mice. Nutrient 2020;12(11):3416.
14. Munawar R, Jehangir A, Waheed A. Comparing hepatoprotective effects of aqueous extract of Cassia Fistula (Amaltas) leaves and silymarin. J Islamic Int Med Coll 2018;13(3):137–40.
15. Ltaif M, Gargouri M, Soussi A. Protective effects of A. Sativa against oxidative stress-induced liver damage in Ovariectomized mice. Biomed Res Int 2021 Jul 15;2021:5577498.
16. Kazemifar AM, Hajaghamohammadi AA, Samimi R, Alavi Z, Abbasi E, Asl MN. Hepatoprotective property of oral silymarin is comparable to N-Acetyl Cysteine in acetaminophen poisoning. Gastroenterology Res 2012;5(5):190–4.
17. Freitag AF, Cardia GF, da Rocha BA, Aguiar RP, Silva-Comar FM, Spironello RA, *et al*. Hepatoprotective effect of Silymarin (Silybum marianum) on hepatotoxicity induced by acetaminophen in spontaneously hypertensive rats. Evid Based Complement Alternat Med 2015;2015:538317.
18. Fahan Rashid HM, Syed U, Ahmad Z, Chauhdary KK, Musharraf U, Kumar S. Comparison of Different Formulations of Vitamin D. J Ayub Med Coll Abbottabad 2017;29(4):650–3.
19. Aliakbarian M, Nikeghbalian S, Ghaffaripour S, Bahreini A, Shafiee M, Rashidi M, *et al*. Effects of N-Acetylcysteine addition to the university of Wisconsin Solution on the rate of ischemia-reperfusion injury in adult orthotopic liver transplant. Exp Clin Transplant 2017;15(4):432–6.
20. El-Yamany MF, Zaki ES, Shaltout SA, Saad MA. Bone marrow mononuclear cells boost the anti-cytogentical aberration effect of N-acetylcysteine and α-lipoic acid in rat's liver and bone marrow: implication of oxidative and inflammatory pathways. Toxicol Mech Methods 2021;31(6):437–49.
21. Targher G, Scorletti E, Mantovani A, Byrne CD. Nonalcoholic fatty liver disease and reduced serum vitamin D(3) levels. Metab Syndr Relat Disord 2013;11(4):217–28.
22. Zhai LL, Zhu AK, Tang ZG. Effect of supplementation with calcitriol versus cholecalciferol on insulin resistance in patients with nonalcoholic fatty liver disease: Several issues of concern. Clin Nutr 2021;40(8):4845–6.
23. Özdemir-Kumral ZN, Erkek BE, Karakuş B, Almacı M, Fathi R, Yüksel M, *et al*. Potential effect of 1,25 dihydroxy vitamin D3 on thioacetamide-induced hepatotoxicity in rats. J Surg Res 2019;243:165–72.

Address for Correspondence:

Dr Farzana Munir, Department of Pharmacology, Islamic International Medical College, Rawalpindi, Pakistan. **Cell:** +92-332-0823318

Email: doc_farzana@hotmail.com

Received: 15 Nov 2022 **Reviewed:** 12 Mar 2023 **Accepted:** 20 Mar 2023

Contribution of Authors

FM: Concept, Data acquisition and analysis, Manuscript writing
UN: Concept, Data analysis and Guidance
SS: Data acquisition and analysis
AC: Study design, Methodology, Critical review

Funding: None
Conflict on Interest: None