

## The Effect of Receiving L-Glutamine on the Reduction of Renal Tissue Damages and Renal Function Recovery Following Gentamicin-Induced Nephrotoxicity in Rats

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### ABSTRACT

**BACKGROUND AND OBJECTIVE:** Gentamicin is one of the aminoglycoside antibiotics that commonly causes nephrotoxicity. Antioxidants are effective in reducing gentamicin-induced nephrotoxicity. In this study, we investigated the possible effect of L-Glutamine on the reduction of renal tissue damages and renal function recovery in gentamicin-induced nephrotoxicity in rats.

**METHODS:** In this experimental study, 32 rats were divided into four groups of eight: Sham group, Gentamicin group (100 mg/kg i.p for 12 days), L-Glutamine group (30 mg/kg by gavage for 12 days), Gentamicin+L-Glutamine group. Then through anesthesia, the blood sample was collected via cardiac and its serum was used to measure the BUN and creatinine levels. The kidney of the rat was used to measure malondialdehyde (MDA), glutathione peroxidase (GPX), catalase (CAT), glutathione (GSH) and determine histopathological parameters.

**FINDINGS:** Receiving gentamicin causes significant elevation in BUN, Creatinine, MDA of the kidney, tubular necrosis, eosinophilic cast, and leukocyte infiltration compared to sham group ( $p<0.05$ ). CAT, GPX, and GSH in gentamicin group caused significant decrease compared to the sham group ( $p<0.05$ ). Glutamine has significantly decreased the MDA level, and leukocyte infiltration compared to gentamicin group ( $p<0.05$ ). Glutamine caused significant increase in GPX (CAT in group 4) compared to gentamicin group ( $p<0.05$ ) and in group 3, BUN, tubular necrosis, eosinophilic caused significant decreases compared to gentamicin group ( $p<0.05$ ).

**CONCLUSION:** Receiving L-Glutamine, 30 mg/kg orally for 12 days has an effective role in the reduction of kidney injuries induced by gentamicin.

**KEY WORDS:** *L-Glutamine, Gentamicin, Nephrotoxicity.*

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## Introduction

Gentamicin, an aminoglycoside antibiotic, has widely been used in the treatment of gram-negative infections. The renal injuries are its most common side effects and approximately 10-20% of patients receiving this drug develop this condition (1, 2). There are other antibiotics known to be used against gram-negative bacteria, causing less nephrotoxicity compared to gentamicin; however, gentamicin is still used against resistant microorganisms against positive beta-lactam due to its low cost and positive effects (1, 2).

Pathological injuries induced by gentamicin include necrosis of kidney tissue and apoptosis caused by oxidative stress, increase of endothelin level, monocyte and macrophage infiltration levels. In addition, gentamicin causes an increase in reactive oxygen species (ROS) such as hydrogen peroxide, superoxide anions, hydroxyl radicals, and reactive nitrogen species in kidney tissue (3). ROS cause irreparable damages in cells and tissues of the body. There is a dynamic balance between ROS and endogenous enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), in a way that these enzymes cause detoxification of ROS and protection of the body against their destructive effects (4).

Most of the studies have shown that different antioxidant factors can halt or decrease the harmful effects of gentamicin in rats. The use of antioxidants in rats being injected with gentamicin can decrease kidney tissue injuries such as tubular necrosis, tubular cell edema, and apoptosis (3, 5, 6). Glutamine, as a precursor for glutathione, leads to antioxidant defense in the body and seems essential for some of the key routes of stressful reactions (7-9). Although glutamine has traditionally been considered as an unessential amino acid, during the rapid growth of the body, stress, or acute disease, it becomes necessary and its use will increase in the body. Thus, it can be defined as a conditionally essential amino acid (10).

L-glutamine has been reported to be effective in reducing gentamicin-induced liver damage, reducing cerebral infarction after cerebral ischemia, and treating sickle cell anemia (11-13). Clinical studies on L-glutamine in the treatment of sickle cell anemia indicate that L-glutamine is tolerable for a short period of time and because these patients are prone to liver, kidney, etc. failures, the use of L-glutamine by these people should be done with caution (13). In many cases, the use of gentamicin is necessary for patients for a long time and on the other hand, kidney damage caused by the use

of this drug is inevitable, so prevention of kidney damage with natural and antioxidant compounds seems necessary. Thus, the purpose of this study is to investigate the possible effect of L-Glutamine on the reduction of renal tissue damages of gentamicin-induced nephrotoxicity in rats by examining kidney function tests, antioxidant enzymes and tissue parameters.

## Methods

**Animals and drug treatment:** All animal experiments in this study are approved by the ethics committee of Dezful University of Medical Sciences, Iran (Ethical number: DURS= 119) based on internationally accepted guidelines for the use of animals. In this experimental study, thirty-two Wistar rats weighting between 200-220 g were provided from the animal lab of Dezful University of medical sciences. These animals were kept at a constant room temperature between 22 and 24°C, and a humidity of 50-60 % and 12 hours of the light/dark cycle. They had free access to water and food.

**Experimental groups:** Rats were randomly divided into four groups of eight:

**1. Sham group**

**2. Gentamicin group:** only received gentamicin 100 mg/kg intraperitoneally for 12 days

**3. L-Glutamine group:** received 30 mg/kg L-Glutamine daily by gavage for 12 days

**4. Gentamicin+L-Glutamine:** received gentamicin and L-Glutamine concomitantly for 12 days

24 hours after the final dose, the animals were anesthetized by injection of 60 mg/kg ketamine and 2.5 mg/kg diazepam. The blood samples were taken, then the right kidney was excised for histopathological studies and left kidney was removed for biochemical experiments and was kept at -80 °c until homogenizing.

**Serum analysis:** The blood sample was centrifuged with 3000 rpm for 10 minutes, and its serum was separated. Urea and creatinine levels were measured by Kits of Pars Azmoon (Pars Azmoon, Tehran, Iran) and biotechnical instruments (BT 1000, Italy).

**Renal Biochemical Analysis:** To measure renal biochemical parameters, kidney tissues were removed from the freezer and homogenized with a homogenizer. Then, tenfold Phosphate Buffered Saline (PBS) solution was added. The solution was centrifuged with (5000 rpm) at 4 °c for 15 min. After removing the supernatant, the solution was used to evaluate parameters such as malondialdehyde (MDA), CAT, glutathione (GSH), and GPX. To measure lipid

peroxidation, briefly, a mixture of homogeneous kidney tissue and trichloroacetic acid was centrifuged, then its supernatant was mixed with Thiobarbituric acid (TBA), and after boiling for 30 minutes, its absorption was read at 530 nm by spectrophotometry (14). To measure glutathione, a mixture of phosphate buffer (PH=7.1), glutathione reductase, EDTA, and kidney sample was prepared and then its adsorption was read at 412 nm by ELISA (15). A mixture of kidney sample with hydrogen peroxide and tert-butyl hydroperoxide was used to measure GPX and was read by ELISA at 420 nm (16). CAT was measured based on the previous study by Aebi. Briefly, a mixture of phosphate buffer (PH=7.4), hydrogen peroxide and kidney sample was prepared and then its absorption at 240 nm was read by a spectrophotometer in 30 seconds for 3 minutes (16, 17).

**Histopathological assessments:** The kidney was fixed inside formaldehyde 10% for 24 hours. After tissue processing and embedding in paraffin, it was cut into 5  $\mu$ m sections and then stained with hematoxylin and eosin. From each kidney, 40 fields at \*400 magnification were evaluated by microscope to analyze leukocyte infiltration, tubular necrosis, and eosinophilic casts (18). The data were collected semi-qualitatively as follows: No damage= 0, Mild (unicellular)= 1; Moderate (damage less than 25%)= 2; Severe (damage between 25-50%)= 3; highly severe (more than 50% damage)= 4.

**Statistical analysis:** The data analysis was performed by SPSS 21.0. Values were assessed for normality using the Shapiro–Wilk normality test. Data were analyzed by ANOVA test. Data were represented as mean $\pm$ standard deviation of means, the level of significance was determined as p-value<0.05. The graph of data was illustrated by Graph Pad Prism 6 software.

## Results

**Effect of L-Glutamine on Renal function:** The BUN levels in the gentamicin group compared to the sham has significantly increased (54.63 $\pm$ 10.23 vs. 57 $\pm$ 6.48, p=0.036). BUN levels were reduced in the glutamine-receiving groups, but this decrease was statistically significant only in group 3 (in group 3: 38 $\pm$ 7.56 vs. 57 $\pm$ 6.48, p<0.0001 and in group 4: 49 $\pm$ 6.82 vs. 57 $\pm$ 6.48, p=0.20) (Figure 1-A). Creatinine in the gentamicin group has significantly increased compared to the sham (0.7 $\pm$ 0.08 vs. 0.58 $\pm$ 0.07, p=0.036). In this study,

treatment with glutamine could decrease creatinine levels in the group receiving gentamicin but this difference was not statistically significant (0.61 $\pm$ 0.06 vs. 0.7 $\pm$ 0.08 p = 0.14) (Figure 1-B).

**Effect of L-Glutamine on Renal biochemical parameters:** The MDA level of kidney tissue in the gentamicin group compared to the sham group has increased and this rise was significant (62.09 $\pm$ 6.73 vs. 45.78 $\pm$ 9.79, p=0.003). Receiving of glutamine in two groups 3 and 4 in comparison with gentamicin (group 2) has decreased the level of MDA and this reduction was significant (in group 3: 40.26 $\pm$ 9.55 vs. 62.09 $\pm$ 6.73, p<0.0001 and in group 4: 36.01 $\pm$ 6.84 vs. 62.09 $\pm$ 6.73, p<0.0001) (Table 1). Although gentamicin has decreased the glutathione level, this reduction was not significant. The glutathione level has increased in groups receiving glutamine compared to gentamicin group but this elevation was not significant (Table 1). Catalase level in gentamicin group significantly decreased compared to the sham group (25.74 $\pm$ 10.17 vs. 40.91 $\pm$ 10.65, p=0.042) (Table 1).

Gentamicin has significantly decreased the renal GPX compared to sham group (77.66 $\pm$ 11.44 vs. 123.68 $\pm$ 19.55, p<0.0001). Glutamine has increased the GPX level in both groups receiving glutamine and this increase was significant compared to gentamicin group (in group 3: 107.03 $\pm$ 12.39 vs. 77.66 $\pm$ 11.44, p=0.005 and in group 4: 102.6 $\pm$ 18.39 vs. 77.66 $\pm$ 11.44, p=0.019) (Table 1).

**Effect of L-Glutamine on Renal Histopathology parameters:** Gentamicin caused an increase in tubular necrosis in group 2. This increase was significant compared to the sham group (2.1 $\pm$ 0.24 vs. 1.26 $\pm$ 0.32, p<0.0001). Treatment with glutamine in groups 3 significantly decreased the tubular necrosis compared to gentamicin group (1.6 $\pm$ 0.28 vs. 2.1 $\pm$ 0.24, p=0.003) (group 3) (Figure 2) (Figure 3).

Eosinophilic casts in the gentamicin group have significantly increased compared to the sham group (1.26 $\pm$ 0.32 vs. 2.1 $\pm$ 0.24, p<0.0001). Eosinophilic casts in group 3 have significantly decreased compared to the gentamicin group (1.6 $\pm$ 0.28 vs. 2.1 $\pm$ 0.24, p=0.003) (Figure 2) (Figure 3).

Gentamicin has significantly increased the leukocyte infiltration in kidney tissue compared to the sham group (2.16 $\pm$ 0.46 vs. 1.13 $\pm$ 0.28 p<0.0001). Glutamine in groups 3 and 4 has decreased the leukocyte infiltration (in group 3: 1.39 $\pm$ 0.25 vs. 2.16 $\pm$ 0.46, p=0.002, in group 4: 1.53 $\pm$ 0.47 vs. 2.16 $\pm$ 0.46, p=0.013) (Figure 2) (Figure 3).

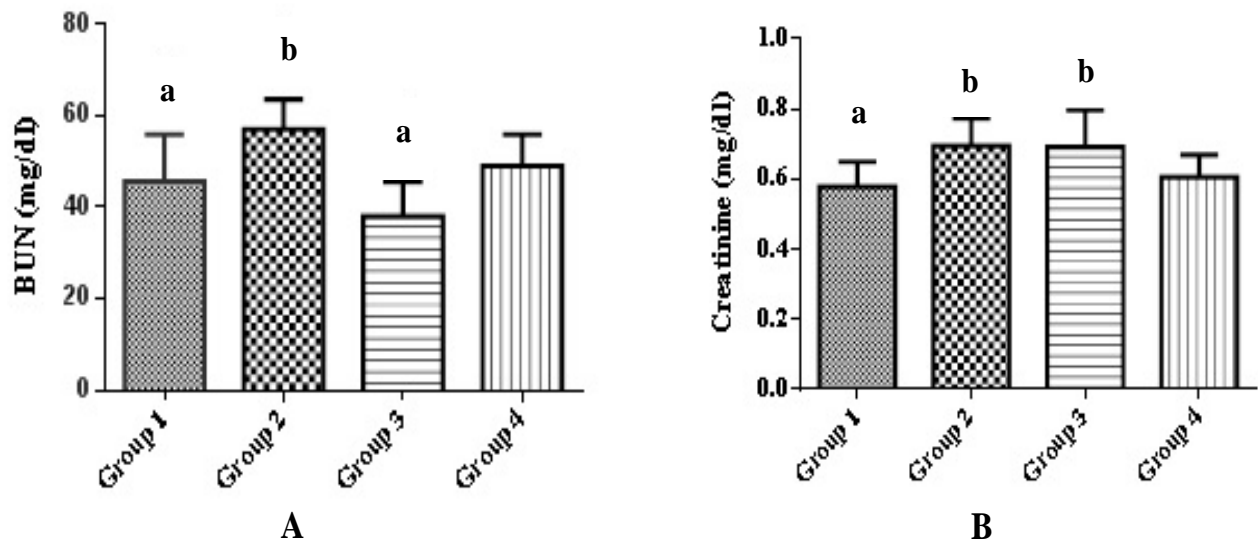


Figure 1. Effect of L-Glutamine on renal function test in gentamicin induced nephrotoxicity. Each figure represents the Mean±SD. \*p<0.05 as compared with Group 2 (gentamicin group), #p<0.05 as compared with Group 1 (Sham group).

Table 1. Effect of L-Glutamine on renal biochemical parameter in gentamicin-induced nephrotoxicity

Group	MDA	CAT	GSH	GPX
	(nm/mg protein)	(U/mg protein)	(U/mg protein)	(U/mg protein)
	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Sham	45.78±9.79*	40.92±10.65*	10.40±3.23	123.68±19.55*
Gentamicin	62.08±6.73#	25.74±10.17#	9.22±2.90	77.66±11.44#
Glutamine	40.26±9.55*	38.61±10.83	11.76±2.63	107.03±12.39*
Gentamicin+Glutamine	36.01±6.84*	41.24±12.36*	9.35±2.14	102.60±18.39*

\*p<0.05 as compared with Group 2 (gentamicin group), #p<0.05 as compared with Group 1 (Sham group).

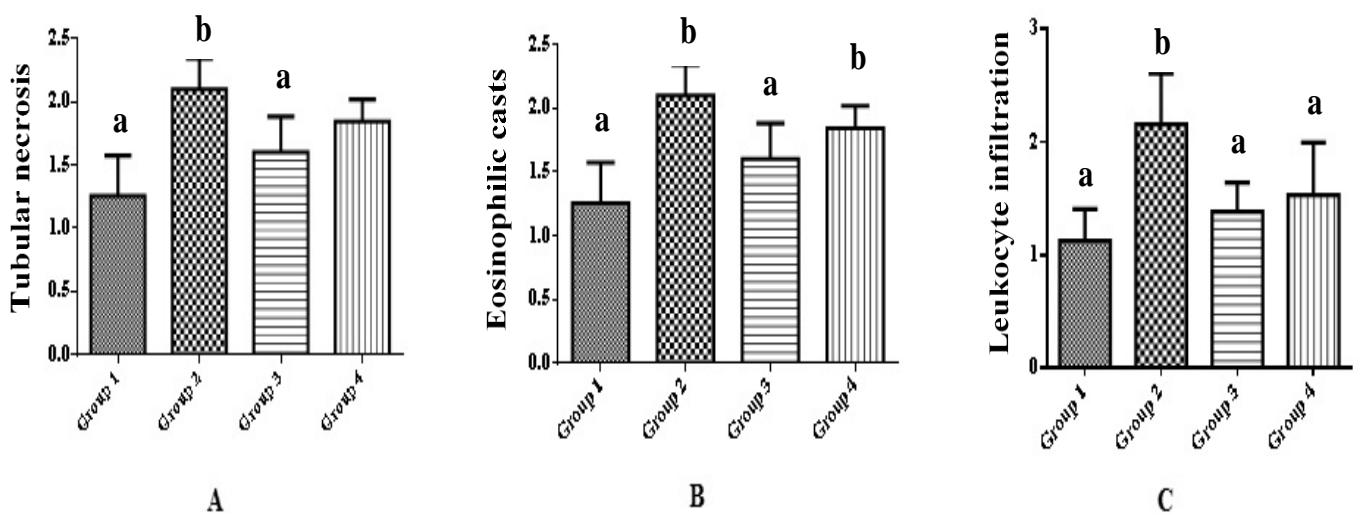
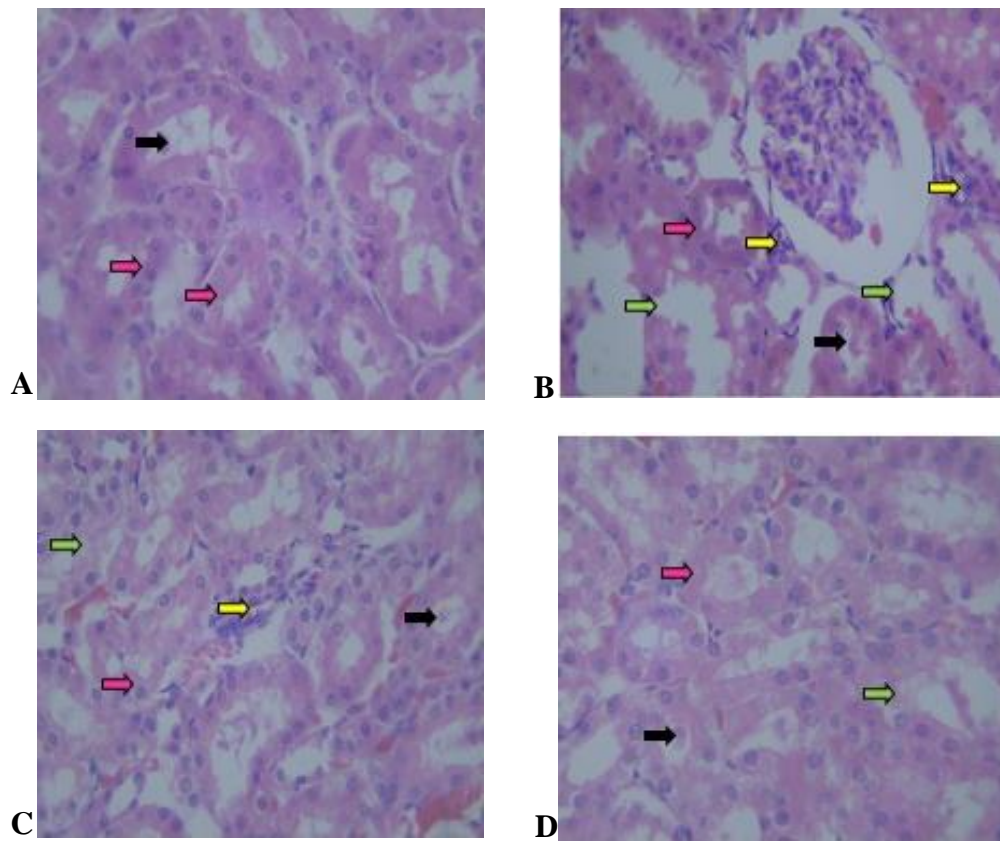


Figure 2. Effect of L-Glutamine on renal histopathology parameter in gentamicin-induced nephrotoxicity. Each figure represents the Mean±SD. \*p<0.05 as compared with Group 2 (gentamicin group), #p<0.05 as compared with Group 1 (Sham group).



**Figure 3. Effect of L-Glutamine on histopathological changes of rat kidney in different groups.** A: Sham group, B: Gentamicin group, C: L-Glutamine group, D: Gentamicine+L-Glutamine. Pink arrow shows normal histological structure of renal tubular. Green arrow shows renal epithelial degeneration and tubular necrosis. Black arrow shows epithelial cast of renal. Yellow arrow shows leukocytes infiltration in the interstitial area (H&E,  $\times 400$ ).

## Discussion

In this study, a dose of 100 mg/kg gentamicin increased oxidative stress, kidney damage, and L-glutamine reduced oxidative stress, increased antioxidant activity, and decreased kidney tissue damage. Other studies have shown that gentamicin 40 mg/kg or a higher dose causes acute renal failure (19). The increase in serum BUN and creatinine levels is a sign of renal dysfunction (20, 21). In our study, gentamicin injection has increased the BUN and creatinine levels. Histopathological analysis of group injected with gentamicin showed increase in acute tubular necrosis, leukocyte infiltration, and eosinophilic casts. With regard to biochemical parameters, gentamicin injection has increased the MDA level and has decreased the antioxidant enzymes (GSH, GPX and CAT) of kidney tissue. The use of some compounds such as diclofenac in combination with gentamicin due to reduced blood flow and inhibition of prostaglandins has caused more severe tissue damage in kidney and olive leaf extract, *Lavandula officinalis* and

rosmarinic acid improve kidney tissue due to their antioxidant properties (5, 22-24). In the conducted study, although receiving glutamine has decreased BUN and creatinine levels, this decline was not significant and it has functioned weaker than previous antioxidant inflammatory drugs such as rosmarinic acid, glutathione and garlic juice (5, 25, 26). It may be due to its weaker antioxidant properties than these compounds or the glutamine reduction effect may increase if the dose of the drug increases. Glutamine treatment in rats receiving gentamicin caused a significant decrease in MDA level of kidney tissue due to reduced lipid peroxidation. Some of the previous studies conducted on the use of antioxidants have shown the decline of MDA level kidney and liver tissue (5, 11, 23). Thus, it is possible that the antioxidant activity of glutamine reduces oxidative stress in tissues. Glutamine, due to its antioxidant gene HSP70, has antioxidant properties and also reduces lipid peroxidation (27). In the present study, glutathione, catalase, GPX levels of kidney tissue of rats receiving glutamine along with gentamicin have

significantly increased and this elevation is probably due to the oxidative stress activities of glutamine which resulted in reinforcement of antioxidant system. In the previous studies, some antioxidants such as hydraulic extract of olive leaf, rosmarinic acid have controlled gentamicin-induced nephrotoxicity by increasing the number of antioxidant enzymes of kidney tissues (5, 23).

In some of the studies, it has been mentioned that glutamine increases the antioxidant capacity of tissues, and copes with oxidative stresses by enhancing SOD, glutathione, and GPX activities (11, 27, 28). In our study, it was also shown that glutamine is effective in preventing histopathological changes caused by gentamicin such as reduction of inflammation in the kidney tissue and reduction of inflammation of epithelial tissue tubules. However, the reduction of cellular damage due to its antioxidant and amino acid properties is less than the observations of other studies in this field, which may be related to the dose of this drug and the number of days it is received by rats (5, 29).

However, any increase in the number of days and the dose received should also be carefully considered. Because, very high doses or long-term use may be dangerous. A study found that the rate of glutaminolysis increases in the blood of patients with heart disease and sickle cell anemia, which usually improves with the administration of glutamine and improves these patients. However, appropriate dose, short-term use or the use of a glutaminolysis inhibitor is recommended in these patients (30). The use of glutamine (due to

its antioxidant properties) or glutaminase inhibitor has also been shown to be effective in curing malignancies (31).

Overall, the results of this study showed that L-glutamine has promising effects of antioxidant and anti-oxidative stress in a gentamicin-induced nephrotoxicity model. The findings of this study indicated that 30 mg/kg glutamine by gavage along with gentamicin for 12 days can play an effective role in the decrease of kidney tissue damages and renal function recovery by reducing oxidative stresses due to its anti-inflammatory and antioxidant properties. Based on the results of this study, L-glutamine can be considered as a relatively suitable antioxidant and anti-inflammation in the treatment of renal injuries. It is suggested that effective mechanisms in reducing cellular damage and molecular studies in this field be considered in future research.

**Conflicts of interest:** The authors declared no competing interests.

**Ethical considerations:** Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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