

Effects of L- glutamine on oxidative stress in gentamicin induced hepatotoxicity rats

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Abstract

Introduction: Liver is a vital organ and one of its functions is to release the harmful materials from the body. Gentamicin is an aminoglycoside used to treat gram-negative bacteria infections, though it causes renal and liver injuries. Antioxidants play effective roles in decreasing gentamicin-induced liver injuries. L-glutamine has antioxidant properties and is used to decrease gentamicin-induced liver injuries.

Methods: In this study, thirty two wistar rats were randomly divided into four groups of eight as follows: 1) control group, 2) gentamicin, 100 mg/kg for 12 days, 3) L-glutamine, 25 mg/kg by gavage for 12 days, 4) group received both gentamicin and L-glutamine after 12 days. The blood sample of the heart of rats was taken through anesthesia and its serum was used to evaluate alanine transaminase (ALT) and aspartate transaminase (AST). The livers of rats were also isolated to evaluate malondialdehyde (MDA), glutathione peroxidase GPX, catalase (CAT) and glutathione (GSH).

Results: Treatment with gentamicin caused some changes in the liver function. We observed an increase not only in AST and ALT, but also in MDA. In addition a decrease was seen in antioxidant enzymes such as CAT, GPX and GSH. In group treated with glutamine, the amount of AST and MDA has significantly decreased compared to group treated with gentamicin. Glutamine significantly increased GPX activity and the level of GSH compared to gentamicin group.

Conclusion: The findings of this study showed that the oral use of L-glutamine can moderately decrease gentamicin-induced liver injuries.

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Introduction

Liver is a vital organ and its cells can perform wide biochemical and metabolic activities. One of these activities is

the release of harmful materials such as poisons and drugs from the body (1). Hepatic or liver injury causes abnormal functions in this organ. In this regard, disorders of mitochondrial function

have been reported as one of its causes. Some drugs can induce disorder in the function of mitochondria; consequently, it leads to necrosis and hepatitis (2). The injured liver cannot perform its activities properly; therefore, poisons can be accumulated in this tissue (1). Amino glycoside antibiotics induce nephrotoxicity and cochlear toxicity despite their beneficial effects. Gentamicin causes the release of oxygen free radicals which can induce pathological processes such as nephrotoxicity and cochlear toxicity (3). Gentamicin also causes liver injury (4). It was reported that amino glycoside antibiotics cause liver injury by inducing renal injuries (5). The defensive mechanism of antioxidant enzymes as well as the release of reactive oxygen species (ROS) are the causes of liver injuries due to oxidative stress (6). Based on some conducted researches, rosmarinic acid and dipyridamole have antioxidant properties and can be effective in reducing the hepatorenal injuries induced by gentamicin (7, 8). In this study, another antioxidant, glutamine, has been used to decrease gentamicin-induced liver injuries. Glutamine serves as a precursor to glutathione (GSH), and leads to antioxidant defense in the body. Thus, it seems necessary for some key routes of stressful reactions (9, 10). Glutamine, as an amino acid, has traditionally been considered unnecessary; however, during the rapid growth of the body as well as stress or acute diseases, more attention is paid to it and its benefits increase in the body. Therefore, it may become a conditionally essential amino acid (11, 12). As glutamine causes biosynthesis of glutathione, it causes attenuated oxidative stress in body (13). Glutamine plays a pivotal role in the survival, differentiation and decrease of stress in normal cells in the context of tumorigenesis (14). Glutamine catabolism in hepatocytes causes ammonia detoxifying in the body. Therefore, it can treat hyperammonemia (15) and decrease the growth of prostate cancer cells (13). Glutamine with its antioxidant properties can protect renal cells during

carcinoma (16). Glutamine could be used in impairment of injured cells like Amino acids that use in treatment of illness and trauma. In this study, it was supposed that L-glutamine as an amino acid with its antioxidant properties can play an effective role in decreasing hepatotoxicity induced by gentamicin; therefore, the purpose of the current study was to investigate the function of liver enzymes as well as the level of liver tissue antioxidants after receiving L-glutamine.

Materials and Methods

The drugs such as gentamicin from Alborz Darou (Pharmaceutical Co, Iran) and L-glutamine powder from Merck (Darmstadt, Germany) were used in this study.

Animals

Thirty two wistar rats, weighing 180-220 g were bought from Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran. The rats were kept in the animal lab of Dezful University of Medical Sciences in a similar environmental condition at 25° c in 12 hour light/dark cycle with easy access to food and water for two weeks prior to and during experimentation.

Experimental design

Wistar rats were randomly divided into four groups, eight per each group as follows: 1) control group 2) Gentamicin group, received gentamicin, 100 mg/kg for 12 days 3) (7); L-glutamine group, received L-glutamine, 25 mg/kg for 12 days 4) gentamicin + glutamine group, received gentamicin 100 mg/kg as well as L-glutamine, 25 mg/kg for 12 days (17). Gentamicin and glutamine were taken at one time (two hours between medications). After this period, the blood samples were taken from the rats' hearts and then centrifuged with 3000 rpm for 10 minutes and their serums were separated. The animal livers were separated to evaluate catalase (CAT),

glutathione, glutathione peroxidase (GPX), and malondialdehyde and then the animal livers were kept in the refrigerator at -80 c.

Analytical method

To evaluate the liver function, liver enzymes such as aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured with the automatic analyzer (Biotechnia instrument BT 1000) and by the application of specific kits from Pars Azmoon Company. After homogenizing the liver tissue, some indexes such as CAT with Sinha method (18), GSH with Ellman method (19), the activity of glutathione peroxidase enzymes with fielding method (20), malondialdehyde level (MDA), and a lipid peroxidation index with Uchiyama method were measured (21). The results of GSH and MDA levels were expressed as nmol/mg-gr and the results of GPX and CAT levels were expressed as Unit/mg-gr.

Statistical analysis

All the values were expressed as mean and the standard error of mean. The group differences were performed by Mann-Whitney test using SPSS software version 23. The statistical significances were at the 5% level ($P < 0.05$).

Results

The findings of the current research revealed a significant increase in AST level in Gentamicin group in comparison with the control group. The AST in glutamine group was significantly decreased compared to gentamicin group. The AST in Gentamicin + Glutamine decreased; however, this reduction was not statistically significant compared to Gentamicin group. Although the ALT serum in gentamicin group increased, but it was not significant. In addition, the ALT in glutamine and gentamicin + glutamine groups showed no significant difference compared to gentamicin group (Table 1).

Table 1. Treatment with glutamine and AST, ALT levels in different groups

Groups	AST (U/L)	ALT (U/L)
Control	165.88±15.42*	43.87±4.89
Gentamicin	329.62±40.61#	52.75±9.16
Glutamine	192.88±19.52*	45.5±3.35
Gentamicin + Glutamine	250.75±31.39	54.63±7.11

Values are expressed as mean ± S.E.M.

* $P < 0.05$, compared with the gentamicin treated groups

$P < 0.05$, compared with the control groups

The MDA level in Gentamicin group indicated a significant increase compared to the control group. Treatment with glutamine in glutamine and gentamicin + glutamine groups could significantly reduce the MDA level compared to gentamicin group (Table 2).

Table 2. Treatment with glutamine and the amounts of liver antioxidant enzymes and MDA level of liver tissue in different groups

Groups	MDA (nmol/ mgpr)	GPX (nmol/min/mgpr)	CAT (pmol/ mgpr)	GSH (μmol/ mgpr)
Control	45.78±3.46*	103.99±4.48×	48.09±2.15*	9.85±0.61*
Gentamicin	62.08±2.38#	89.81±2.79#	36.99±3.17#	6.49±0.34#
Glutamine	47.74±2.09*	103.94±3.86×	32.22±2.6#	8.66±0.75*
Gentamicin + Glutamine	45.7±3.38*	104.34±4.72*	35.91±2.32#	8.8±0.49*

Values are expressed as mean ± S.E.M.

* $P < 0.05$, compared with the gentamicin treated groups

$P < 0.05$, compared with the control groups

The GPX level of liver issue in gentamicin group showed a significant decrease compared to the control group. GPX level in group 4 (gentamicin + glutamine) and glutamine group indicated a significant increase compared to gentamicin group (Table 2).

The catalase level in gentamicin group indicated a significant difference compared to the control group. No significant differences were observed between glutamine group and gentamicin group (Table 2).

The GSH level in gentamicin group indicated a significant difference compared to the control group. Treatment with glutamine in groups 3 and 4 could significantly increase the GSH level compared to gentamicin group (Table 2).

Discussion

The results of this study indicated that the use of gentamicin, 100 mg/kg for 12 days, induces liver toxicity. Hepatotoxicity usually increases the levels of AST and ALT. In the current study, the levels of these two enzymes also increased. Gentamicin, as one of the aminoglycoside, has been used against gram-negative bacteria. It has been reported that this drug induces hepatorenal toxicity despite its clinical benefits (7). The AST level significantly increased in gentamicin group.

Although the AST level in gentamicin + glutamine group decreased compared to gentamicin group, this reduction was not significant. Similar studies conducted on the pretreatment effects of natural antioxidants in herbal medicine such as methanolic leaf extract of caesalpinia, dietary inclusion of garlic, and rice berry bran extract have shown a significant decrease in AST and ALT in treated groups compared to Gentamicin group (1, 7, 22). This reduction may be attributed

to their potential antioxidant properties as well as their uses as pretreatment. It has been reported that amino glycosidic drugs such as gentamicin leads to the oxidative stress both in the liver and in the kidney of rats. This also causes an increase in the formation of free radicals and conversely, a decrease in the inherent antioxidants such as superoxide dismutase (SOD), GST, CAT, and GPX. In addition, during oxidative stress, lipid peroxidation can increase (1, 7, and 23). In the current study, the levels of GPX, CAT, and glutathione decreased in gentamicin group and MDA level of this group increased. This finding is in line with previous studies. According to previous studies, glutamine is an amino acid that has an antioxidant effect which can reduce the damage caused by physical injuries, diseases like prostate cancer, and kidney cancer (13, 16). Glutamine with decreases in ROS and by enhancing glutathione and GPX activity can protect rabbit spermatozoa (24). Glutamine in intestinal ischemia-reperfusion model causes reduced oxidative stress, AST, ALT, ALP, IL-6 and increases SOD, GPX, CAT, and GSH in the liver tissue (17). Also, glutamine in portal hypertension model can decrease lipid peroxidation and gut mucosal injury in rats (25). Receiving glutamine in groups 3 and 4 could significantly increase the levels of glutathione and GPX compared to Gentamicin group; however, the level of catalase in these two groups revealed no significant increase compared to gentamicin group. The MDA level in groups 3 and 4 receiving glutamine, significantly decreased compared to gentamicin group. This difference may be due to the antioxidant properties of glutamine. *Fagonia olivieri*, the native plant of Pakistan, has antioxidant properties. This plant is useful for treating hepato, renal, and urology system disorders (23). In a study, this plant decreased the hepato renal disorder induced by gentamicin by enhancing antioxidant

enzymes and decreasing the MDA level in the liver and kidney tissues. This can be attributed to its antioxidant property (26). Rice berry bran extract (22), methanolic leaf extract of caesalpinia (7), olive leaves extract (20), dipyrindamole (8), vitamin E (27), Satureja khuzestanica essential oil (28), rosmarinic acid (29), coenzyme Q10 (30), oleuropein (31, 32), and homocysteine (33) are some antioxidants that play effective roles in decreasing hepatorenal damages by reinforcing the antioxidant system and decreasing the peroxidation of fatty acids.

Conclusions

According to the current research and previous studies conducted on this subject, it can be concluded that liver

disorders induced by gentamicin can be reduced by the use of antioxidants; especially, if they are used as pretreatment before the administration of gentamicin. L-glutamine not only increased GPX and GSH, but also decreased MDA and AST. Our investigation showed that glutamine could be moderately beneficial for the reduction of liver injuries induced by gentamicin. This positive effect probably could be attributed to its antioxidant property.

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