

Possible beneficial effects of lithium chloride on cerulein-induced acute pancreatitis in mice

A. Abed¹, M. Minaiyan^{1,*} and P. Mahzouni²

¹Department of Pharmacology & Toxicology and Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.

²Department of Clinical Pathology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.

Abstract

One of the most important and serious disorders of gastrointestinal tract is acute pancreatitis which in severe form is associated with high mortality rate particularly in the presence of systemic inflammatory response and multiple organ failure. Apoptosis linked to oxidative stress has been shown in the pancreas of the patients with acute pancreatitis. Lithium, one of the most effective drugs for the treatment of bipolar disorder, also has dramatic effects on preventing cell damage and apoptosis. Also lithium has shown anti-inflammatory effects in some animal studies. This study was designed to investigate the possible effect of lithium chloride in acute pancreatitis. Induction of acute pancreatitis was performed in male mice (25-30 g) by five intraperitoneal (i.p.) injection of cerulein (50 µg/kg) with 1 h intervals. Lithium chloride (10, 20 and 30 mg/kg) was administered i.p. 15 min before the induction of pancreatitis. Six h after the last injection of cerulein, the animals were sacrificed and biochemical as well as histopathological analysis was performed. Pretreatment with 20 mg/kg i.p. of lithium chloride reduced significantly the inflammatory response in cerulein-induced acute pancreatitis by ameliorating pancreatic edema and leukocyte infiltration, attenuating amylase and lipase serum levels, and myeloperoxidase activity compared to control group ($p < 0.05$). Two other administered doses namely 10 and 30 mg/kg were found ineffective. In this study our findings demonstrate that lithium can dose dependently exhibit protective effect against cerulein-induced acute pancreatitis.

Keywords: Acute pancreatitis; Lithium chloride; Cerulein; Mice

INTRODUCTION

Acute pancreatitis is a sudden inflammation of the pancreas that suffers from the lack of specific therapy. Pathophysiology of the acute pancreatitis is poorly understood. Therefore, pancreatitis continues to be associated with significant mortality and morbidity (1).

Inflammatory mediators are released from damaged pancreatic cells and systemic immune cells during pancreatitis. Interleukin-1 (IL-1) and tumor necrosis factor- α (TNF- α) are two major cytokines that play an important role in progression of acute pancreatitis (2).

Identification of intracellular signal transduction pathways that regulate duration of the inflammatory response in the course of the pancreatitis has been shown by protein kinases and has suggested a major role for glycogen

synthase kinase-3 (GSK-3) (3). It has been demonstrated that inhibition of GSK-3 reduces the degree of cerulein-induced acute pancreatitis and mortality rate in mice (4). Lithium which is mainly used medically as a mood-stabilizing drug, in the treatment of bipolar disorder, acts as a specific inhibitor of the GSK-3 family of protein kinases (5). Also it has been demonstrated that lithium has significant neuronal protective actions against apoptosis (6). High concentration of cerulein promoted the expression of proapoptotic gene bax and p53 and DNA fragmentation in AR42J cell line, which was mediated by intracellular Ca^{2+} (7). Lithium inhibits inositol lipid linked signaling pathway which causes remission in intracellular calcium levels (8). These data suggest that lithium might possess potential protective effects against

*Corresponding author: M. Minaiyan
Tel. 0098 311 792 2623, Fax. 0098 311 6680011
Email: minaiyan@pharm.mui.ac.ir

pancreatitis. The present study was performed to investigate the protective effects of lithium chloride in a murine model of acute pancreatitis which was induced by cerulein administration. To gain access to better insight into the possible mechanism (s) of action of the observed protective effects of lithium on pancreatitis, we have investigated the effects of lithium on pancreatic edema, leukocyte infiltration, amylase and lipase serum levels and also myeloperoxidase activity.

MATERIALS AND METHODS

Animals

Male mice weighing 25–30 g bred in the animal house of School of Pharmacy at Isfahan University of Medical Sciences (Isfahan, Iran) were used in this study. Animals were kept in uniform environment of temperature (21 ± 2 °C), and light/dark cycles (12/12 h) and allowed free access to pelleted rodent chow and tap water. Before induction of cerulein-induced acute pancreatitis, animals were fasted over the night for at least 12 h. The study was approved by the Ethics Committee for Animal Care and Uses of Isfahan University of Medical Sciences, Isfahan, Iran.

Induction of pancreatitis

Acute pancreatitis was induced by five consecutive intraperitoneal (i.p.) injections of 50 µg/kg body weight of cerulein (Sigma, St. Louis, MO, USA) with 1 h intervals (9).

Experimental design

In the current investigation, effect of lithium chloride (10, 20 and 30 mg/kg,) on acute pancreatitis was studied. Test doses of lithium were chosen based on the study performed by Egashira and coworkers (10). Lithium chloride was given 15 min before pancreatitis induction.

Control group (healthy mice) received i.p. injection of 5 ml/kg normal saline as the vehicle. In negative control group, mice with acute pancreatitis were pretreated with 5 ml/kg normal saline as i.p injection. Six mice in each group were used. Blood samples were collected 6 h after the last injection of cerulein

and stored at -70 °C for biochemical analysis. Thereafter mice were sacrificed by decapitation and their pancreases were rapidly removed and fixed in formaldehyde (10%) for histological examination. Besides, portions of this organ were promptly frozen in liquid nitrogen and stored at -70 °C until assayed.

Amylase and lipase serum level analysis

Serum lipase and amylase activities were determined using commercially available lipase and amylase kits (Pars-Azmoon Company, Tehran, Iran) (11).

Myeloperoxidase activity assay

Myeloperoxidase (MPO) activity, an index of polymorphonuclear cell accumulation, was measured according to the method of Bradley and coworkers with brief modification (12). Pancreas tissue was homogenized in 1 mL of 50 mM potassium phosphate buffer containing 0.5% hexadecyl trimethylammonium bromide (HTAB). The homogenate was then sonicated in an ice bath for 10 s and freeze thawed twice with sonication between cycles. Afterward, the suspensions were centrifuged at 15000 rpm for 15 min at 4 °C and the supernatant (0.1 mL) was allowed to react with dianisidine dihydrochloride (0.167 mg/ml) and 0.005% hydrogen peroxide contained in 2.9 mL of 50 mM potassium phosphate buffer (pH 6.0) . The absorbance of the reaction mixture was measured at 450 nm using a UV-Vis spectrophotometer (UNICO[®], uv-2100). MPO activity was expressed in units (U) per gram of wet tissues.

Histological examinations

Paraffin-embedded pancreas samples were sectioned (5 µm), stained with hematoxylin and eosin, and examined by a pathologist unaware of the experimental protocol.

The histological grading of edema was made using a scale ranging from 0 to 3 (0=no edema, 1=interlobular edema, 2=interlobular and moderate intralobular edema, and 3=interlobular edema and severe intralobular edema). Leukocyte infiltration was also graded from 0 to 3 (0=absent, 1=scarcely perivascular infiltration, 2=moderate perivascular and scarce diffuse infiltration, and 3=abundant

diffuse infiltration). Grading of vacuolization was based on the appropriate percentage of acinar cells involved: 0=absent, 1=less than 25%, 2=25–50% and 3=more than 50% of acinar cells (13).

Statistical analysis

Biochemical results are expressed as mean \pm SEM. Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Nonparametric data was analyzed by Mann-Whitney *U* test. The minimal level of

significance was considered at $P < 0.05$.

RESULTS

Effects of lithium chloride on the serum levels of amylase and lipase

Cerulein-induced pancreatitis in vehicle-treated mice was associated with significant rises in the serum levels of amylase and lipase. The increase in amylase and lipase was noticeably reduced in cerulein-treated mice which were pretreated with i.p. injection of lithium at the dose of 20 mg/kg (Figs. 1 and 2).

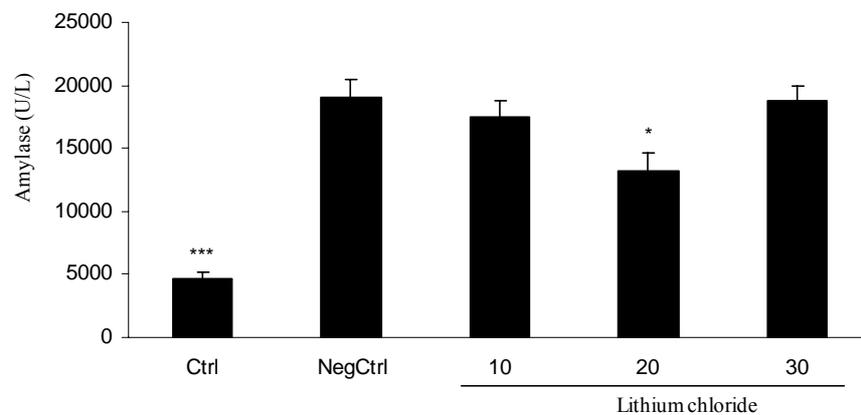


Fig. 1. Effect of lithium chloride on serum amylase levels (U/L) of cerulein-induced acute pancreatitis in mice. Control (Ctrl), normal mice treated with normal saline (5 ml/kg); negative control (NegCtrl), mice with pancreatitis treated with normal saline (5 ml/kg), lithium chloride (10, 20, 30 mg/kg); all treatments were made intraperitoneally. Data are shown as mean \pm SEM of 6 animals in each group. * $P < 0.05$, *** $P < 0.001$ versus negative control (ANOVA).

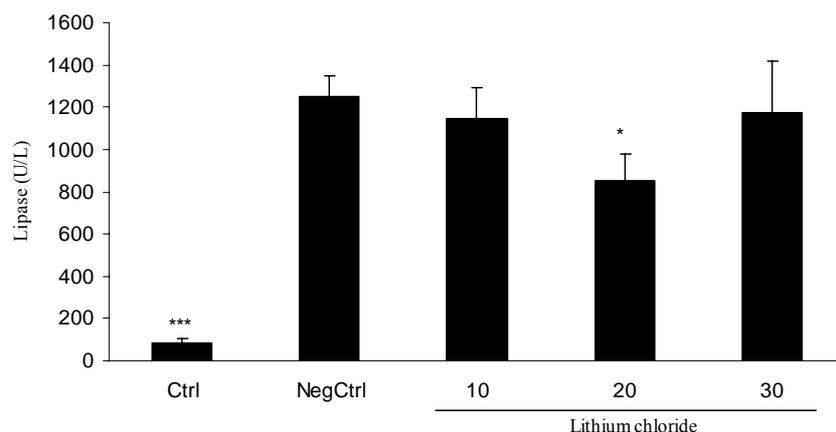


Fig. 2. Effect of lithium chloride on serum lipase level (U/L) of cerulein-induced acute pancreatitis in mice. Control (Ctrl), normal mice treated with normal saline (5 ml/kg); negative control (NegCtrl), mice with pancreatitis treated with normal saline (5 ml/kg), lithium chloride (10, 20, 30 mg/kg); all treatments were made intraperitoneally. Data are shown as mean \pm SEM of 6 animals for each group. * $P < 0.05$, *** $P < 0.001$ versus negative control (ANOVA).

Effects of lithium chloride on myeloperoxidase activity

Myeloperoxidase activity as a marker of leukocyte accumulation was obviously enhanced in the pancreas tissue following the cerulein administration. Pretreatment with lithium at the dose of 20 mg/kg by i.p. injection significantly diminished MPO activity, in comparison with normal saline treated mice ($P<0.05$) (Fig. 3).

Effects of lithium chloride on the histological parameters

As expected, in normal saline treated mice, pancreas did not show any tissue injuries at

light microscopic level ($\times 10$ magnification). Administration of cerulein was able to induce acute edematous pancreatitis with severe leukocyte infiltration and vacuolization in all mice tested.

In lithium-pretreated group at a dose of 20 mg/kg the severity of edema and leukocyte infiltration was significantly reduced compared to normal saline treated group ($P<0.05$) while vacuolization was not affected significantly. On the other hand, two lower and higher doses of lithium (10 and 30 mg/kg) were not effective in protecting pancreas against injuries (Table 1, Fig. 4).

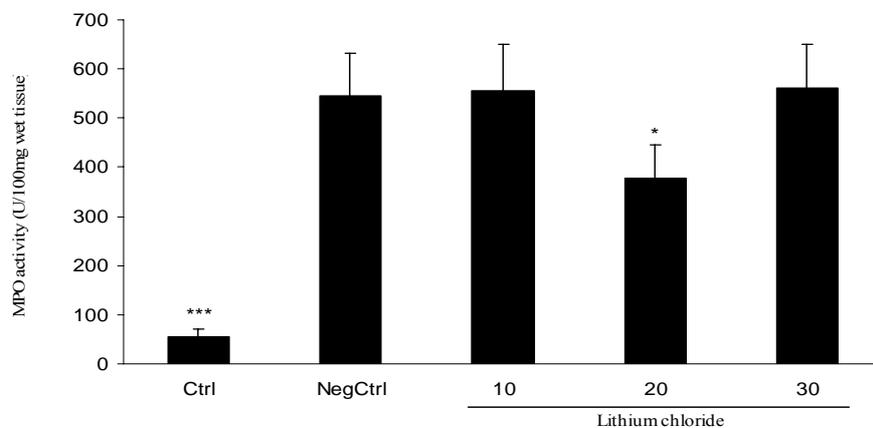


Fig. 3. Effect of lithium on myeloperoxidase activity of cerulein-induced acute pancreatitis in mice. Control (Ctrl), normal mice treated with normal saline (5 ml/kg); negative control (NegCtrl), mice with pancreatitis treated with normal saline (5 ml/kg), lithium (10, 20, 30 mg/kg); all treatments were made intraperitoneally. Data are shown as mean \pm SEM of 6 animals for each group. * $P<0.05$, *** $P<0.001$ versus negative control (ANOVA).

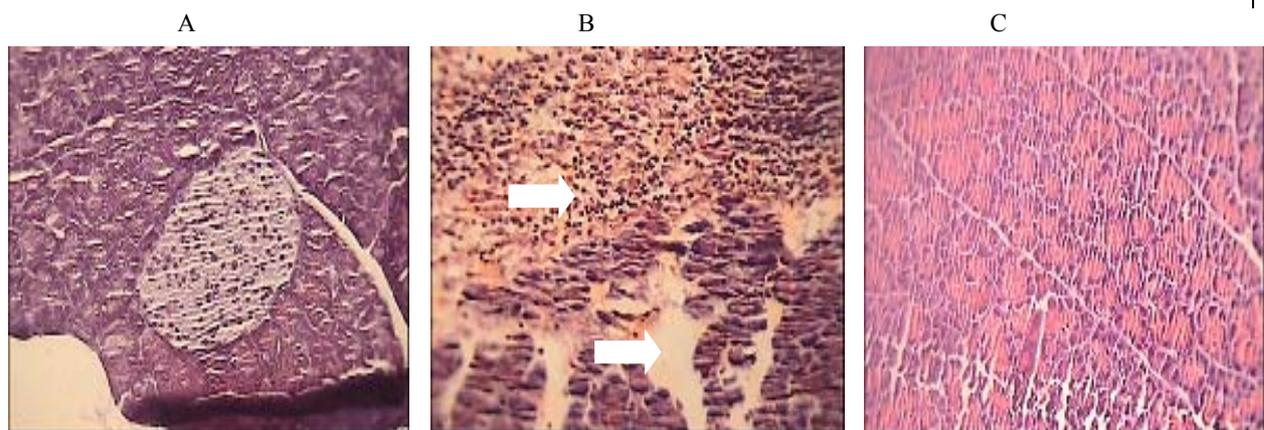


Fig. 4. Representative illustrations of normal pancreas and acute pancreatitis in mice. A. Normal pancreatic tissue. B. Acute pancreatitis induced by cerulein with severe intralobular edema and abundant diffuse leukocyte infiltration (white arrows). C. Acute pancreatitis treated with lithium (20 mg/kg) with moderate intralobular edema and scarce perivascular infiltration. Hematoxylin and eosin staining with low ($\times 10$) power.

Table 1. Effect of lithium on pathological scores of pancreas in cerulein-induced acute pancreatitis in mice.

Group	Edema	Leukocyte infiltration	Vacuolization
Ctrl	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
NegCtrl	2.50 ± 0.22	2.16 ± 0.30	0.62 ± 0.22
Li (10 mg/kg)	2.16 ± 0.16	2.00 ± 0.25	0.53 ± 0.21
Li (20 mg/kg)	1.80 ± 0.25*	1.66 ± 0.21*	0.57 ± 0.20
Li (30 mg/kg)	2.24 ± 0.24	1.9 ± 0.23	0.56 ± 0.16

Control (Ctrl), normal mice treated with normal saline (5 ml/kg); negative control (NegCtrl), mice with pancreatitis treated with normal saline (5 ml/kg); all treatment were made intraperitoneally. Data are shown as mean ± SEM of 6 animals for each group, * $P < 0.05$: significant difference compared to negative control (Mann's Whitney U test).

DISCUSSION

We investigated here the effects of lithium chloride on cerulein-induced acute pancreatitis in mice. Cerulein caused a substantial increase in the serum levels of amylase and lipase as well as tissue damage characterized by inflammatory cell infiltration, edema and increased myeloperoxidase activity.

Lithium chloride reduced serum amylase and lipase activities and myeloperoxidase activity in pancreas tissues. Pancreatic tissue inflammation was also decreased significantly ($P < 0.05$) at the dose of 20 mg/kg of lithium carbonate. But intraperitoneal injection of lithium at doses of 10 and 30 mg/kg did not significantly alter the degree of inflammation and serum lipase and amylase activities elevated in mice with pancreatitis. This is in accordance with the results obtained by Matasis and coworkers (14). They suggested that lithium in high doses caused a rise in the serum amylase concentration which was in accordance with low therapeutic window of lithium activity and suggested more detailed studies to explore the underlying mechanism of action.

Lithium also inhibits GSK-3 which was first identified as ubiquitous serine-threonine protein kinase involved in glycogen metabolism (15,16). GSK-3 has been implicated in cell membrane to nucleus signaling, gene transcription and translation, cytoskeletal organization, and cell cycle progression and survival (3,17,18). Also GSK-3 is a part of many intracellular signaling pathways such as insulin phosphatidylinositol-3 kinase (PI-3K). Activation of these pathways inhibits GSK-3 which has been linked to the neuroprotection and activation of cell survival GSK-3 contributes to the pathogenesis of

signaling pathways (5). It has been shown that cerulein-induced pancreatitis in mice and that treatment with the GSK-3 inhibitor decreases the inflammatory responses associated with the pancreatitis (4).

The nuclear factor- κ B (NF- κ B) is involved in the regulation of many pro-inflammatory genes, including those for TNF- α , IL-1 β and vascular endothelium growth factor (VEGF) (19). The discovery that inhibition of NF- κ B activity may be useful in conditions associated with local and systemic inflammation stimulated the search for agents that prevent its activation. It has also been proven that interfering with the NF- κ B pathway leads to a substantial amelioration of experimental acute pancreatitis (20). GSK-3 β has profound effects on the transcription in a gene-specific manner through a mechanism involving in the control of promoter-specific recruitment of NF- κ B (21).

Lithium has been shown to reduce inflammatory cytokine production by inhibiting GSK3-dependent activation of NF- κ B transcriptional activity (22-24).

It has been reported that prevention of neutrophil infiltration into the pancreas consequently attenuates pancreatic injury during acute pancreatitis (25). It was demonstrated that lithium markedly decreased MPO activity in an animal model of colitis in rat which has been further supported by the results obtained from the present study (26). Cerulein is able to induce apoptosis in pancreatic cells (27). Some studies have been shown that lithium increases cell survival by stimulating activity in anti-apoptotic pathways, including the PI-3K /Akt and the mitogen-activated protein kinase pathways. In addition, lithium reduces pro-apoptotic function by directly and indirectly inhibiting GSK-3 β

activity and indirectly inhibiting N-methyl-D-aspartate (NMDA) receptor-mediated calcium influx which may be partially or fully responsible for protective effect of lithium against cerulein induced acute pancreatitis (28).

Taken together these findings demonstrate that lithium marginally exhibits anti-inflammatory property in animal model of cerulein-induced acute pancreatitis but this effect is critically dependent to dose and needs an accurate estimation of the dosage.

CONCLUSION

We demonstrated that lithium chloride indicated protective activity against cerulein-induced acute pancreatitis in mice which might suggest therapeutic or protective potential under this inflammatory disease condition.

ACKNOWLEDGMENTS

We would like to thank the Isfahan Pharmaceutical Sciences Research Center Isfahan University of Medical Sciences, Isfahan, I.R. Iran for financial support of this research.

REFERENCES

1. Cecil RL, Andreoli TE. Cecil essentials of medicine, 7th ed., Philadelphia: W.B. Saunders; 2007. p. 435-437
2. Surco Y, Huerta Mercado J, Pinto J, Piscoya A, De Los Ríos R, Prochazka R, *et al.* Early prediction of severity in acute pancreatitis. *Rev Gastroenterol Peru.* 2012;32:241-250.
3. Woodgett JR. Judging a protein by more than its name: GSK-3. *Sci STKE* 2001;(100):re12.
4. Cuzzocrea S, Malleo G, Genovese T, Mazzon E, Esposito E, Muià C, *et al.* Effects of glycogen synthase kinase-3beta inhibition on the development of cerulein-induced acute pancreatitis in mice. *Crit Care Med.* 2007;35:2811-2821.
5. Bertsch S, Lang CH, Vary TC. Inhibition of glycogen synthase kinase 3beta activity with lithium in vitro attenuates sepsis-induced changes in muscle protein turnover. *Shock.* 2011;35:266-274.
6. Matsebatlela T, Gallicchio V, Becker R. Lithium modulates cancer cell growth, apoptosis, gene expression and cytokine production in HL-60 promyelocytic leukaemia cells and their drug-resistant sub-clones. *Biol Trace Elem Res.* 2012;149:323-330.
7. Yu JH, Kim H, Kim KH. Calcium-dependent apoptotic gene expression in cerulein-treated AR42J cells. *Ann N Y Acad Sci.* 2003;1010:66-69.
8. De Meyer I, Martinet W, Van Hove CE, Schrijvers DM, Hoymans VY, Van Vaeck L, *et al.* Inhibition of inositol monophosphatase by lithium chloride induces selective macrophage apoptosis in atherosclerotic plaques. *Br J Pharmacol.* 2011;162:1410-1423.
9. Mazzon E, Genovese T, Di Paola R, Muià C, Crisafulli C, Malleo G, *et al.* Effects of 3-aminobenzamide, an inhibitor of poly (ADP-ribose) polymerase, in a mouse model of acute pancreatitis induced by cerulein. *Eur J Pharmacol.* 2006;549:149-156.
10. Egashira N, Abe M, Shirakawa A, Niki T, Mishima K, Iwasaki K, *et al.* Effects of mood stabilizers on marble-burying behavior in mice: involvement of GABAergic system. *Psychopharmacology.* 2013;226:295-305.
11. Minaiyan M, Ghannadi AR, Mahzouni P, Abed AR. Preventive Effect of Cichorium Intybus L. Two extracts on cerulein-induced acute pancreatitis in mice. *Int J Prev Med.* 2012;3:351-357.
12. Bradley PP, Priebe DA, Christensen RD, Rothstein G. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. *J Invest Dermatol.* 1982;78: 206-209.
13. Dembiński A, Warzecha Z, Ceranowicz P, Warzecha AM, Pawlik WW, Dembiński M, *et al.* Dual, time-dependent deleterious and protective effect of anandamide on the course of cerulein-induced acute pancreatitis, Role of sensory nerves. *Eur J Pharmacol.* 2008;591:284-292.
14. Matsis PP, Fisher RA, Tasman-Jones C. Acute lithium toxicity-chorea, hypercalcemia and hyperamylasemia. *Aust N Z J Med.* 1989;19:718-720.
15. Frame S, Cohen P. GSK3 takes centre stage more than 20 years after its discovery. *Biochem J.* 2001;359:1-16.
16. Van Wauwe J, Haefner B. Glycogen synthase kinase-3 as drug target: From wallflower to center of attention. *Drug News Perspect.* 2003;16:557-565.
17. Cohen J, Abraham E. Microbiologic findings and correlations with serum tumor necrosis factor-alpha in patients with severe sepsis and septic shock. *J Infect Dis.* 1999;180:116-121.
18. Embi N, Rylatt DB, Cohen P. Glycogen synthase kinase-3 from rabbit skeletal muscle: Separation from cyclic-AMP-dependent protein kinase and phosphorylase kinase. *Eur J Biochem.* 1980;107:519-527.
19. Virlos I, Mazzon E, Serraino I, Di Paola R, Genovese T, Britti D, *et al.* Pyrrolidine dithiocarbamate reduces the severity of cerulein-induced murine acute pancreatitis. *Shock.* 2003;20:544-550.
20. Virlos I, Mazzon E, Serraino I, Genovese T, Di Paola R, Thiemerman C, Siriwardena A, Cuzzocrea S. inhibitor ameliorates the indices of disease severity in a murine model of cerulein-induced acute pancreatitis. *Intensive Care Med.* 2004;30:1645-1651.
21. Steinbrecher KA, Wilson W 3rd, Cogswell PC, Baldwin AS. Glycogen synthase kinase 3beta

- functions to specify gene-specific, NF-kappa B-dependent transcription. *Mol Cell Biol.* 2005;25:8444-8455.
22. Steinbrecher KA, Wilson PC, Cogswell W, Baldwin AS. Glycogen synthase kinase 3 β functions to specify gene-specific, NF- κ B-dependent transcription. *Mol Cell Biol.* 2005;25:8444-8455.
 23. Hu X, Paik PK, Chen J, Yamilina A, Kockeritz L, Lu T, *et al.* IFN-gamma suppresses IL-10 production and synergizes with TLR2 by regulating GSK3 and CREB/AP-1 proteins. *Immunity.* 2006;24:563-574.
 24. Rehani M, Jope R, Michalek S. Toll-like receptor-mediated cytokine production is differentially regulated by glycogen synthase kinase 3. *Nat Immunol.* 2005;6:777-784.
 25. Abed A, Minaiyan M, Ghannadi A, Mahzouni P, Babavalian MR. Effect of *Echium amoenum* Fisch. et Mey a traditional iranian herbal remedy in an experimental model of acute pancreatitis. *ISRN Gastroenterol.* 2012;2012:141548.
 26. Daneshmand A, Mohammadi H, Rahimian R, Habibollahi P, Fakhfouri G, Talab SS, *et al.* Chronic lithium administration ameliorates 2,4,6-trinitrobenzene sulfonic acid-induced colitis in rats; potential role for adenosine triphosphate sensitive potassium channels. *J Gastroenterol Hepatol.* 2011;26:1174-1181
 27. Yu JH, Lim JW, Kim KH, Morio T, Kim H. NADPH oxidase and apoptosis in cerulein-stimulated pancreatic acinar AR42J cells. *Free Radic Biol Med.* 2005;39:590-602.
 28. Lowthert L, Leffert J, Lin A, Umlauf S, Maloney K, Muralidharan A, *et al.* Increased ratio of anti-apoptotic to pro-apoptotic Bcl2 gene-family members in lithium-responders one month after treatment initiation. *Biol Mood Anxiety Disord.* 2012;2:2-11.