



INTERNATIONAL JOURNAL OF RESEARCH IN SCIENCE & TECHNOLOGY

e-ISSN:2249-0604; p-ISSN: 2454-180X

The Protective Potential of Metformin against Liver Acetaminophen Toxicity

Dina Suhail

PhD in Al-Yarmouk University College, Department of Pharmacy, Iraq

Paper Received: 29th May, 2021; **Paper Accepted:** 07th July, 2021;
Paper Published: 17th July, 2021

DOI: <http://doi.org/10.37648/ijrst.v11i03.001>

How to cite the article:

Dina Suhail, The Protective Potential of Metformin against Liver Acetaminophen Toxicity, IJRST, Jul-Sep 2021, Vol 11, Issue 3, 1-6, DOI: <http://doi.org/10.37648/ijrst.v11i03.001>



ABSTRACT

The inadequacy to replace acetaminophen (APAP) with a more effective analgesic continues its use in therapeutic interventions, upholding the risk of hepatotoxicity. Depletion of glutathione reserves by a metabolic intermediate of acetaminophen, N-acetyl-p-benzoquinone imine (NAPQI), is the major reason. The current study presents the combinatorial effect of metformin, a biguanide, in ameliorating the APAP toxicity. HepG2 cells were used for in vitro studies and MTT and LDH leakage assays were used for viability assessment. 10 μ M of metformin improved the cell viability and membrane integrity of cells treated with a high antioxidant enzymes and reduced glutathione were significantly increased in cells co-administrated with metformin and acetaminophen

Keywords: *metformin, acetaminophen toxicity, liver toxicity, HepG2 cells, necrosis*

INTRODUCTION

Acetaminophen (N-acetyl-p-aminophenol, APAP) was characterized as a most common used secure and beneficial painkiller and antipyretic. Despite of its beneficial effects on inflammation, Acute overdose and chronic use of APAP lead to APAP poisoning with serious complications, including necrosis of the centrilobular cells of the liver [1]. In 2009, the American Association of Poison Control Center offered about 56,000 emergency room visits, 26,000 hospitalizations, and over 400 deaths caused by misuse of APAP and/or drugs containing the compound [2]. The adverse reactions of APAP were associated with the reactive metabolite derived from hepatic cytochrome P-450 metabolism. At the initial stage of metabolism, APAP was oxidized to a reactive substance, N-acetyl-p-

benzoquinone imine (NAPQI), which was then conjugated and detoxified with glutathione (GSH) to produce nontoxic metabolites [3]. At noxious amount of APAP, the excessive generation of NAPQI eventually results in discharge of glutathione. In rodents, higher doses of APAP can saturate the sulfation and glucuronidation pathways, leading to excessive formation NAPQI [2]. Glutathione depletion was only a cascade of intracellular events including mitochondrial oxidative stress, production of reactive oxygen and nitrogen species, activation of stress proteins and gene transcription mediators, and mobilization of the liver's essential immune system. Ultimately, imbalance of the innumerable pathways can lead to hepatic cell loss [4]. That condition was due to the increment of liver enzymes levels after 12 – 36 h of the drug overdose [5]. Metformin, a

widely used dimethylbiguanide anti-hyperglycemic agent, was shown to exert antioxidant effects [6], along with an increase in the reduced liver and blood levels of glutathione (GSH) in diabetes [7]. Moreover, metformin reduces the activities of plasma xanthine oxidase and erythrocyte glutathione peroxidase, suggesting its protective effect against oxidative stress [8]. According to high incidence of APAP poisoning and the resulted severe liver damages, finding hepatoprotective agents seems to be necessary. Therefore, the current study aimed to examine the hepatoprotective effect of metformin in APAP-induced toxicity in mice.

MATERIALS AND METHODS

Animal performance

Body weight gained as well as 24 hr water and feed intake, measured 24 hr post acetaminophen toxicity. All animals given drinking water and standard laboratory diet before and during the experiment. All test animals were subjected to a one-week experimented condition

Study design

Three group with tow mice were chosen the entire study took 7 days to finish.

G1-served as control

G2-served as positive given acetaminophen 250mg/ kg by oral gavage on the fourth day to induce liver damage

G3-frome the first day to the seventh day given metformin 200mg/ kg as hepatoprotective while given acetaminophen 250mg/ kg oral gavage on the fourth day to induce liver damage

Histopathologic studies

The animal then dissected and the livers carefully removed and washed with 0.9% saline solution part of liver sample was preserved in formalin solution for histopathologic study this study could be carried out to assess the degree of damage. This done by staining the fine section of liver isolates and examine under microscope

RESULTS

Liver weight and lesions:

In the current study the absolute mean weight in all groups group2 and group3 relative liver weight were significantly heavier than group1 after hours post aminophenol toxicity. when acetaminophen was given to mice in group2 extensive necrosis was associated with hemorrhage and seen in the liver section. When metformin taken through a week pre and post 1 acetaminophen dose hemorrhage and necrosis of liver tissue was less the finding indicates possible recovery from initial

acetaminophen toxicity It has been demonstrated that metformin at dose of 200mg/ kg significantly reverses oxidant/ antioxidant imbalance induced by acetaminophen toxicity .in this regard we have shown that metformin protect hepatocyte against lipid peroxidation which induce disruption of the phospholipids bilayer membrane and cell integrity and inhibition leakage of cytoplasmic components and enzymes to the blood and the subsequent cell necrosis there for the importance to finding a protective agent against mitochondrial dysfunction in acetaminophen over dose. To assess the effect of metformin on acetaminophen induce liver injury without inhibit its metabolic activation metformin was given 2h post acetaminophen at which time the

metabolic activation of ApAp is almost completed

Histopathologic examination

G1 sample: gross: size 2×2cm

Section show normal hepatic tissue with normal hepatocytes cords, no portal inflammation, no necrosis seen

G2 sample: gross size 2*2cm

Section show portal inflammation mainly lymphocytes, spotty necrosis mainly near for the portal triad and focal hemorrhage

G3 sample: gross size 2.5*2cm

Section show diffuse steatosis, mild focal portal inflammation (mainly lymphocytes), no necrosis seen (figure 1)

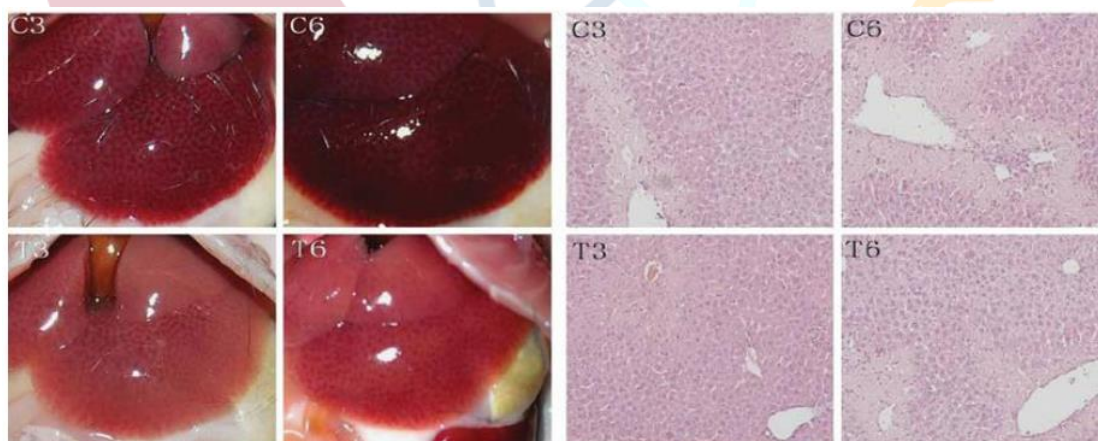


Figure 1. Necrosis stages in area of the liver

DISCUSSION

Acetaminophen overdose was considered a common cause of acute liver failure. The hepatotoxicity leads to mitochondrial oxidative stress and subsequent necrotic hepatocellular death. The current study proposed the possibility of metformin to protect hepatocytes upon acute acetaminophen toxicity.

A study in Iran to examine the protective effect of metformin on acetaminophen-induced oxidative stress, inflammation and subsequent hepatotoxicity in Male BALB/c mice, administered (250 mg/kg/d) acetaminophen for one week then metformin was given (100 and 200 mg/kg/d, p.o.) for three weeks, their results indicated that acetaminophen caused focal hepatocyte necrosis, inflammation and fatty degeneration, in addition elevation of tissue levels of AST, ALT, ALP and MDA, and reduces SOD and GSH activities, while Metformin (200 mg/kg/d) significantly normalized MDA, SOD and GSH levels ($p < 0.001$), and exerted a hepatoprotective effect by significant decreasing ALT, AST and ALP concentrations ($p < 0.001$) (Saravi et al, 2016). Although liver regeneration was commonly done by hepatocytes, there were still various other factors contributing to liver recovery from APAP hepatotoxicity, including hepatic microvasculature

reconstitution and interactions between different cell types (Yan et al, 2018). Metformin had AMPK-independent effects on the liver that may include inhibition of fructose-1,6-bisphosphatase by AMP. As cell and tissue responses are not only a product of dose, but also of treatment duration and model used, we suggest that the physiological relevance of the effects of metformin identified in cells is best validated through studies carried out in vivo, ideally in humans given metformin by the oral route (Rena et al, 2017).

CONCLUSION

The study was shown that metformin can exert an oxidant/ antioxidant balance and limit the inflammation and hepatocyte injury and subsequent increase in cell survival in the ApAp induced liver toxicity. The investigation was performed to present a new treatment to attenuate the hepatotoxicity induced by ApAp over dose for further interpretation to humans. Metformin not inhibit JNK activation or mitochondrial JNK translocation but reduced PAPA protein adducts formation in mitochondria and the mitochondrial oxidant stress and subsequent mitochondrial dysfunction through inhibition of mitochondrial complex 1 activity.

REFERENCE

- [1] Anon. 2010. Tylenol professional product information. Fort Washington (MD): McNeil Consumer Healthcare, Division of McNEIL-PPC, Inc. Bajt ML, Knight TR, Farhood A, Jaeschke H. Scavenging peroxynitrite with glutathione promotes regeneration and enhances survival during acetaminophen-induced liver injury in mice. *J PharmacolExpTher.* 307:67–73, 2003
- [2] R. Dart, A. Erdman, K. Olson, G. Christianson and A. Manoguerra. 2006. Acetaminophen poisoning: an evidence based consensus guideline for out-of-hospital management. *ClinToxicol (Phila).* 44:1–18, 2006
- [3] N. Kaplowitz, M. Shinohara, Z. Liu, and D. Han. How to protect against acetaminophen: don't ask for JUNK. *Gastroenterology.* 135:1047–1051, 2008.
- [4] M. McGill, C. Williams, M. Taha, S. Curry and H. Jaeschke. The mechanism underlying acetaminophen induced hepatotoxicity in humans and mice involves mitochondrial damage and nuclear DNA fragmentation. *J Clin Invest.* 122:1574–1583, 2012
- [5] M. Poon, P. Chiu, D. Mak and K. Ko. Metformin protects against carbon tetrachloride hepatotoxicity in mice. *J Pharmacol Sci.* 93:501–504, 2003.
- [6] A. Reid, R. Kurten, S. McCullough, R. Brock and J. Hinson. Mechanisms of acetaminophen-induced hepatotoxicity: role of oxidative stress and mitochondrial permeability transition in freshly isolated mouse hepatocytes. *J PharmacolExpTher.* 312:509–516, 2005.
- [7] M. Tavafi. Complexity of diabetic nephropathy pathogenesis and design of investigations. *J Renal Inj Prev.* 2:59–62, 2013a
- [8] M. Tavafi. Diabetic nephropathy and antioxidants. *J Nephrothol.* 2:20–27, 2013b.
- [9] S. Saravi, A. Hasanvand, K. Shahkarami and A. Dehpour. The protective potential of metformin against acetaminophen induced hepatotoxicity in BALB/C mice, *Pharmaceutical Biology,* vol54:pp12, 2830-2837, 2016.
- [10] M. Yana, Y. Huob, S. Yina and H. Hu. Mechanisms of acetaminophen-induced liver injury and its implications for therapeutic interventions. *Redox Biology* 17, 274–283, 2018.
- [11] G. Rena, D. Hardie and E. Pearson. The mechanisms of action of metformin. *Diabetologia.* vol 60, pp 9 :1577-1585, 2017 Sep.