Islamic Azad University
Urmia Branch

Journal of Basic and Clinical Veterinary Medicine

2024; 5(1): 19-31

Official Journal of Veterinary Faculty of Islamic Azad University
Urmia Branch

Journal Homepage: https://sanad.iau.ir/journal/jbcvm/

Original Article

Stereological and biochemical study of effects of thiamine and ZnO NPs on cecum after l-arginine-induced experimental pancreatitis in rats

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ARTICLE INFO

Received: 03 January 2024

Accepted: 13 February 2024

DOI: 10.82561/jbcvm.2024.1086115

KEYWORDS:

Pancreatitis
Rats
Histomorphometry
Biochemical evaluation
ZnO NPs

ABSTRACT

Acute pancreatitis (AP) is a critical disease with a high mortality rate due to the necrosis of the pancreas and other organs. We aimed to study the effects of thiamine and ZnO nanoparticles (NPs) in treating AP. Fifty male rats, with an average weight of 28 to 32 grams, were randomly divided into five groups: 1: Control group, 2: L-arginine group (300 mg/100g), 3: Thiamine group (70 mg/kg), 4: ZnO NPs group (1 mg/kg), 5: Combination treatment group: L-arginine (300 mg/100g) + thiamine (70 mg/kg) + ZnO NPs (1 mg/kg). After stereological examination of tissue sections and measurement of biomarkers, the data were analyzed at a significance level of $p \leq 0.05$. According to the results, in the L-arginine treatment group, the thickness of the mucosal, muscular, and adventitial layers decreased in comparison to the total thickness ($p \leq 0.05$). Additionally, values of the surface area and density of the mucosal and muscular layers decreased, as well as the total values of these, compared to the control group. The mentioned parameters increased in the combination treatment group. Evaluation of cholesterol showed a significant increase between the control group and groups 4 and 5. Analysis of ALT and AST factors indicated a significant increase in the level of AST in L-arginine group compared to the control group, and a significant decrease in the level of ALT in comparison between the thiamine and ZnO NPs groups and the L-arginine group. Based on the results of the present research, thiamine and ZnO NPs manifested enhancing effects on the values of thickness, surface area, volume density, and level of ALT.

بررسی استریولوژیک و بیوشیمیایی اثرات تیامین و اکسید روی بر روی سکوم پس از پانکراتیت تجربی ناشی از ال- آرژنین در موش صحرایی

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چکیده

پانکراتیت حاد (AP) یک بیماری شایع و بحرانی با میزان مرگ و میر بالا به دلیل نکروز پانکراس و برخی از اندام ها است. هدف ما بررسی اثر تیامین و نانو ذره اکسید روی در درمان پانکراتیت حاد بود.روش کار :۵۰ موش صحرایی با میانگین وزن ۲۸ تا ۳۲ گرم به طور تصادفی به پنج گروه تقسیم شدند: گروه ۱: کنترل، ۲: گروه ال-آرژنین (۳۰۰ میلی گرم/۱۰۰ گرم)، ۳: گروه تیامین (۷۰ میلی گرم بر کیلوگرم)، ۴: گروه نانوذرات اکسید روی (۱ میلی گرم بر کیلوگرم)، ۵: گروه درمان ترکیبی: ال-آرژنین (۳۰۰ میلی گرم/۱۰۰ گرم) + تیامین (۷۰ میلی گرم بر کیلوگرم) + نانوذرات اکسید روی (۱ میلی گرم بر کیلوگرم). پس از بررسی استریولوژی برش های بافتی و اندازه گیری بیومارکرها، داده ها در سطح معنی داری $p\leq ۰/۰۵$ تجزیه و تحلیل شدند. بر اساس نتایج، در گروه تیمار ال-آرژنین ضخامت لایه‌های مخاطی، عضلانی و اضافی به همراه ضخامت لایه‌های کل کاهش معنی‌داری را نشان داد ($p\leq ۰/۰۵$). همچنین در مقایسه با گروه کنترل ارزیابی سطح و تراکم لایه های مخاط و عضلانی، کاهش را همچون سطح و حجم کلی لایه ها نشان داد در حالی که افزودن تیامین و نانوذرات اکسید روی به ال-آرژنین این پارامترها را افزایش داده است. ارزیابی کلسترول افزایش سطح معنی داری را بین گروه کنترل با گروه ۵ و گروه ۴ ذکر کرد. تجزیه و تحلیل فاکتورهای ALT و AST حاکی از افزایش معنی دار سطح AST در گروه ال-آرژنین در مقایسه با گروه کنترل و کاهش معنی دار در سطح ALT در گروه تیامین و نانوذرات اکسید روی در مقایسه با گروه ال-آرژنین بود. بر اساس نتایج تحقیق حاضر، نانوذرات تیامین و اکسید روی اثرات افزایشی بر ضخامت، سطح و تراکم حجمی و میزان فاکتور بیوشیمیایی ALT نشان دادند.

واژه های کلیدی: التهاب پانکراس، موش صحرایی،هیستومورفومتری، ارزیابی بیوشیمیایی، نانوذرات اکسید روی

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INTRODUCTION

Pancreatitis (pancreas inflammation) is associated with side effects and high mortality due to necrosis of the pancreas and some organs outside the pancreas, as well as subsequent infection of the gland and multifactorial failure of the organ [1]. The management strategy of the disease is promising in more than half of the patients who suffer from mild to moderate pancreatitis. The rest of the patients struggle with one or more chief symptoms and need special medical attention [2]. However, in recent years, despite many advances in the recognition of pancreatic exocrine cytology and the epidemiology of pancreatitis, our knowledge of the treatment or prevention of pancreatic disease is still limited [3]. In experimental studies on animal models, pancreatitis can be induced using different doses of intraperitoneal injection of L-arginine. The results of other studies show that a dose of 500 mg can cause pancreatitis [4]. According to research, various compounds such as cholecystokinin, caerulein, bile acids, and essential amino acids can cause pancreatitis [5, 6]. One of the most important amino acids in the body is L-arginine, which plays an effective role in the growth of some organs and can have various effects on tissues, including the Liver, pancreas, and kidney, but little evidence of physiopathological lesions in intestinal, testicular, splenic, lung, heart, and thymus tissues is available [7]. Pancreatitis causes fibrosis, inflammation of the pancreas, and destruction of the Langerhans islets [8, 9]. Based on the results of electron microscopy, it was found that the changes started with irregularities in the endoplasmic reticulum and after 24 hours followed by fundamental deformation [7]. Changes in the acinar cells and the next stage, necrosis, and replacement of interstitial tissue with fibroblasts and leukocytes were observed after 48 hours [10]. Although the mechanism of action of L-arginine is not fully understood in inducing pancreatitis, the evidence suggests that arginine, free oxygen radicals (ROS), inflammatory mediators, and nitric oxide (NO) mediators play a key role in disease progression [11]. Thiamine or vitamin B1, in free form or combined with other elements, plays an essential role in evolution as a biological co-factor [12]. Thiamine is involved in carbohydrate metabolism as well as in functioning as a neuromuscular transporter [13, 14]. Thiamine also can cross the blood-brain barrier [15]. Anorexia, depression, muscle atrophy, dementia, and Beriberi disease are all caused due to deficient levels of this vitamin [16, 17]. Blood levels of this vitamin depend on glomerular filtration, tubular reabsorption, and plasma concentration [18, 19]. Thiamine is absorbed in the small intestine and thiamine esters are hydrolyzed by pancreatic nucleotide pyro-phosphatase or pancreatic alkaline phosphatase, and subsequently de-phosphorylated thiamine is absorbed in enterocytes (cells of the small intestine) through thiamine transporters at low concentrations as well as at high concentrations of thiamine through diffusion [19, 20]. Zinc is one of the most abundant and important trace elements, used in body tissues [12]. ZnO NPs play a key role at the highest concentration in this [21]. Due to the role of zinc in the body's antioxidant system, its importance in immune responses and oxidative stress can be defined [22]. ZnO NPs increase protein oxidation, cell death, Leydig cells secretory activity, FSH and LH, and star gene expression decreases Pregnenolone, and destroys the DNA of Leydig cells [23]. Among the applications of zinc oxide are the treatment of skin lesions, anti-dandruff shampoos, anti-inflammatory agents for the skin, and relief of allergic reactions [24]. Despite the optical, catalytic, and magnetic applications of ZnO NPs, they cannot be claimed as applicable for sure in terms of toxicology and biosafety. Some studies have shown that nausea, vomiting, and

diarrhea occur in mice treated with zinc nanoparticles [25, 26]. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), the main circulating enzymes in the body, are synthesized by the liver and have half-lives of approximately 18 and 36 hours in healthy young adults, respectively [27]. Transaminase enzymes are commonly considered as monitoring criteria for the evaluation of liver function; however, in addition to the liver, AST is produced in other tissues, such as the heart, muscles, and so on [28]. Serum ALT activity has long been used as an inflammatory marker to assess liver injury related to multiple etiologies including hepatitis, tumors, liver cirrhosis, and alcohol consumption, and the level of ALT/AST ratio (LSR) in the serum has been generally accepted as a better predictor of liver injury [8]. Cholesterol is the main sterol in mammals and has an important role in the plasma membrane where it is responsible for modulating membrane fluidity, permeability, and signaling [29, 30]. It is also found in the endoplasmic reticulum membrane in small amounts where it is essential for its metabolic regulation [31]. Urea is a highly water-soluble, polar molecule with a neutral charge, with one oxygen and two nitrogen atoms, involved in the hydrogen bonds, and two amine groups, providing a total of four hydrogen bonds. A solution of urea is odorless, colorless, and neither acidic nor alkaline. In 1932, the biosynthesis pathway of urea in mammalian liver in vitro was discovered, and this pathway was subsequently named as the urea cycle (also known as the ornithine cycle) [32, 33].

MATERIALS AND METHODS

Animals

Fifty adult male BALB/C mice, weighing 28–32 g, were used in this study. The animals were fed commercial mouse food and tap water and kept in standard temperature (around 21 °C) and humidity (21–33%) conditions with a 12–12 h light-dark-cycle. To create a suitable environment during the test period, the cage floors were covered with fresh and soft sawdust and the cages were cleaned every three days to maintain hygiene. Mice were randomly divided into the experimental groups described as follows.

Chemicals

L-arginine hydrochloride powder (Sigma, UT, USA) was dissolved in 0.9% saline and the pH set at 7.4, using 0.1 M sodium hydroxide (NaOH) solution. Before each injection, an L-arginine solution was prepared which these processes were taken two weeks. Acute pancreatitis was induced by intraperitoneal injections (I.P.) of L-arginine hydrochloride in an animal model (mice). The doses of 300 mg/100 g B.W. of L-arginine were chosen for administration to mice through I.P. injection (at an interval of 2 h). After experimental induction of pancreatitis (72 h), they were injected with thiamine at a dose of 70 mg/kg and ZnO NPs at a dose of 1mg/kg. Animals in the control group were injected with normal saline intraperitoneal as a placebo. Several samples were selected and after tissue slide preparation, pancreatitis was confirmed by a pathologist microscopically.

Grouping

After adaptation of the mice to the new environment, they were randomly divided into 5 groups (10 samples in each group): Group 1: injected (I.P.) with normal saline solution as a placebo (control group).

Group 2: injection of thiamine at a dose of 70 mg/kg (with intervals of 2h). Group 3: injection of ZnO NPsat a dose of 1 mg/kg (with intervals of 2h). Group 4: injection of L-arginine at the dose of 300 mg/100g B.W. (experimental pancreatitis); afterward, administration of thiamine at a dose of 70 mg/kg and ZnO NPsat a dose of 1 mg/kg (with the interval of 2h) for treatment. Group 5: the experimental pancreatitis group: administration of L- L-arginine at a dose of 300 mg/100 g B.W. (with intervals of 2h). At the end of the experiment, 6 hours after the last treatment, blood samples were collected from the heart under general anesthesia. Blood sampling was performed to determine levels of ALT, and AST. The samples were centrifuged for 10 minutes at 3000 rpm at 4 °C, using a Universal centrifuge (Hettich, Tuttlingen, Germany) device. The final clear sera were stored at -70 °C until use. In addition, pancreatic tissue was removed by laparotomy after dissection of surrounding tissues. Specimens were sectioned and fixed in 10% neutral buffered formalin solution for histopathological examinations and the serial microscopic slides were stained with H&E. Finally, the pathological analysis was performed under light microscopy by an expert pathologist.

Histological evaluation of the pancreatic islets' diameter

The preparation of tissue sections was performed using histological techniques. Thus, according to our previous study, the quantitative technique was defined based on preparing serial sections and investigating the random selection from sections for measuring the diameter of pancreatic islets. The mean diameter of the islets was measured by tracing the cross-sectional boundary between the pancreatic islets and the pancreatic exocrine glands. Since the islands lacked a regular geometric shape, the mean of the smallest and largest diameter was considered as the average diameter, and then the measured diameter was introduced as the final diameter.

Pathology examinations

Pancreatic histological slides were prepared according to the histological protocols of the tissue section preparation and stained with Hematoxylin and Eosin method. Pancreatic tissue sections were evaluated by an expert pathologist according to the study protocol. The severity of the injuries was interpreted based on edema, inflammation, and histopathological changes.

Statistical analysis

To compare data between control and treatment groups, SPSS software version 23 was used. Data analysis was performed using One-Way Analysis of Variance (ANOVA), followed by an LSD test as a post-hoc test, and were recorded as Mean ± SD. Significant differences were observed between the groups at a 95% confidence interval with a significance level of $p \leq 0.05$.

RESULTS

The thickness of the cecum layers

The layers' thickness of the cecum wall was measured using histological sections (Figure 1) Stained sections show the cecum layers and the structures specific to the cecum layers, which are used as a guide to identifying the cecum layers. According to the obtained results, the thickness of mucosal, muscular, and adventitial layers along with the thickness of the total layers (Table 1) indicated a significant decrease in the experimental pancreatitis group (G-5)

compared to the control group (G-1) ($p \leq 0.05$). The morphometric study showed that the addition of thiamine and ZnO NPs to the L-arginine group (G-4) increased the values of the mentioned parameters compared to the experimental pancreatitis (G-5); nevertheless, the difference related to only two parameters, including mucosa and total thickness was significant ($p \leq 0.05$). Significant differences were not observed in the thickness of mucosal, muscular, and adventitial layers in groups of thiamine and ZnO NPs, compared to the control group ($p \leq 0.05$).

Surface area and volume density of different layers of the cecum

As shown in Tables 2 and 3, differences within the control and thiamine groups were not observed for either surface area or volume density of the different layers of the cecum ($p > 0.05$). Additionally, no changes induced by ZnO NPswere observed in the surface and volume of cecal tissue layers in the mice compared to the control group ($p > 0.05$). In L-arginine-treated rats, values of the surface area and volume density of the mucosal and muscular layers as well as the total value, indicated significant differences in comparison with the control group ($p \leq 0.05$). Simultaneous administration of the thiamine and ZnO NPs, in mice treated with L-arginine (group-4), had a positive effect on the mentioned parameters; therefore, the values of the surface area and volume density changed significantly in L-arginine + thiamine + ZnO NPs group (Group-4) compared to the L-arginine group (Group-5), ($p \leq 0.05$). By calculating the mean of group 4, it was found that the values of surface area and volume density were increased to some extent approaching the value in the control group (G-1) ($p \leq 0.05$). The surface area and volume density of adventitia layer values in animals treated with L-arginine + thiamine + ZnO NPs (Group-4) was higher compared to the L-arginine group (Group-5); however, the statistical study did not reveal a significant difference ($p > 0.05$). The results of the BUN parameter measurement revealed that there was a significant difference between the control group and other studied groups ($p \leq 0.05$). The comparison of the thiamine group (group) with group 4 and L-arginine group (group5) indicated a significant decrease in urea level. Besides, the results indicated that there was a significant difference between groups 4 and 5 ($p \leq 0.05$). According to the evaluation of the cholesterol, the increase was significant between the control group with L-arginine (G-5) and group 4. The analysis of ALT and AST factors indicated a significant increase in the level of AST in comparison of the control group and L-arginine group; in addition, a significant difference was found between the L-arginine group and groups 2, 3, and 4. ($p \leq 0.05$). On the other hand, the results of the ALT evaluation manifested significantly lower levels in the comparison of the thiamine group (G-2) and ZnO NPs group (G-3) with the L-arginine group (Table 4). Meanwhile, the normal range for measured biochemical factors has been presented in Table 5.

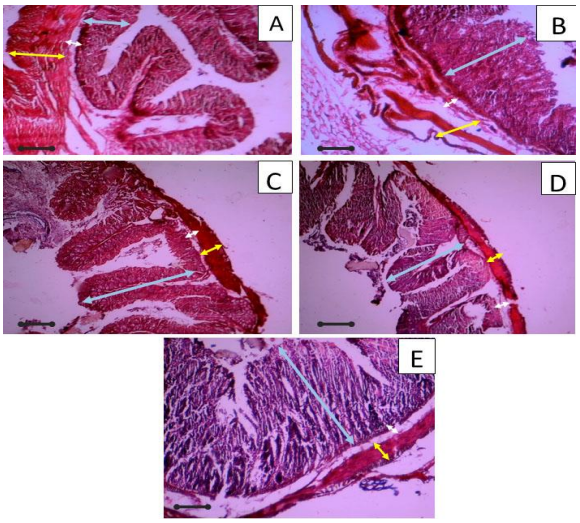

Figure 1: Photomicrograph of the layers' thickness of the cecum wall in different groups. (A): Control. (B): L-arginine. (C): L-arginine + thiamine + zinc oxide NPs. (D): ZnO NPs. (E): Thiamine. H&E; Scale bar = 15µm.

Table 1: Morphometric data on the thickness of cecal intestinal layers in control and treatment groups in rats.

Grouping		Thickness of layers (µm)				p value
		Thickness of mucosa layer	Thickness of muscle layer	Thickness of the adventitia layer	The thickness of the total layers	
G-1	Control	151.90±14.44	143.75±13.75	17.16±1.62	311.82±11.93	p≤0.05
G-2	Thiamine	150.01±12.70	136.01±15.09	16.02±1.84	302.04±9.87	p≤0.05
G-3	ZnO NPs	153.20±13.76	138.80±13.82	16.96±1.63	308.96±9.73	p≤0.05
G-4	L-A+ZnO NPs +Th	142.1±14.17**	118.02±11.83*	13.03±1.47*	283.15±8.49**	p≤0.05
G-5	L-A	107.80±9.81*	114.60±11.51*	10.76±1.05*	233.16±7.45*	p≤0.05

Differences between groups are shown with * than to controls and ** than to the 5th group in the same column which is statistically significant (p≤0.05).

Table 2: Effects of thiamine and Zn NPS on the surface area of cecum tissue layers in rats.

Grouping	Surface area of layers (µm^2)				p value
	The surface area of the mucosal layer	The surface area of the muscular layer	Surface area of adventitia layer	The total Surface area of the layers	
Group-1	0.69±0.035	0.30±0.04	0.058±0.006	1.098±0.027	p≤0.05
Group-2	0.70±0.039	0.28±0.02	0.046±0.004	1.026±0.028	p≤0.05
Group-3	0.65±0.042	0.26±0.05	0.043±0.008	0.933±0.033	p≤0.05
Group-4	0.68±0.025**	0.22±0.03**	0.028±0.005*	0.922±0.021**	p≤ 0.05
Group-5	0.56±0.032*	0.16±0.01*	0.026±0.001*	0.746±0.014*	p≤0.05

Differences between groups are shown with * than to controls and ** than to the 5th group in the same column which is statistically significant (p≤0.05).

Table 3: Effects of thiamine and Zn NPS on the volume of cecum tissue layers in rats.

Grouping	Volume of layers (μm³)				p value
	The volume of the mucosal layer	The volume of the muscular layer	The volume of the adventitia layer	The total Volume of the layers	
Group-1	3.45±0.17	1.75±0.20	0.29±0.05	5.49±0.32	p≤0.05
Group-2	3.5±0.19	1.40±0.17	0.23±0.03	5.13±0.41	p≤0.05
Group-3	3.15±0.21	1.30±0.12	0.21±0.04	4.66±0.28	p≤0.05
Group-4	3.40±0.12**	1.20±0.15**	0.16±0.02*	4.80±0.26**	p≤0.05
Group-5	2.80±0.16*	0.80±0.05*	0.13±0.005*	3.43±0.17*	p≤0.05

Differences between groups are shown with * than to controls and ** than to the 5th group in the same column which is statistically significant (p≤0.05).

Table 4: Biochemical factors values in different groups.

Grouping	Biochemical factors				p value
	Urea (mg/dl)	Cholesterol (mg/dl)	AST (u/l)	ALT (u/l)	
Group-1	29.28±0.96	75.00±4.07	49.28±2.46	185.85±13.81	p≤0.05
Group-2	30.60±1.83**	85.00±8.38	48.20±3.45	166.00±15.52**	p≤0.05
Group-3	32.50±2.53*	83.25±14.40	40.00±2.04	145.75±24.56**	p≤0.05
Group-4	35.16±1.27*	77.66±6.00*	42.00±5.53	198.66±20.29	p≤0.05
Group-5	49.40±1.12*	109.25±12.92*	60.80±2.74*	243.50±5.67	p≤0.05

Differences between groups are shown with * than to controls and ** than to the 5th group in the same column which is statistically significant (p≤0.05).

Table 5: Biochemical factors values in different groups of rats.

Biochemical factors	Urea (mg/dl)	Cholesterol (mg/dl)	AST (u/l)	ALT (u/l)
Normal range	10-43	80-200	0-31	0-31

DISCUSSION

Pancreatitis is an inflammatory disease that not only causes high mortality and numerous complications but also imposes a heavy cost on human societies. The side effects of this disease include septic shock, respiratory failure, kidney failure and heart problems [6]. Studies show that administration of high doses of L-arginine causes pancreatitis. The pathophysiology of AP is very complex and any damage to the intracellular and extracellular structure and the generation of free radicals would result in developing acute pancreatitis [34]. Besides being a substrate for nitric oxide synthetase, L-arginine also plays a role in inducing nitrostatic and oxidative stress. Although the exact mechanism of L-arginine-induced pancreatitis is not known, some studies indicate the role of l-

arginine metabolites, oxidative stress, and metabolic acidosis in pancreatic damage [8]. The obtained results demonstrated that after 3 days, injection of L-arginine with a dose of (500 mg/100 g) causes severe necrotic pancreatitis. Also, they have shown that L-arginine with a dose greater than (500 mg/100 g) will cause the death of most mice after a few hours [1]. The important role of mononuclear cells and free radicals in the development of pancreatitis is evident. L-arginine-induced pancreatitis is considered a well-known model of pancreatitis that is similar to acute pancreatitis in humans. [11]. Researchers illustrated that in pancreatitis induced by L-arginine, the amount of oxygen free radicals (ROS), especially malondialdehyde (MDA), increases in the pancreas and other organs away from the pancreas and the number of protective antioxidants such as glutathione peroxidase and superoxide dismutase are reduced. An increase in MDA and a decrease in antioxidants together cause oxidative stress in the Pancreas and other organs of the body. It has been proven that oxidative stress is an important factor in the development of pancreatitis [35]. Abdominal cavity syndrome (ACS) is one of the problems caused by acute pancreatitis syndrome (SAP) [36]; in which, increased intra-abdominal pressure (IAH) caused by ASC, causes a decrease in blood supply to the abdominal cavity organs [37], resulting in releasing cytokines into the general bloodstream and affecting the abdominal organs. Subsequently, general inflammation and accumulation of exudate fluid in the abdominal and the retroperitoneal cavity, followed by IAH and decreased blood supply occurs [37]. Following pancreatitis, phospholipase A2 is released from the pancreas and accumulates in the convoluted tubules of the kidney, which causes certain changes in their tissue structure [35]. Phospholipase A2 activates the lipo-oxygenase pathway, which reduces kidney blood pressure and oxygen supply to the convoluted tubules, and shows its destructive effect on the tissue structure of these tubules. It has been reported that IAH causes a decrease in blood supply to the renal cortex and thus decrease in its volume is observed [38]. Researchers have reported that pancreatitis causes fat necrosis in the testicular cord and tunica vaginalis. They claimed that the occurrence of fat necrosis includes the accumulation of a large number of white blood cells with polymorphous nuclei, and amorphous and basophilic calcium deposits on the surface of tunica vaginalis and testicular cord [38]. The release of inflammatory fluids and pancreatic secretions into the intestine and the abdominal cavity is one of the consequences of pancreatitis, which occurs due to the accumulation of inflammatory fluids in the pancreatic tissue and the space around the pancreas that causes the formation of cysts, abscesses, inflammation, and necrosis of other body organs. It is thought that the lipase enzyme, present in pancreatic secretions causes necrosis in tissues and organs far from the pancreas [39]. Other researchers showed that pancreatitis causes dysfunction of organs, including the intestines. In one of the studies, it was found that L-arginine-induced pancreatitis had different effects on the mean area of different cecum layers of the rats compared to the control group [5]. According to the results obtained in the present study, destruction and reduction of the thickness of the mucosal, muscle, and adventitia layers were observed in the L-arginine group (G5). It should also be noted that the reduction in the level of area and overall volume of the layers in this group is evident compared to the control group. The research results have proven that thiamine is essential for the normal function of pancreatic beta cells. Some studies show the effect of antioxidants in plants and drugs on reducing the effects of pancreatitis [13]. Thiamine functions as a crucial cofactor in numerous enzymatic processes that are present in almost all major

metabolic pathways. Within living organisms, thiamine dependent enzymes play a vital role in the decarboxylation of a-ketoacids, utilizing both non-oxidative and oxidative mechanisms. Through the investigation of the non-enzymatic decarboxylation of a-ketoacids, either by thiamine itself or by simpler thiazolium derivatives, scientists have gained extensive knowledge about the chemistry of thiamine. Building upon these findings, Breslow1 proposed a mechanism for the catalytic function of thiamine, which is widely accepted as the standard model today [40] In the group treated with thiamine, ZnO NPs, and L-arginine, a positive increasing effect on the thickness of the mucosal layer and the overall thickness of the layers was observed, compared to the L-arginine group. Studies also mentioned the toxic effects and changes in pancreatic cells caused by ZnO NPs nanoparticles [41]. Zinc oxide (ZnO) NPs induced oxidative DNA damage-mediated necrosis and increase basic ROS levels of macro-phages. Therefore, clinically, ZnO NPs, through this mechanism, can help the immune system in the clearance of inhaled particulates during inflammation. In fact, acute cationic nanocarrier-induced necrosis occurs via an interaction with the Na+/K+-ATPase and is associated with theexposure of molecular patterns dependent on mitochondrial damage that lead to inflammatory response. A novel mechanism by which cationic nanocarriers such as polyethylenimine(PEI), cationic liposomes, and chitosan, lead to rapid necrosisis related to their positive surface charges [42] From the results obtained in this study, the reduction in the volume and surface of cecum layers in the group treated with ZnO NPs has been observed compared to the control group. Meanwhile, the healing effects including the increase in the surface and volume of all layers except the adventitial layer were seen in group 4 compared to group 5. The physiological and hemodynamic functions of the liver and pancreas are closely related to each other due to their anatomical position. As a result of this anatomical relationship and the proximity of the bloodstream, the distribution of oxygen-free radicals in pancreatitis enters the liver and the process of liver damage begins [41]. In the present study, the evaluation of the serum level of AST shows an increase in this biochemical factor in the group treated with L-arginine compared to the control group. Also, a significant difference can be observed among groups 2, 3, and 4; While in the measurement of the serum level of ALT, a significant difference is seen in groups 2 and 3 compared to the L-arginine group. Body nitrogen balance is controlled via regulation of the urea production. In ureotelic animals such as mammals, the physiological significance of the urea cycle in the liver is to convert the cytotoxic ammonia to much less toxic urea, even though the synthesis has a net energy requirement. Urea is not further metabolized by mammalian tissues ,but urea markedly inhibits itself agenesis as negative feedback regulation. The complete urea cycle also exists in the enterocytes of mammals. Urea biosynthesis is susceptible to regulation by hormones. Insulin decreases the capacity for urea synthesis. In insulin-dependent diabetes mellitus, the generation of urea in rat liver is elevated through upregulation of CPS-1 and OTC, causing negative nitrogen balance. When cultured hepatocytes are supplemented with amino acids, high insulin preconditioning downregulates urea synthesis using downregulating CPS-1, OTC, ASS, and ARG-1 [26]. The urea synthesis rate is significantly reduced in patients with chronic pancreatitis whose glucagon secretion is impaired. The effect of glucagon administration on the urea cycle relies on up-regulating CPS-1, ASL, and ARG-1 in cultured fetal hepatocytes. Hyper-insulinemic-induced hypoglycemia patients, whose glucagon concentration is doubled, have a high rate of urea biosynthesis [43]. BUN, as a

single marker, is a useful, routine, easy-to-perform sensitive index to predict the severity and mortality of acute pancreatitis in early assessments. Pancreaticoduodenectomy, which is performed on patients with pancreatic carcinoma and some other conditions, has a high morbidity rate as a complication. A BUN level of 20 mg/dL or greater can help surgeons identify patients with an increased risk of morbidity and mortality after pancreaticoduodenectomy. another study showed that high BUN on the first postoperative day is associated with an increased occurrence and severity of complications, including the occurrence of pancreatic fistula [44]. The analysis of biochemical data in the present study showed that the amount of urea factor increased significantly in all groups compared to the control group. It should be noted that the amount of urea factor in the thiamine group was significantly lower than in groups 4 and 5. Also, the amount of this factor in the L-arginine group was higher than the normal range. Some studies indicated that the dysfunction of pancreatic beta cells can be caused by the accumulation of cholesterol. The accumulation of cholesterol and fatty acids in pancreatic beta cells plays a key role in the degeneration of pancreatic islets. these studies show that inhibition of cholesterol biosynthesis and overexpression of srebp2 in beta cells increases absorption and accumulation of cholesterol and disrupts cell function [45]. The evidence shows that dysfunction of beta cells can be due to disruption of membrane transporters involved in the removal of excess cholesterol (ABCA1) from cells. On the other hand, the accumulation of cholesterol in the endoplasmic reticulum reduces the necessary calcium deposits and disrupts the pancreas [46]. The results of another study demonstrated that the majority of dogs with naturally occurring pancreatitis (≤70%) had serum triglyceride and cholesterol concentrations within the normal range. Additionally, a small percentage of dogs showed an increase in serum triglyceride or cholesterol concentrations or both, and the increases were generally mild. Therefore, marked increases in serum triglyceride or cholesterol concentrations or both may not be related to pancreatitis. In contrast, evaluation of lipoprotein profiles in dogs with pancreatitis and the control group showed important differences; for instance, dogs with pancreatitis showed higher LDL amounts (mainly LDL2, LDL3, and LDL4) and lower TRL and HDL amounts (mainly HDL2a and HDL3c) in comparison to the control dogs [30]. A different study demonstrated a U-shaped association between cholesterol level and severity in acute pancreatitis. Patients with low total cholesterol levels (≤160 mg/dL) and high total cholesterol levels (≤240 mg/dL) were at a higher risk of SAP in comparison to patients with moderate total cholesterol levels (160-240 mg/dL). Therefore acute pancreatitis patients with high total cholesterol levels suffered from more inflammation [47]. The results of the cholesterol evaluation among the treated groups indicated a significant increase of this factor in groups 4 and 5 compared to group 1, in which a higher rate of cholesterol was observed.

CONCLUSION

Induction of experimental pancreatitis and subsequent administration of thiamine and ZnO NPs in the mice indicated that the thiamine and ZnO NPs not only improved the conditions by increasing values of the mucosa and total thickness significantly but also increased the values of surface area and volume density up to the normal level in comparison with the L-arginine treated group (G-5). In addition, the results revealed that thiamine and ZnO NPs had positive effects by decreasing the biochemical factor ALT level in comparison with the L-arginine group.

ACKNOWLEDGMENTS

The authors of this article would like to express their gratitude to the research assistant of Shahrekord University and the histology laboratory experts of Shahrekord University, Mr. Hatampour and Mr. Ahmadi.

ETHICS

Approved.

CONFLICT OF INTEREST

None.

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