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Regulation of bacterial lipopolysaccharide in liver toxicity caused by chlorpromazine and Z24 in Sprague-Dawley rats

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[Abstract] Objective To determine the characteristics and manifestations of liver toxicity induced by chlorpromazine (CPZ, a phenothiazine antipsychotic drug) and Z24 (an anticancer candidate drug) and to study the relationship between short-term inflammation and drug-induced liver toxicity through monitoring inflammation induced by low-dose lipopolysaccharides (LPS) and the hepatic toxicants CPZ and Z24.

Methods A specific inhibitor of Kupffer cells, $GdCl_3$, was used to probe the role of these cells in the development of liver toxicity. Interleukin-1 alpha (IL-1α) was evaluated to study the roles of hepatic non-parenchymal cells and inflammatory mediators in liver toxicity induced by CPZ and Z24, as well as the effect of LPS on drug-induced liver toxicity. In addition, the mechanism, process, steps and pathways of the influence exerted by hepatic non-parenchymal cells and inflammatory mediators are discussed.

Results Treatment with 75 mg/kg CPZ caused significant changes in the blood biochemical index of liver toxicity, but this dosage did not have an effect on liver pathology. When 75 mg/kg CPZ was administered 2 h after an inflammatory response had been induced by 2 mg/kg LPS, the blood biochemical index of liver toxicity increased further, and liver injury lesions occurred. Treatment with 200 mg/kg Z24 significantly increased the blood biochemical index of liver toxicity and induced a significant pathological change in liver injuries. However, after 2 h of pretreatment with 2 mg/kg LPS followed by treatment with Z24, the blood biochemical index was normal, and pathological change was significantly reduced. Pretreatment with 10 mg/kg $GdCl_3$ significantly inhibited the enhancement of CPZ-induced liver toxicity by LPS; however, the same treatment did not alter the protective effect of LPS against Z24-induced liver toxicity. Two hours of pretreatment with 1.25 μg/kg IL-1α significantly antagonized the liver toxicity induced by Z24; however, compared with the control group, a degree of liver toxicity was still observed.

Conclusions When an organism is experiencing an inflammatory response, activation of Kupffer cells enhances CPZ-induced liver toxicity. The inflammatory response induced by LPS could be used to establish and study a screening model for idiosyncratic liver toxicity. Under inflammatory response

conditions, activating endothelial cells and IL-1α might have a protective effect against Z24-induced liver toxicity.

[Key words] *chlorpromazine, lipopolysaccharide, liver toxicity, protective effect*

Introduction

Drug-induced liver toxicity has attracted increasing attention, and idiosyncratic liver toxicity (ILT) is one of the major causes of drug development failure and listed drug withdrawal. Compared with the inherent liver toxicity of drugs, the most prominent problems for ILT are as follows: ILT is difficult to predict; there is a lack of experimental animal models for ILT; and the course of ILT is acute and severe. Because of the lack of suitable animal models for ILT, it is difficult to predict and screen for its occurrence during the early stages of drug development. Existing research has shown that the condition of immune function and inflammatory responses are closely related to the occurrence of ILT [1]. Inflammatory responses often occur during the development and progression of some types of ILT. In addition, mild inflammation may change the susceptibility of a patient to ILT that is induced by certain drugs. Lipopolysaccharide (LPS) is a complex of lipids and polysaccharides that is closely related to the occurrence of infectious hepatitis; through a reaction with toll-like receptors on the surfaces of inflammatory cells, LPS can induce a systemic inflammatory response [2,3]. The interactions among hepatocytes, Kupffer cells (KCs), endothelial cells and fat-storing cells in the liver play an important role in mediating liver dysfunction[4,5]. Because they are the first immune cells that are attacked upon intestinal endotoxin translocation and largest macrophage colony in the body, KCs play an important role in the occurrence of uncontrolled systemic inflammatory response after trauma[6]. After a small amount of LPS enters the liver, it can be directly phagocytized by activated KCs or removed by inflammatory mediators that release cytokines. The effect of the LPS-induced inflammatory response against drug-induced liver toxicity, especially ILT, has two aspects. A mild inflammatory response increases drug-induced liver toxicity and produces an idiosyncratic response such that during drug treatment, the inflammatory response of the patient can reduce the threshold of drug toxicity, which renders the patient sensitive to liver toxicity even if they had not previously been sensitive[7]. Studies have also shown that the levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and γ-glutamyltransferase (γ-GT) all significantly increased in rats that had been administered ranitidine and LPS simultaneously; however, the same serum indexes were normal in rats that received ranitidine or LPS alone. Two hours before a non-toxic dose of monocrotaline was administered, a

small dose of LPS was administered to induce an inflammatory response that could result in ILT[8]. When a small dose of LPS and allyl alcohol or aflatoxin B1 were administered simultaneously, the liver-damaging effects of the latter two chemicals both increased significantly [9, 10]. This type of study is beneficial to solving the problem of a lack of animal models for studies of ILT. In addition, based on this type of study, drug screening or evaluation models for ILT can be designed for use in the optimization of new chemical entities or structural modifications to existing chemical entities at the drug discovery stage. However, LPS-induced inflammatory responses can also reduce the liver toxicity induced by certain drugs. For instance, LPS has a definite protective effect against liver toxicity that is induced by acetaminophen and carbon tetrachloride [12]; this type of protective effect may be related to the release of interleukin-1 alpha (IL-1α) and inhibition of cytochrome P450.

Chlorpromazine (CPZ) is a phenothiazine antipsychotic drug, and its main side effect is ILT reaction. Z24 is a tumor-related protein inhibitor synthesized through computer-aided drug design that is based on the three-dimensional structure of the anti-apoptotic protein B-cell lymphoma 2 (Bcl-2). Z24 has good in vivo and in vitro antitumor activity; however, a previous study by our research team discovered that Z24 exhibited significant liver toxicity [16], which curbed its further development.

To determine the effect of LPS-induced inflammation on ILT, we used the phenothiazine antipsychotic drug CPZ and anticancer candidate drug Z24 as the treatment and rats as the model to study the mechanism underlying inflammation-mediated ILT and the key role of liver KCs in the mechanism. In addition, we investigated the potential impacts of such factors as the LPS treatment time and mediating effect of IL-1α on the experimental results. We also explored the feasibility of using LPS-induce inflammation models to establish animal models for research on ILT that could be used in the screening studies on potential ILT drugs to reduce the probability of failure of new drugs and attempted to determine whether induction of inflammation had a protective effect against Z24-induced liver toxicity, which would be beneficial to the modification and optimization of Z24 as a new antitumor drug.

Materials and Methods

Experimental animals

Healthy male Sprague-Dawley (SD) rats were provided by Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) (certificate number of animal quality: SCXK (Beijing) 2007-0003). The rats, which were graded specific-pathogen-free (SPF), weighed between 180 and 200 g and were raised in the Animal

Room of the National Beijing Research Center for Safety Evaluation of Drugs, which has been accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) and meets international animal welfare requirements. The rats were allowed to adapt to the environment for 7d before intoxication.

Growth conditions: the animals were raised in a fenced animal room (certificate number of animal room: SYXK-(Army) 2002-016) with the temperature controlled at 20~26˚C. Humidity was maintained at 40~70%, and the light/dark cycle was12 h/12h. The animals were fed routinely and allowed free access to water.

Main reagents

CPZ, LPS (from Escherichia coli, serotype O128:B12) and $GdCl_3 \cdot 6H_2O$ were purchased from Sigma-Aldrich (St. Louis, MO, USA). The purity of each reagent was >99%. Z24 (patent number: CN1365972) (purity>99.0%) was provided by Professor Song Li from Institute of Pharmacology and Toxicology. ALT, AST, Alkaline phosphatase (ALP), total cholesterol (TC), direct bilirubin (Dbil), total bilirubin (Tbil), triglyceride (TG) and total bile acid (TBA) detection kits were all purchased from SHANGHAI KEHUA BIO-ENGINEERING CO., LTD. IL-1α (batch number: 090204; purity>98%) was purchased from PeproTech (Rocky Hill, NJ, US).

Animal treatment

Effect of LPS pretreatment against CPZ- and Z24-induced liver toxicity

Thirty male SD rats were randomized based on their weights into the following six groups: blank control group, LPS pretreatment control group, CPZ-alone treatment group, Z24-alone treatment group, CPZ-combined treatment group and Z24-combined treatment group. There were five rats in each group, and the rats were fasted for 24h before drug administration. The rats in the pretreatment and combined treatment groups were pretreated by intravenous administration of 2 mg/kg LPS; the rats in the CPZ- and Z24-alone treatment groups were intravenously administered an equal amount of normal saline. Two hours later, the rats (pretreatment and combined treatment groups) were intraperitoneally administered 75 mg/kg CPZ or 200 mg/kg Z24 by gavage. Twenty-two hours after drug administration, blood samples were collected from the femoral arteries, and sera were prepared. Afterwards, the animals were sacrificed. Partial liver tissue was collected and subjected to fixation in 10% neutral formalin, tissue slicing, paraffin embedding, conventional sectioning, hematoxylin and eosin (HE) staining, dehydration in graded ethanol,

clearing, enveloping and observation under an optical microscope. A 7020 Biochemical Analyzer was used to determine the following serum biochemical indexes: ALT, AST, ALP, TC, Dbil, Tbil, TG and TBA.

Effect of $GdCl_3$ treatment on the regulation of LPS on CPZ- and Z24-induced liver toxicity

Thirty-five male SD rats were randomized based on their weights into seven groups: blank control group, LPS-alone control group, CPZ-alone treatment group, $GdCl_3$-alone treatment group, LPS+CPZ-combined treatment group, $GdCl_3$+CPZ-combined treatment group and $GdCl_3$+LPS+CPZ-combined treatment group. There were five rats in each group. The rats fasted for 24h before drug administration. Twenty-two hours after 10 mg/kg $GdCl_3$ was intravenously administered, the rats were intravenously administered 10 mg/kg LPS; 2h later, the rats were administered 75 mg/kg CPZ. The design of the study to determine the effects of LPS against Z24-induced liver toxicity was the same as described above, although the dose of Z24 was 200 mg/kg (by gavage). All of the other steps were the same as those described in Section 1.3.1.

Effect of IL-1α treatment against Z24-induced liver toxicity

Twenty male SD rats were randomized based on their weights into four groups, and there were five rats in each group. The four experimental groups were the solvent control group, iv.1.25μg/kg IL-1α 2h+ig.200 mg/kgZ24 group, iv.1.25 μg/kgIL-1α24h+ig.200 mg/kgZ24 group and ig.200 mg/kgZ24 group. The method for determining the serum biochemical indexes was the same as the method described in Section 1.3.1.

Statistical analysis

Data were expressed in the form of mean ± standard deviation. In addition, the Statistical Package for the Social Sciences (SPSS) 10.0 software was used to perform the one-way analysis of variance (ANOVA). Tukey's test was performed for multiple comparisons among groups.

Results

Enhancing effect of LPS against CPZ-induced liver toxicity

Figure 1 shows that compared with the control group, the 2 mg/kg LPS group did not exhibit significant changes in the serum biochemical indexes for ALT, AST and TC, but the levels of serum TC and Dbil increased significantly. The five serum biochemical indexes ALT, AST, TC, Tbil and TG of the animals in the 75 mg/kg CPZ group all significantly increased, with the levels of ALT and AST increasing by approximately 2-3 fold. The five serum biochemical indexes ALT, AST, TC, Tbil and TG of the animals in the LPS+CPZ-combined group not only significantly increased compared with the solvent control group but were also significantly higher than those of the animals in the 75 mg/kg CPZ group. Therefore,

inflammation induced by 2 mg/kg LPS pretreatment significantly aggravated CPZ-induced liver injury.

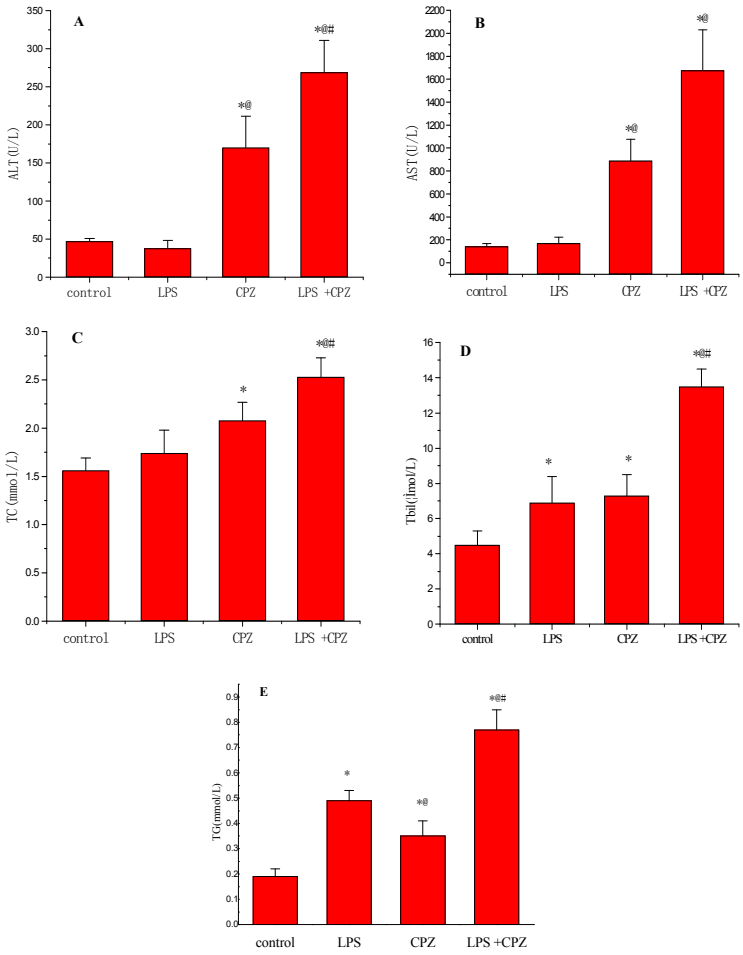

Fig. 1 Effects of LPS/CPZ on blood serum biochemistry of rats. A: ALT; B: AST; C: TC; D: Tbil; E: TG; n=5, *P<0.05 (compared with control group), @P<0.05 (compared with 2mg/kg LPS group), #P<0.05 (compared with75mg/kg CPZ group).

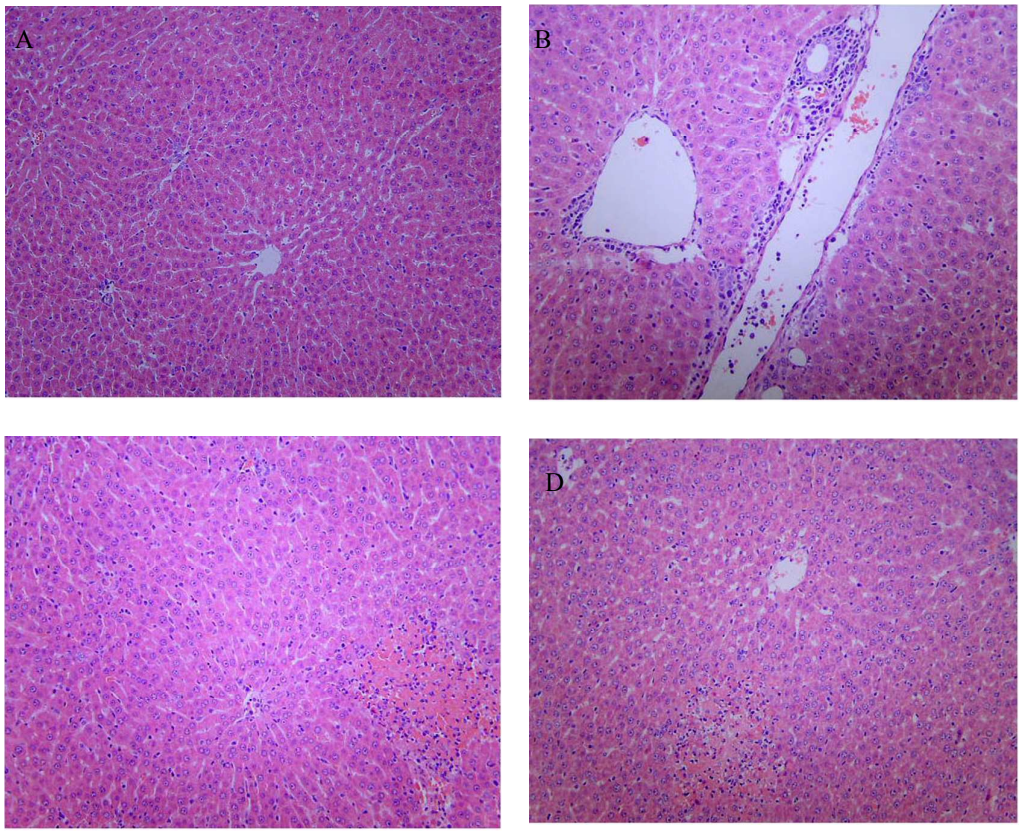

Fig.2 Liver histopathology (HE. 150×).
A, control group; B, LPS-treated group; C, CPZ-treated group; D, LPS+CPZ-treated group.

The pathological findings showed that for the rats in the control group, the structure of their hepatic tissue was distinguishable, and their hepatic sinusoids and hepatic cords were regularly arranged; in addition, no necrosis and apoptosis of the hepatocytes or abnormal substance depositions were found in the rats in the control group (Figure 2A). For the rats in the 2 mg/kg LPS group, monocyte aggregations were found in the small blood vessel lumens in their livers (Figure 2B), chronic inflammatory cell infiltration was found in the portal areas, acidophilic degeneration or eosinophilic necrosis of the hepatocytes was occasionally observed in their hepatic lobules, and coagulation necrosis occurred in small areas of the hepatocytes in the peripheral and central zones of the hepatic lobules of certain individual animals. The rats in the 75 mg/kg CPZ group had basically normal hepatic tissue structure, and no apparent degeneration, apoptosis or necrosis of the hepatocytes was observed (Figure 2C). The hepatic tissue structure was basically normal in the rats in the LPS+CPZ-combined group, and small areas of coagulative necrosis lesions were observed in individual animals (Figure 2D). Therefore, 75 mg/kg CPZ had no apparent impact on the rats' liver pathology, whereas 2 mg/kg LPS could induce inflammation in the portal areas of the livers and their hepatic lobules. Even through 2 mg/kg LPS could enhance changes in enzymology

indicators caused by 75 mg/kg CPZ, the hepatic pathology of the rats in the combined drug group did not change significantly because CPZ did not cause hepatic pathological changes in the rats.

2.2 Protective effect of LPS against Z24-induced liver toxicity

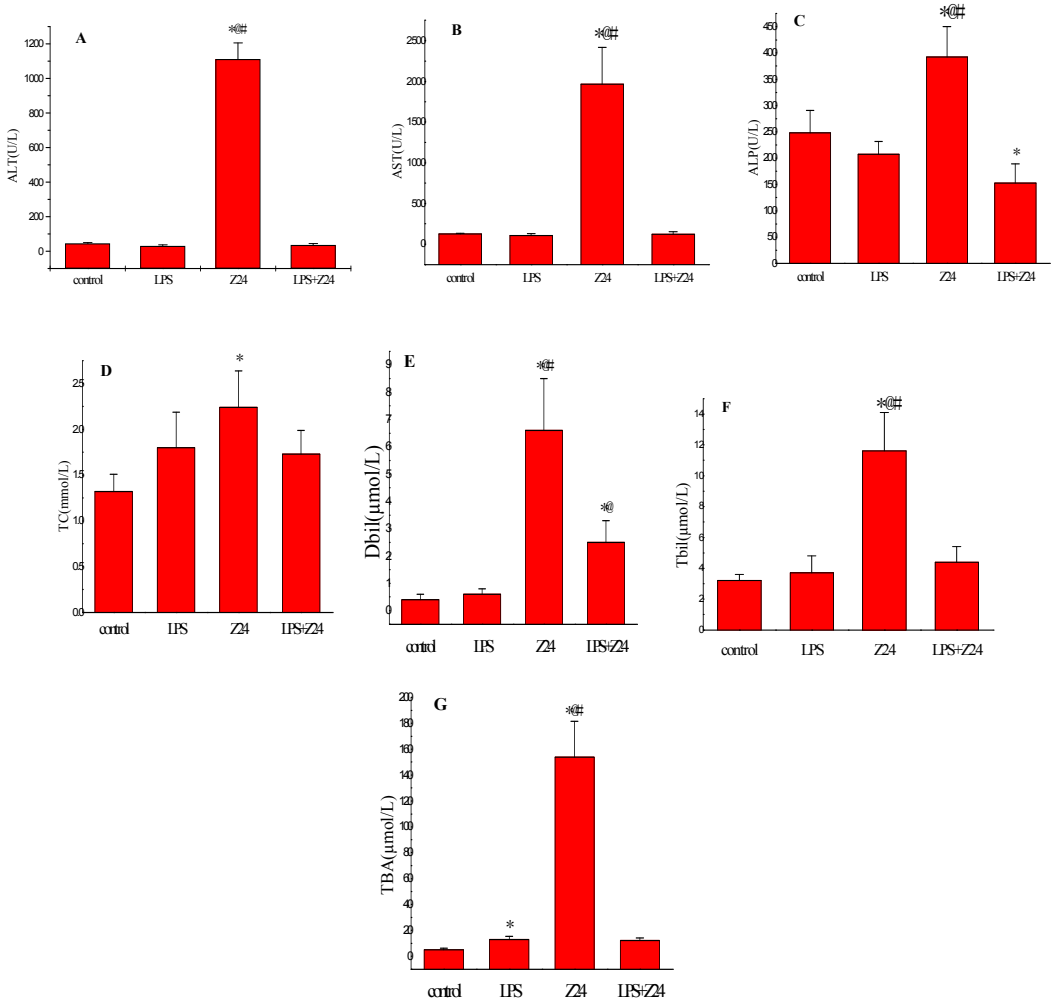

Fig. 3 Effects of LPS/CPZ on blood serum biochemistry of rats. n=5, *P<0.05 (compared with control group), @P<0.05 (compared with 2mg/kg LPS group), #P<0.05 (compared with 75mg/kg CPZ group).

Figure 3 shows that the seven biochemical indexes ALT, AST, ALP, Dbil, Tbil, TC and TBA of the animals in the 200 mg/kg Z24 group all increased significantly, with the levels of ALT, AST and TBA increasing by 20-30 times. The five biochemical indexes ALT, AST, Tbil, TC and TBA of the animals in the LPS+Z24-combined group all returned to normal and were not significantly different from those of the animals in the solvent control group. In addition, the ALP level of the animals in the LPS+Z24-combined group was slightly lower than that of the animals in the solvent control group, whereas the Dbil level of the animals in the LPS+Z24-combined group was slightly higher than that of the animals in the solvent control

group. Therefore, 2 mg/kg LPS pretreatment-induced inflammation showed an obvious ability to protect against liver injury caused by CPZ.

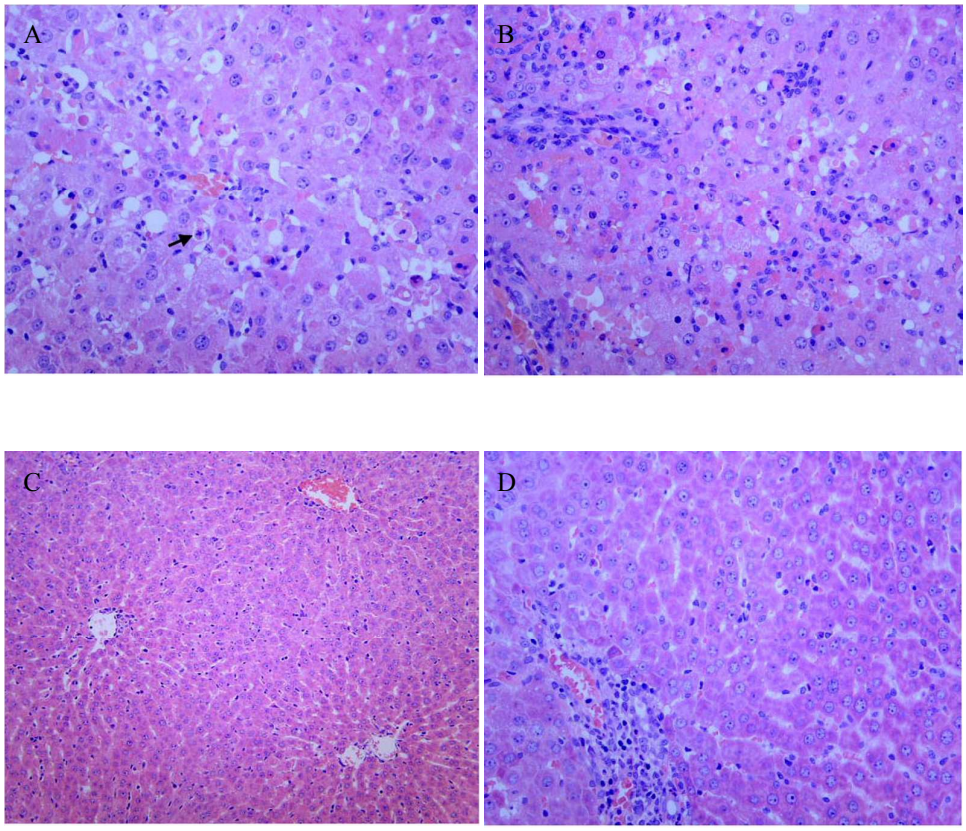

Figure 4. Liver histopathology (HE. 150× OR 300×). A, B: z24 treated group; C, D: LPS+Z24 treated group.

The rats in the 200 mg/kg Z24 group had diffuse livers, the hepatocytes in the peripheral and central zones of their hepatic lobules exhibited cloudy swelling, and ballooning degeneration of the hepatocytes occurred. In addition, apoptosis (Figure 4A) or eosinophilic necrosis (Figure 4B) of a large number of cells occurred, and neighboring lesions merged and formed large areas. The structure of the hepatic lobules of the rats in the LPS+Z24-combined group was normal, and no necrosis or apoptosis of the hepatocytes was observed (Figure 4C). In addition, the liver lesions were significantly reduced, and chronic inflammatory cell infiltration could only be found in a few portal areas (Figure 4D).

Effect of $GdCl_3$ treatment on the regulation of LPS on CPZ- and Z24-induced liver toxicity

Table 1 Effects of (iv.10mg/kg$GdCl_3$, iv.2mg/kg LPS and ip.75mg/kg CPZ) on blood serum biochemistry of male rats

groups	ALT	AST	ALP	TC
	(U/L)	(U/L)	(U/L)	(mmol/L)
Control	54.6 ± 7.9	112.4 ± 9.8	249.2 ± 31.4	1.50 ± 0.12
CPZ	170.4 ± 41.1*	888.6 ± 188.5*@#	261.4 ± 47.4	2.08 ± 0.19*
LPS	37.6 ± 11.0@	171.2 ± 53.8#	200.2 ± 51.6	1.74 ± 0.24
LPS+CPZ	268.8 ± 42.0*	1675.5 ± 355.9*@	266.3 ± 45.9	2.53 ± 0.20*@
$GdCl_3$	57.2 ± 8.0	134.8 ± 19.7#	240.6 ± 46.8	1.59 ± 0.25#
$GdCl_3$+CPZ	217.0 ± 97.8	895.0 ± 218.2*@	282.0 ± 47.7	1.91 ± 0.24
$GdCl_3$+LPS+CPZ	68.8 ± 3.3@#	193.0 ± 65.7#	281.8 ± 68.3	2.19 ± 0.80
groups	Dbil	Tbil	TG	TBA
	(μmol/L)	(μmol/L)	(mmol/L)	(μmol/L)
Control	0.2 ± 0.1	3.0 ± 0.2	0.34 ± 0.08	17.6 ± 3.2
CPZ	1.0 ± 0.3*	6.9 ± 1.5*	0.49 ± 0.04	11.9 ± 3.9
LPS	0.5 ± 0.1@	7.3 ± 1.2*#	0.35 ± 0.06#	15.6 ± 5.8
LPS+CPZ	0.8 ± 0.1*	13.5 ± 1.0*@	0.77 ± 0.08*@	24.4 ± 5.0@
$GdCl_3$	0.3 ± 0.3@	2.6 ± 0.7@#	0.23 ± 0.08@#	14.1 ± 4.1
$GdCl_3$+CPZ	0.5 ± 0.3@	3.1 ± 0.4@#	0.29 ± 0.05#	22.6 ± 2.3@#
$GdCl_3$+LPS+CPZ	0.5 ± 0.2	4.3 ± 0.4@#	0.49 ± 0.23#	20.8 ± 5.1

Note: Data represents mean±SD, n=5. *P<0.05 (compared with control group), @P<0.05 (compared with CPZ group), #P<0.05 (compared with LPS+CPZ group).

Table 1 shows that the animals in the LPS-alone group exhibited no other abnormality than increased Dbil and Tbil, which were significantly higher than those of the animals in the control group. The Tbil, ALT, AST and TC of the rats in the CPZ-alone group were all significantly higher than those of the rats in the control group, and no abnormality was found in all eight serum indexes of the animals in the $GdCl_3$-alone group compared with the control group. The Tbil, ALT, AST, TC and TG of the animals in the LPS+CPZ-combined group were all significantly higher than those of the animals in the control group, and the Tbil, ALT and AST of the animals in the LPS+CPZ-combined group were also higher than those of the animals in the CPZ-alone group, indicating that the interaction between LPS and CPZ enhanced CPZ-induced liver toxicity. Compared with the LPS+CPZ-combined group, the four serum biochemical

indexes ALT, AST, Tbil and TG of the rats in the $GdCl_3$+LPS+CPZ-combined group significantly decreased; in addition, when compared with the control group, seven serum biochemical indexes of the rats in the $GdCl_3$+LPS+CPZ-combined group were normal, indicating that the enhancement of CPZ-induced liver toxicity by LPS could be effectively blocked by the prior administration of$GdCl_3$, which inactivates KC function. When compared with the control group, two indexes (ALT and AST) of the rats in the $GdCl_3$+CPZ-combined group both significantly increased, which indicated that using $GdCl_3$ alone to inhibit liver KCs could not completely eliminate CPZ-induced liver toxicity and indirectly indicated that CPZ-induced liver toxicity had a direct toxic effect on parenchymal hepatic cells and that CPZ-induced liver toxicity was mediated by KCs.

Table 2 Effects of (iv10mg/kg$GdCl_3$, iv2mg/kg LPS, ig200mg/kgZ24) on blood serum biochemistry of male rats

groups	ALT	AST	ALP	TC
	(U/L)	(U/L)	(U/L)	(mmol/L)
Control	46.8 ± 8.1	128.4 ± 13.1	298.4 ± 27.4	1.57 ± 0.21
CPZ	37.6 ± 11.0$^{@}$	171.2 ± 53.8$^{@}$	200.2 ± 51.6$^{@}$	1.74 ± 0.24
LPS	1111.4 ± 94.0*	1964.2 ± 452.7*	392.4 ± 57.6	2.24 ± 0.40*
LPS+CPZ	32.2 ± 10.3$^{@}$	135.0 ± 29.4$^{@}$	159.0 ± 21.8*$^{@\#}$	1.97 ± 0.18
$GdCl_3$	57.2 ± 8.0$^{@}$	134.8 ± 19.7$^{@}$	240.6 ± 46.8$^{@}$	1.59 ± 0.25$^{@}$
$GdCl_3$+CPZ	1314.6 ± 200.6$^{*\#}$	1750.2 ± 492.6$^{*\#}$	451.2 ± 75.5*#	2.36 ± 0.28*
$GdCl_3$+LPS+CPZ	41.4 ± 8.3$^{@}$	124.8 ± 17.7$^{@}$	289.8 ± 66.4	1.98 ± 0.19
groups	Dbil	Tbil	TG	TBA
	(μmol/L)	(μmol/L)	(mmol/L)	(μmol/L)
Control	0.3 ± 0.1	3.2 ± 0.3	0.27 ± 0.13	45.3 ± 12.6
CPZ	1.0 ± 0.3*	6.9 ± 1.5	0.49 ± 0.04	11.9 ± 3.9$^{@}$
LPS	6.6 ± 1.9*	11.6 ± 2.5*	0.37 ± 0.14	154 ± 27.6*
LPS+CPZ	1.0 ± 0.1*	4.5 ± 0.7$^{@}$	0.49 ± 0.15	12.10 ± 2.0$^{@}$
$GdCl_3$	0.3 ± 0.3$^{@}$	2.6 ± 0.7$^{@}$	0.23 ± 0.08	14.1 ± 4.1$^{@}$
$GdCl_3$+CPZ	3.1 ± 1.8	5.3 ± 1.7$^{@}$	0.66 ± 0.47	51.9 ± 19.4$^{@}$
$GdCl_3$+LPS+CPZ	4.8 ± 1.6	4.3 ± 0.7$^{@}$	0.41 ± 0.07	29.4 ± 7.1$^{@}$

Note: Data represents mean±SD, *n*=5. *P<0.05 (compared with control group), $^{@}$P<0.05 (compared with Z24 group), #P <0.05 (compared with $GdCl_3$+LPS+Z24 group).

Table 2 shows that no abnormalities in the indexes were found in the rats in the LPS-alone group other than increased Dbil and Tbil, which were significantly higher than in the rats in the control group. The six indexes Dbil, Tbil, ALT, AST, TC and TBA of the rats in the Z24-alone group were significantly higher than those of the rats in the control group. When compared with the control group, no abnormality was found in the seven serum indexes of the animals in the $GdCl_3$-alone group, but the TBA level slightly decreased, indicating that $GdCl_3$ itself had no apparent liver toxicity. Six indexes (Dbil, Tbil, ALT, AST, TC and TG) of the animals in the LPS+Z24-combined group were equivalent to those of the animals in the control group, but ALP and TBA slightly decreased, indicating that the interaction between LPS and Z24 had a certain protective effect against Z24-induced liver toxicity. When compared with the LPS+Z24-combined group, two serum biochemical indexes, ALP and Dbil, of the rats in the $GdCl_3$+LPS+Z24-combined group increased slightly. When compared with the control group, eight serum biochemical indexes of the rats in the $GdCl_3$+LPS+Z24-combined group were normal, indicating that the protective effect of LPS against Z24-induced liver toxicity could not be effectively blocked by prior administration of $GdCl_3$ to inactivate KCs function. When compared with the control group, six indexes, (Dbil, Tbil, ALT, AST, ALP and TG) of the rats in the $GdCl_3$+CPZ group still increased significantly, which indicated that using $GdCl_3$ to inhibit liver KC function had no apparent impact on Z24-induced liver toxicity and indirectly indicated that Z24-induced liver toxicity was not primarily mediated by KCs but might be caused by direct toxicity to the parenchymal hepatic cells.

Effect of IL-1α against Z24-induced liver toxicity

Table 3 Effects of (iv1.25μg/kgIL-1α2h, ig 200mg/kgZ24 after 24h) on blood serum biochemistry of male rats

groups	ALT	AST	ALP	TC
	(U/L)	(U/L)	(U/L)	(mmol/L)
Control	35.2 ± 1.3	97.2 ± 8.5	258.0 ± 73.1	1.81 ± 0.31
IL-1α2h igZ24	157.6 ± 67.3	321.4 ± 88.6*	268.0 ± 30.2	2.16 ± 0.48
IL-1α24h igZ24	291.4 ± 79.1$^{*\#}$	507.2 ± 141.0*	358.6 ± 59.1	1.94 ± 0.41
Z24	1111.4 ± 94.0$^{*\#@}$	1964.2 ± 452.7$^{*\#@}$	301.8 ± 58.4	1.71 ± 0.28
groups	Dbil	Tbil	TG	TBA
	(μmol/L)	(μmol/L)	(mmol/L)	(μmol/L)
Control	0.4 ± 0.1	2.9 ± 0.5	0.42 ± 0.08	31.5 ± 3.9
IL-1α2h igZ24	1.2 ± 0.3*	3.6 ± 0.6	0.46 ± 0.13	45.6 ± 9.2
IL-1α24h igZ24	5.0 ± 2.2	7.1 ± 1.9*	0.36 ± 0.15	48.5 ± 13.1
Z24	6.6 ± 1.9$^{*\#}$	11.6 ± 2.5$^{*\#}$	0.32 ± 0.13	153.9 ± 27.6$^{*\#@}$

Note: Data represents mean±SD, n=5. *P<0.05 (compared with control group), $^{@}$P<0.05 (compared with IL-1α24h igZ24 group), #P <0.05 (compared with IL-1α2h igZ24 group).

Table 3 shows that when compared with the control group, the serum biochemical indexes ALT, AST, Dbil and Tbil of the IL-1α 2h and IL-1α 24h pretreatment groups all exhibited an increasing trend, and there were significant differences in the indexes between these two pretreatment groups. However, when compared with Z24-alone group, the serum biochemical indexes ALT, AST, Dbil, Tbil and TBA of animals in the IL-1α pretreatment groups all decreased significantly. In addition, the effect of the 2h pretreatment was significantly superior to that of the 24h pretreatment, which indicated that pretreatment with the cytokine IL-1α could have a partial protective effect against liver injury caused by Z24 and indirectly indicated that liver injury caused by Z24 might be mediated by cytokine IL-1α, which is released by non-parenchymal hepatic cells such as KCs.

Discussion

The experimental results showed that a dose of 75 mg/kg of CPZ could cause the serum biochemical indexes ALT and AST to increase significantly in SD rats, and CPZ-induced liver toxicity could be significantly increased if the rats were intravenously administered 2 mg/kg LPS 2h prior to treatment. Using $GdCl_3$ to inactivate the functions of KCs effectively blocked the enhancing effect of LPS against CPZ-induced liver toxicity and reduced CPZ-induced liver toxicity. A dose of 200 mg/kg of Z24 induced extremely significant liver toxicity. Intravenous administration of LPS 2h in advance had a significant

protective effect against Z24-induced liver toxicity. However, using $GdCl_3$ to inactivate the KCs could not effectively block the protective effect of LPS against Z24-induced liver toxicity. IL-1α, which is released by non-parenchymal hepatic cells, such as KCs, also had a partial protective effect against Z24-induced liver toxicity.

In the present study, a non-toxic dose of LPS was used to induce a mild inflammatory response in SD rats. Significant inflammatory cell aggregations were observed, although they did not result in significant liver injury. Because of the effect of this dose of LPS, the liver toxicity generated by CPZ increased significantly. Therefore, a mild inflammatory response might be a related factor that activates CPZ and induces idiosyncratic reactions. Administration of this dose of LPS may enable the animal to induce the expression of tumor necrosis factor alpha (TNF-α) and release it into the blood before contact with CPZ, causing the neutrophils in the liver to aggregate. Although the above-mentioned cotreatment schemes could increase liver injury, other schemes may reduce liver injury. For example, administration of CPZ prior to or 30 min after delivering a large dose of LPS inhibited the synthesis of TNF-α and reduced the lethality of CPZ and CPZ-induced liver toxicity[14]. In addition, CPZ weakens the functions of neutrophils in vitro, including the generation of chemotaxis and superoxide. Therefore, the timing between the administration of LPS and exposure to CPZ may be an important factor that determines the toxicity.

The molecular mechanisms that enable LPS to render animals more sensitive to CPZ toxicity were not explored in present study. However, the ability of LPS to activate inflammatory cells to release proinflammatory mediators is likely to be involved. Previous studies have identified neutrophils, TNF-α, and cyclooxygenase products as critical mediators of liver injury [15]. These mediators precipitate a series of secondary biological events (e.g., promote cells to generate active oxygen and release toxic proteolytic enzymes), which eventually result in changes to the inner balance of the body and significant damage to the parenchymal hepatic cells caused by CPZ. Interestingly, inflammatory mediators that are critical to the enhancement of toxicity by LPS exposure differ for different xenobiotic agents. The inflammatory cytokines produced during LPS exposure also influence the expression of xenobiotic-metabolizing enzymes in the liver. In addition, the inflammatory cytokines caused by exposure to LPS can also affect the expression of drug-metabolizing enzymes in the liver [15]. Thus, LPS can also enhance CPZ-induced liver toxicity by affecting CPZ metabolism. Further studies are necessary to understand the other mechanisms of the interaction between LPS and CPZ. The present study also determined that using $GdCl_3$ to inactivate KCs effectively blocked the enhancing effects of LPS against CPZ-induced liver toxicity and partially

reduced CPZ-induced toxicity, indicating that CPZ-induced liver toxicity had a direct toxic effect on parenchymal hepatic cells and was mediated by KCs.

In short, the present study proved that the simultaneous administration of CPZ and a small dose of LPS could induce an inflammatory response in rats and replicate the main symptoms of the human phenothiazine idiosyncratic reaction. This result indicates that the simultaneous occurrence of mild inflammation may be a key factor that promotes idiosyncratic drug reactions. Therefore, it is possible to predict human idiosyncratic drug reactions and better study their mechanisms through the establishment of animal models.

Contrary to the experimental results in which prior intravenous administration of LPS 2h enhanced CPZ-induced liver toxicity, prior intravenous administration of 2 mg/kg LPS 2h resulted in an apparent protective effect against 200 mg/kg Z24-induced liver toxicity. Z24-induced liver toxicity primarily manifested as partial hepatic diffuse necrosis and inflammation with a dose-response effect, and the mechanism of liver toxicity is related to apoptosis of hepatocytes[16,17]. After LPS was intravenously administered in advance, a complete protective effect was observed in the serum biochemical indexes and pathological manifestations. This type of research result can promote new ideas for successful research and the development of Z24 as an antitumor drug.

Previous research showed that LPS-mediated inflammation had a protective effect against certain drugs that could induce liver toxicity. For instance, N-acetyl-p-aminophenol (APAP) exhibited a dual direction effect, and short-term pretreatment with LPS prior to drug administration could significantly increase APAP-induced liver toxicity [18]. However, Liu et al. [12] discovered that pretreating mice with LPS 24h prior to drug administration could significantly protect the liver from injury caused by APAP. Possible protective mechanisms include down-regulating the expression of cytochrome P450 (CYP 450) and reducing the generation of the toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI). The main difference between the doubling of APAP-induced liver toxicity in response to LPS is reflected in the LPS pretreatment time. Acute inflammation caused by a short-term pretreatment can result in the release of inflammatory mediators, which promotes or even induces the activation of cell death pathways and enhances drug-induced liver toxicity. Chronic inflammation caused by a long-term pretreatment can cause cells to change adaptively, which reduces the toxic damage caused by drugs. In regard to the protective effect against LPS on Z24-induced liver toxicity, its time relationship is just the opposite, meaning that acute inflammation caused by LPS has a protective effect. Therefore, we inferred that the mechanism

underlying this type of protective effect might not be the hepatic adaptive response but rather an acute pro-inflammatory mediator such as IL-1.

The present study showed that using $GdCl_3$ to inactivate KCs could not effectively block the protective effect of LPS against Z24-induced liver toxicity. In addition, pretreating with cytokine IL-1α also had a partial protective effect against liver injury caused by Z24, indicating that Z24-induced liver toxicity was not mediated by KCs but might be mediated by other non-parenchymal hepatic cells (e.g., endothelial cells and stellate cells) or may be a result of the direct toxic effect of Z24 on the parenchymal hepatic cells. One possible cause for the protective effect against Z24-induced liver toxicity was that the release of endothelial cells and IL-1α was activated under overwhelming inflammatory response conditions.

Although KCs had a beneficial effect on the elimination of LPS, inhibition of the functions of KCs had a protective effect against LPS-induced lethality and liver injury. This superficial contradiction can be partially explained as follows: the excessive growth of Gram-negative bacteria or intravenous administration of LPS resulted in a high concentration of LPS in the portal circulation; when the concentration of LPS was high enough to exceed the detoxification ability of the KCs, the KCs could be activated. When responding to the activation caused by LPS, the KCs released many inflammatory chemical mediators, including cytokines, prostaglandins, leukotriene, active oxygen, platelet activating factor, carbon monoxide, etc. These mediators might have a side effect on neighboring cells and worsen tissue damage. Therefore, the mechanism of inhibiting the functions of KCs and protecting animals from generating adverse reactions to LPS might include reducing the release of toxic mediators. In fact, although using $GdCl_3$ to inhibit the activity of KCs could reduce the damaging effect of a large dose of LPS on the liver, neutrophil aggregation in the liver was not reduced, and platelet aggregation only slightly decreased. These results indicate that in liver injury caused by a large dose of LPS, the KCs had no effect on the recruitment of inflammatory cells in the liver but were beneficial for subsequent events, such as the release of soluble mediators, and they eventually resulted in tissue necrosis.

A small dose of LPS can enhance CPZ-induced liver toxicity, and using $GdCl_3$ to inhibit the functions of KCs could significantly reduce the enhancing effect of LPS against CPZ-induced liver toxicity. The protective effect of $GdCl_3$ appears to be mediated through blocking the effect of LPS instead of through blocking the effect of CPZ. These results indicated that although the functions of KCs were not necessary for a large dose of CPZ to induce liver injury, LPS enhanced CPZ-induced liver toxicity via a KC-dependent mechanism.

Compared with CPZ, $GdCl_3$ pretreatment did not have a significant impact on Z24-induced liver toxicity or the protective effects of LPS against Z24-induced liver toxicity, indicating that the KCs might not play a role in Z24-induced liver toxicity or the protective effects of LPS against Z24-induced liver toxicity. However, the antagonizing effect of IL-1α pretreatment on Z24-induced liver toxicity indicated that inflammatory mediators play an important role in the occurrence of Z24-induced liver toxicity. Inhibition of KCs by $GdCl_3$ had little impact on Z24-induced liver toxicity; therefore, it could be inferred that this type of inflammatory mediator might originate from non-parenchymal hepatic cells other than KCs. The existing evidence shows that this type of cell may be hepatic endothelial cells. It was discovered[19] that when stimulated by LPS, hepatic endothelial cells could be activated and release inflammatory mediators and regulate the physiological functions of other hepatocytes.

Conclusions

The present study demonstrated that under overwhelming inflammatory response conditions, CPZ-induced liver toxicity could be enhanced through the activation of KCs and LPS-induced inflammatory response could be used to establish screening models for ILT. In addition, activating endothelial cells and the cytokine IL-1α might have a protective effect against Z24-induced liver toxicity in SD rats.

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Notes and references

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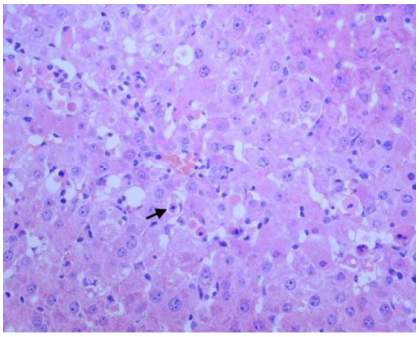
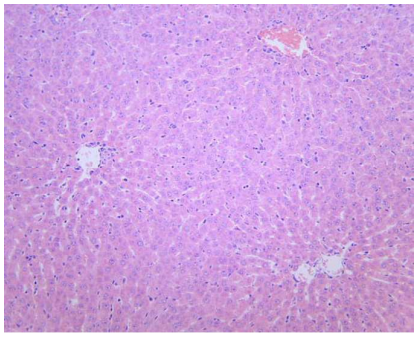
Graphical Abstract: LPS-induced inflammatory response could be used to establish screening models for ILT and provides a new way to reduce liver toxicity of Z24.



Liver histopathology after z24 treated (HE. 150× OR 300×). Z24 has liver toxicity

Z24-induced liver toxicity decreased after 2h of pretreatment with 2 mg/kg LPS followed by treatment with Z24



Liver histopathology after LPS+Z24 treated (HE. 150× OR 300×).