ORIGINAL ARTICLE

HEPATOPROTECTIVE EFFECT OF ZINC COMPLEX OF BETULINIC ACID ON PYRAZINAMIDE INDUCED HEPATOTOXICITY IN MICE

Alia Rehman, Adnan Jehangir*, Fatima Mohammadi, Salma Salim***, Rabia Iftikhar**, Amber Rashid****

Department of Pharmacology, Mohtarma Benazir Bhutto Shaheed Medical College, Mirpur, AJK, * Punch Medical College, Rawlakot, AJK, **Islamic International Medical College, Rawalpindi, ***Mohi-ud-din Islamic Medical College, Mirpur, AJK

Background: Hepatotoxicity is a common side effect of pyrazinamide, a first line anti-tuberculous drug. Objective of this study was to determine the hepatoprotective effect of zinc complex of Betulinic acid (BA) on pyrazinamide induced hepatotoxicity in mice. **Methods:** This experimental**,** randomized control study was conducted at Islamic International Medical College, Rawalpindi, and National Institute of Health Islamabad from 1 Sep 2020 to 31 Aug 2021. Thirty male Balb/c albino mice were divided into three groups each having 10 mice. Group 1 received normal diet with no medication. Group 2 (NC) received pyrazinamide 500 mg/Kg daily for 28 days. Group 3 (DC) received Zinc complex of Betulinic acid 1 mg/Kg/day per oral once daily along with pyrazinamide. Final sampling was done on day 28 by intracardiac puncture for estimation of serum ALT and bilirubin level. Data was analysed on SPSS-21. Comparisons between the groups were analyzed using one way ANOVA (Post Hoc Tuckey test), and $p<0.05$ was considered significant. **Results:** Zinc complex of Betulinic acid treated group had significantly decreased serum ALT (58.00±5.639) and bilirubin (0.617±0.601) in comparison with pyrazinamide treated group. **Conclusion:** Zinc complex of Betulinic acid exerted significant hepatoprotective activity in pyrazinamide induced hepatotoxicity.

Keywords: Hepatoprotective, Pyrazinamide, Betulinic Acid, Zinc

Pak J Physiol 2023;19(3):36–8

INTRODUCTION

Liver is an indispensable organ in the human body which executes a myriad of crucial tasks, e.g., metabolism of fat, proteins and carbohydrates, coagulation factor production, metabolism of xenobiotics, drugs and various nutritional substances.[1] Due to the presence of liver between the site of absorption and systemic circulation, it can be the target of toxic metabolites.[2]

Tuberculosis is the 9th leading cause of deaths throughout the world. Every year 10 million new cases of tuberculosis are notified.[3] Pakistan is ranked at 5th position among first 22 high burden countries of tuberculosis. WHO report about TB incidence published in 2016 states that incidence in Pakistan is 270 per 100,000 population.[4] Treatment given for TB includes a combination regime which involves isoniazid, rifampicin, pyrazinamide, and ethambutol. Among these, isoniazid, rifampicin and pyrazinamide are hepatotoxic and their most common side effect is drug induced liver injury. The incidence of drug induced liver damage in tuberculosis patients taking ATT drugs is about 2–28%.[5] Various studies has documented that pyrazinamide is more hepatotoxic than isoniazid and rifampicin among first line anti-TB hepatotoxic drugs.[6]

Pyrazinamide (PZ) is a nicotinamide pyrazine analogue. It has bacteriostatic as well as bactericidal activity against mycobacterium tuberculosis. Studies on rat model have shown that PZ causes a change in activity of enzymatic as well as non-enzymatic antioxidants. Some of these antioxidants include superoxide dismutase (SOD), Glutathione (GSH), and malondialdehyde. Oxidative stress can be involved in pyrazinamide induced hepatotoxicity. These findings are further supported by the studies which have shown the down regulation of PPAR-α along with its target genes by pyrazinamide and improvement of pyrazinamide induced hepatotoxicity by fenofibrate which is a PPAR-α agonist.[7]

Betulinic acid (BA) is a lupane-type triterpenoid with a natural pentacyclic structure. It is very common and has a broad distribution in the plant kingdom. There are many different plants which contains BA such as *Betulacea* (birch tree), *Ziziphus* (Indian jujube), *Syzygium* (black plum)*, Diospyros* (date plum)*, Paeonia* (garden pony).[8] There are number of biological activities associated with this compound such as anti-tumour activity, anti-oxidant activity, hepatoprotective activity, cardio-protective activity, anti-inflammatory activity as well as nephroprotective effects.[9] Hepatoprotective effects of BA are attributed to the beneficial effects of this compound on antioxidant enzymes such as glutathione, glutathione peroxidase and superoxide dismutase along with betulinic acids ability to curb lipid peroxidation.[10]

Zinc is a trace element which is most common in human body. Zinc reduces oxidative stress by enhanced induction of metallothioneins. Zinc also produces its anti-oxidant effects by enhancing the activation of certain enzymes which are the component

of antioxidant system such as glutathione and catalase. It also causes stabilization of protein sulfhydryl groups against oxidation and also has antagonistic action against the chemical reactions which are catalysed by transition metals. Antioxidant effects of Zn are further enhanced by the ability of Zn to cause exchange of redox actives metals, e.g., copper and iron on certain binding sites.[11] Zn has an established hepatoprotective action.[12] Studies have shown that the hepatoprotective actions of Zn are attributed partly to their above mentioned antioxidant effects.[13]

Studies have shown the hepatoprotective effects of both the betulinic acid[10] and zinc[14] due to their antioxidant actions but limited data is available about their combined effect. This study was done to see the hepatoprotective effects of zinc complex of BA keeping in mind anti-oxidant effects of the compound so that it can be used as an adjunct drug therapy.

METHODOLOGY

It was an experimental randomized control study which was conducted at Pharmacology Department, Islamic International Medical College, Rawalpindi in collaboration with Riphah Institute of Pharmaceutical Sciences and Animal House of National Institute of Health, Islamabad. After approval of the Institutional Review Committee, the study was carried out in one year from 1 Sep 2020 to 31 Aug 2021.

A total of 30 healthy male albino Balb/C mice weighing 30–50 g, and aged 8 weeks, were included in the study. All mice were kept under room temperature of 22±2 °C and 12-hour light-dark cycle for 1 week. Zinc complex of Betulinic acid was prepared at Riphah Institute of Pharmaceutical Sciences, Islamabad. The mice were randomly divided into three groups each containing 10 mice. Group 1, i.e., normal control group was provided with tap water and normal diet. Group 2, was given pyrazinamide in dose of 500 mg/Kg.[15] Group 3 was given zinc complex of Betulinic acid 1 mg/Kg in 1% starch jelly along with pyrazinamide.[10]

On day 0 blood samples were taken from 2 mice in each group for baseline evaluation. Second blood sampling was done from 2 mice in each group on day 15. Final sampling was done on day 28. Sampling was done through cardiac puncture by using 3 cc syringes. Samples were allowed to clot. Serum was separated with centrifugation at 3,000 RPM for 5 minutes. Serum was aspirated in sterile tubes and ALT was estimated using ALT kit (Merck) and bilirubin was estimated using serum Bilirubin kit (Merck) on Chemistry Analyser, MicroLab 200 (Merck).

Data was analysed using SPSS-20. Mean and standard error of mean was calculated for all groups and Post-hoc test was done for comparison between the different groups. Results were considered significant at $p<0.05$.

RESULTS

Table-1 shows the comparison of Mean±SEM of all groups. The results of group 3 are comparable to group 2 and significant of $p<0.05$ was seen. The significant results are verified which are certainly comparable with the disease control group. In zinc complex of Betulinic acid treated group, there was a substantial drop in hepatic enzymes which shows the positive effect of zinc complex of Betulinic acid in the treatment of pyrazinamide induced hepatotoxicity.

Table-1: Comparison of ALT and Bilirubin in all groups (Mean±SEM)

Serum (U/L)	Group 1	Group 2	Group 3	*p*
ALT	41.17±4.238	165.67±7.592	58.00±5.639	0.000
Bilirubin	0.217±0.0477	1.000±0.0577	0.617±0.0601	0.000

DISCUSSION

In the present study it is observed that zinc complex of Betulinic Acid ameliorates the hepatotoxic effect induced by Pyrazinamide. Hepatoprotective effect of zinc complex of Betulinic acid was seen in group 3. Damage to the hepatocellular membrane leads to its disruption and release of hepatic enzymes. ALT and bilirubin are located in the cytosol and increased serum level of these enzymes suggests that the hepatocytes have been damaged.

Use of anti-oxidants has been proposed to combat liver injury caused by oxidative stress. Improvement in the biochemical markers like ALT and bilirubin in this study is supported by the study of Yi J *et al*[10] who studied the hepatoprotective activity of betulinic acid on alcohol induced liver damage in Kunming mice. They established that betulinic acid can cause improvement in the hepatic enzyme levels and a decrease in micro vesicular steatosis in mice administered with alcohol through improvement of tissue redox system, maintenance of antioxidant system and decrease lipid peroxidation in liver.

Reduction in the hepatic enzyme activity by betulinic acid was also studied by Zheng *et al*[16], who proved the hepatoprotective effect of betulinic acid on D-GalN/LPS-induced acute liver damage in mice. They showed that BA pretreatment improved the survival rate of mice administered with D-GalN/LPS, and attenuated serum transaminases. BA administration caused an increase in GSH and CAT activity, and decreased MDA level, which indicates that one of the hepatoprotective mechanisms of BA might be via the antioxidant defence system.

Abdullah *et al*[17] concluded that root extract of *Ziziphus oxyphylla*, a herb traditionally used for the treatment of hepatic diseases in Pakistan, showed improvement in hepatic enzyme levels. It also improved the antioxidant enzymes and decreased the lipid peroxidation in carbon tetrachloride induced hepatic damage in BALB/c mice. That study determined that the

hepatoprotective effects of *Ziziphus oxyphylla* are through improvement in antioxidant defense system and stabilization of membrane. The study also concluded that active components of *Ziziphus oxyphylla* responsible for its hepatoprotective action are pentacyclic triterpines the most important being Betulinic acid.[17]

Al-Jawad FH *et al*, studied the hepatoprotective effects of zinc by subjecting the rats to thallium poisoning. In his study he revealed that zinc caused a significant reduction in hepatic enzyme level such as ALT and AST indicating the hepatoprotective effects of zinc against hepatic injury caused by thallium. There was also preservation of normal hepatic architecture in zinc treated mice.[18]

Improvement in serum ALT and bilirubin by administration of zinc was also documented by Wardah Siddique, *et al*. They documented that administration of zinc to isoniazid and rifampicin induced hepatotoxicity in mice leads to the improvement in not only the biochemical but histological parameters as well.[19]

In the present study improvement in biochemical parameter in group 3, was observed indicating that zinc complex of Betulinic acid can be used in the treatment of pyrazinamide induced hepatotoxicity.

CONCLUSION

Zinc complex of Betulinic acid significantly lowers hepatic enzyme in pyrazinamide induced hepatotoxicity in mice. Zinc complex of Betulinic acid can be used as an adjunct in the prevention of pyrazinamide induced hepatotoxicity.

REFERENCES

1. Shehata AA, Farrag AA, Elhady EE, Elsheikh HH. Effect of Mushroom Fungus Feeding on Induced Hepatotoxicity Rats. Egypt J Appl Sci 2020;35(11):127–42.
2. Thuwaini MM, Al-Derawi KH, Kadhem H. The possible protective effect of carthmustinctorius (Leaves) on anti-tuberculosis (rifampin & isoniazid) drugs-induced hepatotoxicity in rats. Int J Pharm Res 2018;10(4):516–22.
3. Khan MD, Ahmad B, Hussain A, Ishaq T, Haider O, Murtaza HG. Frequency of drug-resistant mycobacterium tuberculosis in Chiniot, Pakistan. Life Sci J Pak 2021;3(1):3–7.
4. Abebe G, Bonsa Z, Kebede W. Treatment outcomes and associated factors in tuberculosis patients at Jimma University Medical Center: A 5-Year retrospective study. Int J Mycobacteriol 2017;6(3):239–45.
5. Marjani M, Fahimi F, Sadr M, Dizaji MK, Moniri A, Khabiri S, *et al*. Evaluation of Silymarin for management of anti-tuberculosis drug induced liver injury: A randomized clinical trial. Gastroenterol Hepatol Bed Bench 2019;12(2):138–42.
6. Hussain Z, Zhu J, Ma X. Metabolism and hepatotoxicity of Pyrazinamide, an antituberculosis drug. Drug Metab Dispos 2021;49(8):679–82.
7. Zhang Y, Guo H, Hassan HM, Ding PP, Su Y, Song Y, *et al*. Pyrazinamide induced hepatic injury in rats through inhibiting the PPARα pathway. J Appl Toxicol 2016;36(12):1579–90.
8. Ríos JL, Máñez S. New pharmacological opportunities for betulinic acid. Planta Med 2018;84(1):8–19.
9. Silva FSG, Oliveira PJ, Duarte MF. Oleanolic, ursolic and betulinic acids as food supplements or pharmaceutical agents for type 2 diabetes: promise or illusion? J Agric Food Chem 2016;64(15):2991–3008.
10. Yi J, Xia W, Wu J, Yuan L, Wu J, Tu D, *et al*. Betulinic acid prevents alcohol-induced liver damage by improving the antioxidant system in mice. J Vet Sci 2014;15(1):141–8.
11. Marreiro DD, Cruz KJ, Morais JB, Beserra JB, Severo JS, de Oliveira AR. Zinc and oxidative stress: Current mechanisms. Antioxidants (Basel) 2017;6(2):24.
12. Aftab K. Hepatoprotective role of zinc sulphate in carbon tetrachloride induced liver toxicity. J Pharmacol Clin Res 2016;1(3):1–4.
13. Liu J, Zhou ZX, Zhang W, Bell MW, Waalkes MP. Changes in hepatic gene expression in response to hepatoprotective levels of zinc. Liver Int 2009;29(8):1222–9.
14. Jarosz M, Olbert M, Wyszogrodzka G, Młyniec K, Librowski T. Antioxidant and anti-inflammatory effects of zinc. Zinc-dependent NF-κB signaling. Inflammopharmacology 2017;25(1):11–24.
15. Taziki S, Khori V, Jahanshahi M, Seifi A, Babakordi F, Nikmahzar E. Protective role of taurine against hepatotoxicity induced by pyrazinamide in rats. Natl J Physiol Pharm Pharmacol 2018;8(6):824–8.
16. Zheng ZW, Song SZ, Wu YL, Lian LH, Wan Y, Nan JX. Betulinic acid prevention of D-galactosamine/lipopolysaccharide liver toxicity is triggered by activation of Bcl-2 and antioxidant mechanisms. J Pharm Pharmacol 2011;63(4):572–8.
17. Abdullah, Khan MA, Ahmad W, Adhikari A, Ibrar M, Rehman MU, *et al*. Exploration of hepatoprotective potential and phytochemicals of ziziphus oxyphylla edgew. Pak Vet J 2020;40(4):431–6.
18. Al-Jawad FH, Sharquie KE, Abu Raghif, Nashtar SB. Hepatoprotective effects of zinc sulphate and silymarin against thallium-induced poisoning in rats. Iraqi Acad Sci J 2016;1:42–57.
19. Siddique W, Rashid N, Warraich NY, Talat H, Talha MU, Siddique H, *et al*. Comparison of hepatoprotective effect of silymarin and zinc sulfate against hepatotoxicity induced by isoniazid and rifampicin in albino rats. Pakistan J Med Heal Sci 2020;14(1):113–9.

Address for Correspondence:

Dr Alia Rehman, Department of Pharmacology, Mohtarma Benazir Bhutto Shaheed Medical College, Mirpur, Azad Jammu & Kashmir, Pakistan. **Cell:** +92-347-5493859

Email: aliarehmanraja@yahoo.com

Received: 7 Sep 2021 **Reviewed:** 20 Sep 2023 **Accepted:** 22 Sep 2023

Contribution of Authors:

AR: Concept, data acquisition, analysis and manuscript writing
AJ: Concept, data acquisition, analysis and manuscript writing
FM: Design, methodology, analysis and manuscript writing
SS: Design, methodology, revision of project and critical review if intellectual contents
RI: Revision of project and critical review of intellectual contents
AR: Revision of project and critical review of intellectual contents

Conflict of Interest: None
Funding: None